GENETIKA 2006

September, 28th – October, 1st, 2006
Biološko središče, Večna pot 111, Ljubljana

4th Congress of Slovenian Genetic Society
and
2nd Meeting of The Slovenian Society of Human Genetics
with International Participation

IV. Kongres Slovenskega genetskega društva
in
II. srečanje Slovenskega društva za humano genetiko
z mednarodno udeležbo

SSHG

THE SLOVENIAN SOCIETY
OF HUMAN GENETICS

SLOVENSKO DRUŠTVO
ZA HUMANO GENETIKO

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Genetika 2006
4th Congress of Slovenian Genetic Society and 2nd meeting of The Slovenian Society of Human Genetics with international participation / IV. Kongres Slovenskega genetskega društva in II. srečanje Slovenskega društva za humano genetiko, z mednarodno udeležbo

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Book of Abstracts

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4th Congress of Slovenian Genetic Society and 2nd Meeting of The Slovenian Society of Human Genetics with International Participation

IV. Kongres Slovenskega genetskega društva in II. srečanje Slovenskega društva za humano genetiko, z mednarodno udeležbo

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WELCOME TO GENETIKA 2006

The congress Genetika 2006, which is organized by Genetic Society of Slovenia (GSS) and The Slovenian Society of Human Genetics (SSHG) is the foremost meeting of the Slovenian genetics community and is held every three years. The first congress of GSS was in 1997 and it contributed considerably to the promotion of genetics in Slovenia and laid down solid foundations for the future activities of the Society. Three years later the second congress of GSS was organized and was named Genetika 2000. The congress was a great success as it reached high »scientific excellence« and the participation nearly doubled. In addition the year 2000 was an important year for geneticists since the sequencing of human genome was completed giving good reason for celebration. The congress Genetika 2003 was marked by 50th anniversary of momentous discovery of the structure of DNA as well as with the rapid advances of »-omic« research. This was clearly reflected through excellent congress contributions and organisation of an even today very actual round table on ethical questions in genetics.

The tangible impact of the advances in genetics in particular in genomic science and biotechnology will make attendance at Genetika 2006 essential for genetic scientists as well as for health professionals, toxicologists, biotechnologists and alike. Genetika 2006 reflects rapid research advances of Slovenian geneticists, which can be seen form excellent contributions divided into seven thematic sessions. The broad scope of the congress will enable the participants to meet and exchange views and experience with colleges from Slovenia and numerous other countries. More than 130 contributions will be presented as invited lectures, oral and poster presentations. Particularly outstanding is the young researchers contest for the »Golden Chromosome« Award that is being presented by the GSS. We look forward to their presentations!

We would like to thank the members of Programme and Organizing committees for their contribution to the preparation of the sound scientific programme and enjoyable meeting. The sponsors' contributions have made the event possible and they are gratefully acknowledged.

Finally, we thank all the congress participants for their valuable contribution to the scientific recognition and success of Genetika 2006.

Welcome to Genetika 2006!

Metka Filipič
President of the Organizing committee of Genetika 2006 and
President of Genetic Society of Slovenia

Damjan Glavač
President of The Slovenian Society of Human Genetics

DOBRODOŠLI NA GENETIKI 2006

Kongres Genetika 2006, ki ga organizirata Slovensko genetsko društvo (SGD) in Slovensko društvo za humano genetiko (SSHG) je najpomembnejše srečanje slovenskih genetikov in se odvija vsake tri leta. Prvi kongres Slovenskega genetskega društva, ki je bil leta 1997, je odločilno vplival na napredek genetike v Sloveniji in postavil temelje za bodoče aktivnosti društva. Tri leta kasneje je bil organiziran drugi kongres SGD, ki je bil poimenovan Genetika 2000. Kongres je bil zelo uspešen saj je bila dosežena visoka "znanstvena odličnost", število udeležencev pa se je skoraj podvojilo. Poleg tega je bilo za genetike leto 2000 posebej pomembno, saj je bilo dokončno določeno zaporedje človeškega genoma, kar je bil dober razlog za praznovanje. Kongres Genetika 2003 je obeležila 50. obletnica pomembnega odkritja zgradbe DNA, kot tudi hiter napredek tako imenovanih "-omic" raziskav. To se je jasno izrazilo prek odličnih kongresnih prispevkov in prek organizacije okrogle mize na temo etičnih vprašanj v genetiki, ki je še danes zelo aktualna.

Zaradi očitnih vplivov napredka genetike, predvsem genomike in biotehnologije bo navzočnost na Genetiki 2006 zelo pomembna tako za genetike, kot za medicince, toksikologe, biotehnologe in podobne. Genetika 2006 odraža hiter raziskovalni napredek slovenskih genetikov, kar je razvidno iz odličnih prispevkov, ki bodo predstavljeni v sedmih tematskih skupinah. Širok okvir kongresa bo udeležencem omogočil, da se srečajo in izmenjajo poglede in izkušnje s slovenskimi kolegi in kolegi iz tujine. Več kot 130 prispevkov bo predstavljenih v obliki vabljenih predavanj ter ustnih in posterskih predstavitev. Posebno pomemben je natečaj za mlade raziskovalce za nagrado "Zlati kromosom" , ki jo podeljuje SGD. Veselimo se njihovih predstavitev!

Radi bi se tudi zahvalili Programskemu in Organizacijskemu odboru za sodelovanje pri pripravi znanstvenega programa in prijetnega srečanja. Prispevki sponzorjev so dogodek omogočili, zato njim dolgujemo posebno zahvalo.

Končno, se zahvaljujemo vsem udeležencem za njihov prispevek k znanstveni prepoznavnosti in uspehu Genetike 2006.

Dobrodošli na Genetiki 2006!

Metka Filipič
Predsednica organizacijskega odbora Genetika 2006 in
Predsednica Slovenskega genetskega društva

Damjan Glavač
Predsednik Slovenskega društva za humano genetiko

CONGRESS PROGRAMME
PROGRAM KONGRESA

Thursday / Četrtek 28. 9. 2006

10:00 - 18:00 Registration / Registracija

14:00 - 16:00 Genetic Resources and Diversity I / Genetski viri in raznolikost I
Chairs / Predsedujoči: Jasna Puizina, Branka Javornik

14:00 - 14:30 **Jasna Puizina**
Understanding meiosis in plants: an integration of cytological and molecular approach. / *Razumevanje mejoze pri rastlinah na osnovi citoloških in molekulskih podatkov.*
University of Split, Split, Croatia

14:30 - 14:50 **Branka Javornik**
Molecular genetic studies in hops. / *Molekularne genetske raziskave hmelja.*
University of Ljubljana, Ljubljana, Slovenia

14:50 - 15:05 **Jelka Šuštar - Vozlič**
Slovene autochthonous collection of crisp lettuce (Lactuca sativa L.): morphological and molecular variability, Bremia resistance. / *Morfološka in molekulska raznolikost slovenske avtohtone solate (Lactuca sativa L.) ter njena odpornost na solatno plesen.*
Agricultural Institute of Slovenia, Ljubljana, Slovenia

15:05 - 15:20 **Maruša Pompe - Novak**
Biological diversity of Grapevine fanleaf virus. / *Biološka raznovrstnost virusa pahljačavosti listov vinske trte.*
National Institute of Biology, Ljubljana, Slovenia

15:20 - 15:35 **Katja Drobnič**
The development of a sry assay useful for sex determination in forensic STR multiplex kits. / *Razvoj testa sry za določanje spola v forenzičnih kompletih STR.*
Ministry of the Interior, Ljubljana, Slovenia

15:35 - 15:50 **Andreja Ramšak**
Revealing of population structure in selected jellyfish species using genetic markers. / *Raziskovanje genetske strukture izbranih meduznih vrst z genetskimi markerji.*
National Institute of Biology, Ljubljana, Slovenia

15:50 - 16:05 **Dragomir Kompan**
Genetic diversity based on pedigree analysis of Slovenian sheep breeds. / *Genetska pestrost na osnovi analize porekla pri slovenskih pasmah ovc.*
University of Ljubljana, Ljubljana, Slovenia

16:30 Opening Ceremony / Otvoritvena slovesnost

17:00 Opening Lecture / Otvoritveno predavanje

17:00 **Ivan Kreft**
The Unfinished Story of the Genetics in Slovenia. / *Nedokončana zgodba o genetiki pri Slovencih.*
University of Ljubljana, Ljubljana, Slovenia

18:00 - 20:00 Welcome Reception / Otvoritveni sprejem

Friday / Petek 29. 9. 2006

8:00 - 10:00 Mutagenesis and Genome Environmental Interactions / Mutageneza in medsebojni vplivi genoma in okolja
Chairs / Predsedujoči: Bo Lambert, Metka Filipič

8:00 - 8:30 **Bo Lambert**
On the causes and nature of HPRT mutation in human T-cells in vivo. / *Vzroki in narava HPRT mutacije v človeških T-celicah in vivo.*
The Karolinska Institute, Sweden

8:30 - 8:50 **Paola Fortini**
Decreased efficiency of Base Excision Repair in terminally differentiated muscle cells. / *Zmanjšana učinkovitost baznega izrezovalnega popravljana v končno diferenciranih mišičnih celicah.*
Istituto Superiore di Sanità, Rome, Italy

8:50 - 9:10 **Raffaella Corvi**
Alternative test methods in genotoxicity and carcinogenicity. / *Alternativne testne metode za ugotavljanje genotoksičnosti in karcinogenosti.*
European Commission Joint Research Centre, Ispra, Italy

9:10 - 9:30 **Tamara Lah**
Autocrine and paracrine effects of downregulation of lysosomal protease genes in normal and neoplastic cells - review. / *Autokrini in parakrini učinki znižanja izražanja genov lizosomskih proteaz v normalnih in neoplastičnih celicah - pregled.*
National Institute of Biology, Ljubljana, Slovenia

9:30 - 9:45 **Francizca Ferk**
Antioxidant and free radical scavenging activities of sumac (Rhus coriaria) and identification of gallic acid as its active principle. / *Antioksidativne lastnosti in sposobnost prestrezanja prostih radikalov ruja (Rhus coriaria) ter identifikacija galne kisline kot aktivne učinkovine.*
Medical University of Vienna, Vienna, Austria

9:45 - 10:00 **Ninoslav Djelić**
Mechanisms of genotoxic and mutagenic effects of hormones. / *Mehanizmi genotoksičnega in mutagenega delovanja hormonov.*
University of Belgrade, Belgrade, Serbia

10:00 - 10:30 Coffe break / Odmor

10:30 - 12:30 Genomic Technologies I / Genomske tehnologije I
Chairs / Predsedujoči: Simon Horvat, Mojca Narat

10:30 - 11:00 **Calvin L. Keeler, Jr.**
Microarray technology and its use in studying avian innate immunity. / Tehnologija mikromrež in njena uporaba pri raziskavah prirojene imunosti pri pticah.
University of Delaware, Newark, Delaware, USA

11:00 - 11:20 **Teresa Lettieri**
DNA Microarray Technology: Application to Ecotoxicology. / *Tehnologija DNA mikromrež: uporaba v ekotoksikologiji.*
European Commission Joint Research Centre, Ispra, Italy

11:20 - 11:40 **Kristina Gruden**
Towards better understanding of plant-pathogen / pests interactions - Expression profiling as a tool in systems biology. / *Analiza interakcij med rastlino in patogenom oz. škodljivcem - ekspresijsko profiliranje kot orodje sistemske biologije.*
National Institute of Biology, Ljubljana, Slovenia

11:40 - 12:00 **Boris Zagradišnik**
Application possibilities for multiplex ligation dependent probe amplification (MLPA). / *Aplikacijske možnosti metode pomnoževanja od ligacije odvisnih sond (MLPA).*
Maribor Teaching Hospital, Maribor, Slovenia

12:00 - 12:15 **Jernej Jakše**
Comparative genetic and sequence analyses of asparagus BACs reveal no microsynteny with onion or rice. / *Primerjalna genetska in sekvenčna analiza umetnih bakterijskih kromosomov (BAC) šparglja v primerjavi s čebulo in rižem potrjuje neohranjeno zaporedje genov na mikro nivoju.*
University of Ljubljana, Ljubljana, Slovenia

12:15 - 12:30 **Andreas Jarrin**
Recent developments in real-time PCR- The Eppendorf Mastercycler EP realplex. / *Najnovejši razvoj pri PCR v realnem času - The Eppendorf Mastercycler EP.*
Eppendorf AG, Hamburg, Germany

12:30 - 14:00 Lunch & Poster view / Kosilo & ogled posterjev

14:00 - 16:00 Bioinformatics and Biostatistics / Bioinformatika in biostatistika
Chairs / Predsedujoči: Gregor Anderluh, Milena Kovač

14:00 - 14:30 **Jure Piškur**
How did Saccharomyces yeasts evolve to become good brewers? / *Kako so se razvijale kvasovke Saccharomyces, da so postale dobre pivovarke?*
Lund University, Lund, Sweden

14:30 - 14:50 **Blaž Zupan**
Computational Phenomics. / *Računska fenomika.*
University of Ljubljana, Ljubljana, Slovenia

14:50 - 15:05 **Uroš Petrovič**
Combination of mutant and expression data to predict the molecular mechanism of action of perturbations to yeast cells. / *Kombiniranje podatkov o rasti mutant in o izražanju genov za napovedovanje molekulskih mehanizmov delovanja perturbacij na celice kvasovke.*
Jožef Stefan Institute, Ljubljana, Slovenia

15:05 - 15:25 **Milena Kovač**
Teoretical aspects - Statistical analysis from crossbreeding schemes. / *Teoretična izhodišča - Statistična analiza podatkov iz križanj.*
University of Ljubljana, Ljubljana, Slovenia

15:25 - 15:45 **Špela Malovrh**
Genetic evaluation for litter size in swine by joint purebred and crossbred data. / *Genetsko vrednotenje velikosti gnezda na podatkih čistopasemskih in hibridnih prašičev.*
University of Ljubljana, Ljubljana, Slovenia

15:45 - 16:00 **Andreja Komprej**
Heritability estimates for milk traits with regression models in dairy sheep in Slovenia. / *Ocene heritabilitet za lastnosti mlečnosti s pomočjo regresijskih modelov pri mlečnih ovcah v Sloveniji.*
University of Ljubljana, Ljubljana, Slovenia

16:00 - 16:30 Coffe break / Odmor

16:30 - 18:45 Golden Chromosome / Zlati kromosom

16:30 - 16:40 **Gašper Berginc**
DHPLC based method using mononucleotide repeats and pentaplex PCR for rapid and accurate analysis of microsatellite instability in colorectal cancer. / *Metoda na osnovi tehnologije DHPLC in uporabe mononukleotidnih tandemskih ponovitev ter multiple PCR za hitro in zanesljivo analizo mikrosatelitne nestabilnosti pri kolorektalnem raku.*
University of Ljubljana, Ljubljana, Slovenia

16:40 - 16:50 **Emanuela Boštjančič**
Molecular characterization of erythropoietic protoporphyria (EPP) in Slovenia - identification of novel mutations in the ferrochelatase gene. / *Molekularna karakterizacija eritropoetske protoporfirije v Sloveniji - odkritje novih sprememb v genu za ferokelatazo.*
University of Ljubljana, Ljubljana, Slovenia

16:50 - 17:00 **Matej Butala**
Temperature dependent colicin K synthesis. / *S temperaturo uravnana sinteza kolicina K.*
University of Ljubljana, Ljubljana, Slovenia

17:00 - 17:10 **Pablo Hirschegger**
Cytogenetical and Morphological Studies of Novel Great Headed Garlic (Allium sp.) Accessions. / *Citogenetske in morfološke raziskave novih akcesij poletnega luka (Allium sp.).*
University of Ljubljana, Ljubljana, Slovenia

17:10 - 17:20 **Sebastijan Hobor**
Genetic variations of the horse kappa casein gene (Csn3) and comparative genomics approach to study conserved regions. / *Genetska variabilnost kapa kazeinskega gena (Csn3) pri konju in pristop primerjalne genomike za študij ohranjenih področji.*
University of Ljubljana, Ljubljana, Slovenia

17:20 - 17:30 **Irena Hreljac**
Genotoxicity of organophosphorous pesticides correlates with the induction of DNA damage responsive genes. / *Genotoksičnost organofosfatnih pesticidov korelira z indukcijo izražanja genov, ki se odzovejo na poškodbe DNA.*
National Institute of Biology, Ljubljana, Slovenia

17:30 - 17:40 **Jana Jelerčič**
Microsatellite marker for homozygosity testing of Mimulus aurantiacus. / *Testiranje homozigotnosti pri vrsti Mimulus aurantiacus s pomočjo microsatelitnega markerja.*
University of Ljubljana, Ljubljana, Slovenia

17:40 - 17:50 **Peter Kozmus**
Genetic characterization of bumblebees (Hymenoptera: Apidae) in Slovenia. / *Genetska karakterizacija čmrljev (Hymenoptera: Apidae) v Sloveniji.*
National Institute of Biology, Ljubljana, Slovenia

17:50 - 18:00 **Miha Lavrič**
Molecular characterization of chicken immunocompetent cell response to Mycoplasma synoviae haemagglutinins. / *Molekularna karakterizacija odziva kokošjih imunsko zmožnih celic na prisotnost hemaglutininov izoliranih iz bakterije Mycoplasma synoviae.*
University of Ljubljana, Ljubljana, Slovenia

18:00 - 18:10 **Marko Maras**
Characterization of Slovene common bean genetic resources by molecular, biochemical and morphological markers. / *Karakterizacija slovenskih genskih virov navadnega fižola z molekulskimi, biokemijskimi in morfološkimi markerji.*
Agricultural Institute of Slovenia, Ljubljana, Slovenia

18:10 - 18:20 **Suzana Mesojednik**
Transfection efficiency of electrically-assisted gene delivery to tumors is tumor type and time dependent. / *Učinkovitost vnosa genov v tumorje s pomočjo elektroporacije in vivo je odvisna od tipa tumorja in časovnega intervala.*
Institute of Oncology Ljubljana, Slovenia

18:20 - 18:30 **Vid Mlakar**
DNA microarrays and hereditary eye diseases. / *DNA čipi in dedne očesne bolezni.*
University of Ljubljana, Ljubljana, Slovenia

18:30 - 18:40 **Andrej Razpet**
The construction of bovine-human synteny map using available mapped bovine markers. / *Izdelava karte sintenije med govedom in človekom na osnovi razpoložljivih označevalcev na genomu goveda.*
University of Ljubljana, Ljubljana, Slovenia

18:40 - 18:50 **Matjaž Simončič**
Physiological and microarray analyses of cholesterol homeostasis in the polygenic mouse model of obesity. / *Fiziološke analize in analize mikromrež homeostaze holesterola pri poligenem modelu miši za debelost.*
University of Ljubljana, Ljubljana, Slovenia

18:50 - 19:00 **Mojca Stražišar**
Sporadic and familial CJD in Slovenia: molecular classification and characterisation. / *Sporadične in družinske oblike CJD v Sloveniji: molekularna klasifikacija in karakterizacija.*
University of Ljubljana, Ljubljana, Slovenia

Saturday / Sobota, 30. 9. 2006

8:00 - 10:00 Biotechnology / Biotehnologija
Chairs / Predsedujoči: Zsusza Bősze, Peter Dovč

8:00 - 8:30 **Mathias Müller**
Studying human pathogens in animal models: Fine tuning a humanized mouse. / *Študij človeških patogenov z živalskimi modeli: natančno prirejena humanizirana miš.*
University of Natural Resources and Applied Life Sciences, Tulln, Austria

8:30 - 8:50 **Peter Dovč**
Molecular mechanisms involved in the regulation of lactoprotein gene expression. / *Molekulski mehanizmi, ki vplivajo na uravnavanje izražanja laktoproteinskih genov.*
University of Ljubljana, Ljubljana, Slovenia

8:50 - 9:10 **Zsusza Bősze**
Transgenic rabbits as models for producing biologically active human recombinant proteins. / *Transgeni kunci kot model za proizvodnjo biološko aktivnih rekombinantnih človeških proteinov.*
Agricultural Biotechnology Center, Gödöllő, Hungary

9:10 - 9:30 **Oscar R: Burrone**
Genetic vaccines for B-cell lymphomas. / *Genetske vakcine za B celične limfome.*
International Centre for Genetic Engineering and Biotechnology, Trieste, Italy

9:30 - 9:45 **Matic Legiša**
Increased productivity of recipient commercial micro-organisms after the insertion of modified pfkA gene from Aspergillus niger. / *Povečanje produktivnosti komercialnih mikroorganizmov po vnosu spremenjenega pfkA gena glive Aspergillus niger.*
National Institute of Chemistry, Ljubljana, Slovenia

9:45 - 10:00 **Francesca Caloni**
Alternative methods to animal use and 3Rs in veterinay toxicology. / *Alternativne metode za nadomeščnje uporabe živali in 3R v veterinarski toksikologiji.*
University of Milan, Milan, Italy

10:00 - 10:30 Coffe break / Odmor

10:30 - 12:30 Pharmacogenomics / Farmakogenomika
Chairs / Predsedujoči: Xavier Gidrol, Irena Mlinarič-Raščan

10:30 - 11:00 **Xavier Gidrol**
Integration of genome-wide data to infer genetic networks. / *Povezovanje genomskih podatkov za izgradnjo genetskih mrež.*
CEA / DSV-Service de Génomique Fonctionnelle Genopole, d'Evry, France

11:00 - 11:20 **Irena Mlinarič - Raščan**
Pharmacogenomic approaches in novel drug discovery. / *Farmakogenomika v procesu razvoja novih zdravil.*
University of Ljubljana, Ljubljana, Slovenia

11:20 - 11:40 **Miho Ohsugi**
The chromokinesin kid specifically safeguards early stage embyonic cleavages. / *Kromokinezin kid specifično varuje cepitve v zgodnji embrionalni fazi.*
University of Tokyo, Tokyo, Japan

11:40 - 11:55 **Mateja Lopuh**
Influence of 118 A>G polymorphism in the OPRM1 gene on the treatment efficiency with transdermal fentanyl. / *Vpliv polimorfizma 118A>G v genu za OPRM1 na učinkovitost zdravljenja bolečine s fentanilom v transdermalnem obližu.*
University of Ljubljana, Ljubljana, Slovenia

11:55 - 12:10 **Barbara Ostanek**
Gilbert's syndrome in Slovenian population - a new case of a $(TA)_8$ allele in the UGT1A1 gene promoter in Caucasians. / *Gilbertov sindrom v slovenski populaciji - nov primer alela $(TA)_8$ v promotorju gena za UGT1A1 pri Kavkazijcih.*
University of Ljubljana, Ljubljana, Slovenia

12:10 - 13:00 Lunch / Kosilo

13:00 - 15:30 Genomic Technologies II / Genomske tehnologije II
Chairs / Predsedujoči: Damjan Glavač, Uroš Potočnik

13:00 - 13:30 **Paolo Gasparini**
Mapping and cloning of disease genes - past and present. / *Mapiranje in kloniranje bolezenskih genov - preteklost in sedanjost.*
University of Trieste, Trieste, Italy

13:30 - 13:55 **Ioannis M Stylianou**
Mining Gene Expression, Proteomics and Haplotypes for Complex Trait Genes. / *Integrirana uporaba podatkov genoma (haplotipov), transkriptoma in proteoma za študije kompleksnih lastnosti.*
The Jackson Laboratory, Bar Harbor, USA

13:55 - 14:15 **Maja Čemažar**
Electrogene therapy of cancer. / *Elektrogenska terapija raka.*
Institute of Oncology, Ljubljana, Slovenia

14:15 - 14:35 **Zlatko Trajanoski**
Can Molecular Mechanisms be Revealed by Large-Scale Expression Profiling? / *Ali je možno odkrivati molekularne mehanizme z obsežnim prerezom prek izražanja genov?*
Graz University of Technology, Graz, Austria

14:35 - 14:55 **Uroš Potočnik**
Gene discovery in Inflammatory bowel diseases- lessons for complex diseases? / *Odkrivanje genov za kronične vnetne črevesne bolezni - model za genetske študije kompleksnih bolezni?*
University of Maribor, Maribor, Slovenia

14:55 - 15:10 **Alenka Erjavec - Škerget**
The use of subtelomeric FISH, MLPA and CGH to investigate chromosomal rearrangements associated with mental retardation. / *Uporaba subtelomerne FISH, MLPA in PGH za iskanje kromosomskih sprememb pri idiopatski duševni manjrazvitosti.*
Maribor Teaching Hospital, Maribor, Slovenia

15:10 - 15:25 **Denis Lobidel**
Ultra-High Throughput SNP Analysis with Beckman Coulter's GenomeLab SNPstream Genotyping System. / *Ultra-visoko zmogljivostna analiza z Beckman Coulter's GenomeLab SNPstream sistemom za genotipizacijo.*
Beckman Coulter, UK

15:25 - 16:00 Coffe break / Odmor

16:00 - 18:30 Genetic Diseases I / Genetske bolezni I
Chairs / Predsedujoči: Metka Ravnik-Glavač, Giorgio Stanta

16:00 - 16:20 **Giorgio Stanta**
New strategies for molecular medicine development. / *Nove strategije na področju razvoja molekularne medicine.*
University of Trieste, Trieste, Italy

16:20 - 16:40 **Metka Ravnik - Glavač**
Genetic screening of microsatellite instability in colorectal cancer. / *Genetsko presejanje mikrosatelitno nestabilnega kolorektalnega raka.*
University of Ljubljana, Ljubljana, Slovenia

16:40 - 17:00 **Nina Canki - Klain**
Clinical, genetic and epidemiologic characteristics of major limb girdle muscular dystrophies (LGMDS). / *Klinične, genetske in epidemiološke značilnosti najbolj pomembnih obročastih mišičnih distrofij (limb-girdle muscular dystrophies - LGMDS).*
Zagreb University Medical School, Zagreb, Croatia

17:00 - 17:20 **Gordana Petruševska**
Evaluation of Her2 gene status in breast cancer by chromogenic in situ hybridization. / *Ocena statusa Her2 gena pri raku dojke z kromogeno in situ hibridizacijo.*
Medical Faculty, Skopje, R. Macedonia

17:20 - 17:40 **Martina Jarc Vidmar**
Rare mutations of the VMD2 gene in Slovenian families with Best's vitelliform macular dystrophy. / *Redke mutacije gena VMD2 pri slovenskih družinah z Bestovo viteliformno makularno distrofijo.*
Eye Clinic, Medical Centre Ljubljana, Slovenia

17:40 - 18:00 **Martina Witsch - Baumgartner**
Population genetics of the Smith-Lemli-Opitz syndrome. / *Populacijska genetika Smith-Lemli-Optiz-ovega sindroma.*
Medical University Innsbruck, Insbruck, Austria

18:00 - 18:15 **Hackemi Zeraia**
NCodeTM miRNA Analysis platform identifies miRNA biomarkers in Colon cancer. / *Analitska platforma NCodeTM miRNA identificira miRNA biomarkerje pri raku črevesa.*
Invitrogen Corporation, Inchinnan, Scotland, UK

18:15 - 19:00 Redna letna skupščina Slovenskega društva za humano genetiko

20:00 Congress Dinner / Svečana večerja

Sunday / Nedelja, 01. 10. 2006

8:30 - 11:00 Genetic Diseases II / Genetske bolezni II
Chairs / Predsedujoči: Matija Peterlin, Nadja Kokalj-Vokač

8:30 - 9:00 **Matija Peterlin**
Central tolerance and AIRE: mutations in APECED patients. / *Splošna toleranca do lastnih antigenov in avtoimunska regulacija: mutacije pri bolnikih z avtoimunskimi endokrinološkimi boleznimi (APECED).*
University of California San Francisco, San Francisco, USA

9:00 - 9:20 **Peter M. Kroisel**
A rare variant of Tietz Syndrome. / *Redka variacija Tietz - ovega sindroma.*
University of Greifswald, Greifswald, Germany

9:20 - 9:40 **Anamarija Meglič**
The molecular diagnostics in children with hereditary hematuria: the differential diagnostic questions and ethical dilemmas in clinical practise. / *Molekularnogenetska analiza pri otrocih z dednimi hematurijami: diferencialno diagnostična vprašanja in etične dileme v klinični praksi.*
Medical Centre Ljubljana, Ljubljana, Slovenia

9:40 - 10:00 **Rubens Jovanović**
Evaluation of telomerase activity in patients with chronic B lymphocytic leukemia versus age matched controls. Correlation between the telomerase activity and bone marrow infiltration. / *Ocena telomerazne aktivnosti pri pacientih s kronično B limfatično levkemijo proti kontrolni skupini. Korelacija med telomerazno aktivnostjo in infiltracijo kostnega mozga.*
Medical Faculty, Skopje, R. Macedonia

10:00 - 10:15 **Mirna Štabuc - Šilih**
Rare VSX1 gene variations in sporadic and hereditary keratoconus patients. / *Redke variacije v genu VSX1 pri sporadični in dedni obliki keratukonusa.*
University Clinical Centre Ljubljana, Ljubljana, Slovenia.

10:15 - 10:30 **Ksenija Geršak**
X chromosome mosaicism in women with premature ovarian failure and recurrent pregnancy loss. / *Mozaicizem kromosoma X pri ženskah s prezgodnjo menopavzo in s ponavljajočimi splavi.*
University Clinical Centre, Ljubljana, Slovenia

10:30 - 10:45 **Špela Stangler Herodež**
Molecular - genetics and molecular-cytogenetics methods in diagnosis of hereditary motor and sensory neuropathy (HNSN) type 1A. / *Molekularno-genetske in molekularno-citogenetske tehnike pri diagnostiki mišično senzorne nevropatije (HMSN) tipa 1A.*
Maribor Teaching Hospital, Maribor, Slovenia

10:45 - 11:00 **Marina Mencinger**
Genetic testing for cystic fibrosis. / *Genetsko testiranje za cistično fibrozo pri odraslih bolnikih.*
The University Clinic of Pulmonary and Allergic Diseases Golnik, Golnik, Slovenia

11:00 - 11:30 Coffe break / Odmor

11:30 - 12:40 Genetic Resources and Diversity II / Genetski viri in raznolikost II
Chairs / Predsedujoči: Levente Emődy, Darja Žgur-Bertok

11:30 - 12:00 **Levente Emődy**
Evolution of Escherichia coli virulence. / *Evolucija virulence bakterije Escherichia coli.*
University of Pécs, Pécs, Hungary

12:00 - 12:20 **Darja Žgur - Bertok**
Heterogeneity in expression of the Escherichia coli colicin K activity gene cka is controlled by the SOS system, LexA binding affinity, and stochastic factors. / *Izražanje gena kolicina K pri bakteriji Escherichia coli je nadzorovano s sistemom SOS, afiniteto vezave proteina LexA in stohastičnimi faktorji.*
University of Ljubljana, Ljubljana, Slovenia.

12:20 - 12:40 **Ines Mandić - Mulec**
Functional and structural polymorphism of a quorum sensing system in Bacillus subtilis strains isolated from a soil aggregate. / *Funkcijska in strukturna variabilnost komunikacijskega sistema talnih izolatov Bacillus subtilis.*
University of Ljubljana, Ljubljana, Slovenia

12:40 - 13:20 Zaključno predavanje / Closing lecture

12:40 **Steve Busby**
Transcriptional regulation in bacteria revisited. / *Uravnavanje transkripcije pri bakterijah.*
University of Birmingham, Birmingham, United Kingdom

13:20 Closing remarks / Zaključek kongresa

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OPENING LECTURE
UVODNO PREDAVANJE

Ivan Kreft
Department of Agronomy, Biotechnical faculty, University of Ljubljana, Ljubljana, Slovenia

Date and place of birth:
23 November 1941, Novo mesto, Slovenija

Bachelor's degree:
1965
University of Ljubljana, Department of Agronomy,

1968
Genetics and plant physiology: University of Lund, Sweden

Master's degree:
1968
Genetics: University of Lund, Sweden

Doctor's degree:
1972
University of Ljubljana, Biotechnical Faculty, Department of Agronomy

From 1983: Professor of Genetics, Biotechnical faculty, University of Ljubljana.
1992 - 1993: visiting professor at Kyoto University, Department for Doctoral Studies.
From 2001: associated member of the Slovenian Academy of Science and Arts.
From 2003: full member of the Slovenian Academy of Science and Arts.

Awards:
1997 – Golden Award of University of Ljubljana
1997 – Honorary member of Ukrainan Academy of science and higher education
2001 – Honorary visiting professorship at Shanxi University, Biotechnology Department, Taiyuan, China
2002 – Jesenko Award of Biotechnical Faculty, University of Ljubljana
2005 – Ministry of Science and Education Award »Ambasador znanosti«

Selected publications:

- Škrabanja, V, Kreft, I. Resistant starch formation following autoclaving of buckwheat (Fagopyrum esculentum Moench) groats. An in vitro study. J. agric. food chem., 1998, 46, 2020-2023.
- Škrabanja, V., Liljeberg, H. G. M., Hedley, C. L., Kreft, I., Björck, I. M. E. Influence of genotype and processing on the in vitro rate of starch hydrolysis and resistant starch formation in peas (Pisum sativum L.). J. agric. food chem., 1999, 47, 2033-2039.
- Kreft, S, Knapp, M, Kreft, I. Extraction of rutin from buckwheat (Fagopyrum esculentum Moench) seeds and determination by capillary electrophoresis. J. agric. food chem., 1999, 47, 4649-4652.
- Kreft, S, Štrukelj, B, Gaberščik, A, Kreft, I. Rutin in buckwheat herbs grown at different UV-B radiation levels: comparison of two UV spectrophotometric and an HPLC method. J. Exp. Bot., 2002, 53, 1801-1804.
- Fabjan, N, Rode, J, Košir, IJ, Wang, Z, Zhang, Z, Kreft, I. Tartary buckwheat (Fagopyrum tataricum Gaertn.) as a source of dietary rutin and quercitrin. J. agric. food chem., 2003, 51, 6452-6455.

THE UNFINISHED STORY OF THE GENETICS IN SLOVENIA

Ivan Kreft
Biotechnical Faculty, University of Ljubljana, Slovenia

Among Slovenians, genetics was involved in many controversies. It started with Fran Jesenko; his workspace was spread from Škofja Loka to Vienna, from Paris to Russia, and from Sweden to Egypt. As well other early developments (mainly those in the first half of the 20th century) in genetics in Slovenia, and by Slovenian scientists working abroad, is presented in the lecture.

NEDOKONČANA ZGODBA O GENETIKI PRI SLOVENCIH

Ivan Kreft
Biotehniška fakulteta, Univerza v Ljubljani, Ljubljana, Slovenija

Pri Slovencih je razvoj genetike povezan z mnogimi spornimi vprašanji. Začenja se že s samim Franom Jesenkom, znanstvenikom, ki je deloval od Škofje Loke do Dunaja, od Pariza do Rusije in menda tudi od Švedske do Egipta. Pri predavanju bo predstavljenih tudi nekaj drugih posebnosti zgodnjega razvoja genetike (s poudarkom na prvi polovici dvajsetega stoletja) pri Slovencih, ki so delovali doma ali v tujini.

CLOSING LECTURE
ZAKLJUČNO PREDAVANJE

Stephen John Williams Busby
School of Biochemistry/Biosciences, University of Birmingham, United Kingdom

Date and place of birth:
5 March 1951, Oldham, UK

1969-1972 – BA
Sidney Sussex College, Cambridge

1972-1975 – PhD
Department of Biochemistry
University of Oxford

1975-1979 – Post-doc
Pasteur Institute, Paris

1979-1980 – Fogarty Visiting Associate
Laboratory of Molecular Biology, National Cancer Institute, National Institute of Health, Bethesda, Maryland, USA

1980-1983 – Staff Scientist, Pasteur Institute, Paris

1983-1988 – Lecturer, Department of Biochemistry, University of Birmingham

1988-1990 – Senior Lecturer, School of Biochemistry, University of Birmingham

1990-1995 – Royal Society Research Fellow and Reader in Biochemistry

1995-present – Professor, School of Biochemistry/Biosciences, University of Birmingham

2000-2002 – Dean of Science, University of Birmingham

2002-2004 – Dean of Life and Health Sciences, University of Birmingham

RECENT PUBLICATIONS (since 2005)

- Spreadbury, C, Pallen, M, Overton, T, Behr, M, Mostowy, S, Spiro, S, Busby, S & Cole, J (2005) Point mutations in the DNA- and cNMP-binding domains of the homologue of the cAMP receptor protein in *Mycobacterium bovis* BCG: implications for the inactivation of a global regulator and strain attenuation. *Microbiology* **151** 547-556
- Kovacs, A., Rakhely, G, Browning, D, Fulop, A, Maroti, G, Busby, S & Kovacs, K (2005) An FNR-type regulator controls the anaerobic expression of Hyn hydrogenase in *Thiocapsa roseopersicina*. *J. Bacteriol.* **187** 2618-2627
- Browning, D, Grainger, D, Beatty, C, Wolfe, A, Cole, J & Busby, S (2005) Integration of three signals at the *Escherichia coli nrf* promoter: a role for Fis protein in catabolite repression. *Molecular Microbiology* **57** 496-510
- Grainger, D, Busby, S, Hurd, D, Holdstock, J & Harrison, M (2005) How do bacteria turn their genes on and off? *Genetic Engineering News* **25** 46-47
- Grainger, D, Hurd, D, Harrison, M, Holdstock, J & Busby, S (2005) Studies of the distribution of *Escherichia coli* cAMP receptor protein and RNA polymerase along the *E. coli* chromosome. *Proc Natl Acad Sci USA* **102** 17693-17698

TRANSCRIPTIONAL REGULATION IN BACTERIA REVISITED

Steve Busby
University of Birmingham, School of Biosciences, UK

The expression of most bacterial genes is principally regulated at the level of the initiation of transcription. The contributions of promoter elements, sigma factors and transcription factors to this regulation at simple promoters are well understood. It is now appreciated that many promoters are directly regulated by several transcription factors. I will present recent work that elucidates some of the mechanisms by which the contributions of different factors are integrated at complex promoters. In some cases, nucleoid-associated proteins play an essential role in this integration. Recent advances that reveal the distribution of transcription factors across bacterial genomes will be discussed.

Viri/References: Browning, D & Busby, S (2004) The regulation of bacterial transcription initiation. *Nature Reviews Microbiology* **2** 57-65
Grainger, D, Hurd, D, Harrison, M, Holdstock, J & Busby, S (2005) Studies of the distribution of *Escherichia coli* cAMP receptor protein and RNA polymerase along the *E. coli* chromosome. *Proc Natl Acad Sci USA* **102** 17693-17698

GENETIC RESOURCES I
GENETSKI VIRI I

UNDERSTANDING MEIOSIS IN PLANTS: AN INTEGRATION OF CYTOLOGICAL AND MOLECULAR APPROACH

Jasna Puizina
Department of Biology, Faculty of Natural Sciences, Mathematics and Education, University of Split, Teslina12, 21000 Split

Meiosis is a complex multistep process that includes chromosome pairing, synaptonemal complex formation and crossing over, recombination and disjunction of homologues chromosomes and cytokinesis. A single round of DNA replication followed by two successive nuclear divisions leads to the chromosome number reduction, accurate chromosome transmission and genetic recombination. Although the process of meiosis in higher plants has been studied cytologically for over a century, and many meiotic mutants have been isolated and described, until recently the resources have not been available for an integration of cytological, genetic and molecular approaches to the analysis of plant meiosis. The situation has changed by the adoption of as a model plant system, very well amenable to such an integrated approach. The complete sequence of its genome, genes identification and characterization based on both forward and reverse genetic approach, publicly available collections of mutants, in combination with improved techniques of molecular cytogenetics and immunogenetics have greatly improved our understanding of meiosis and its control in plants. Particularly advantageous feature of meiosis analyses in is that mutations affecting meiotic genes usually do not trigger a cell-cycle check point, so all stages of aberrant meiosis can be studied, in contrast to yeast and mammals, where functional analyses of meiotic genes are frequently hampered by the lethal consequences of their mutations. This presentation will focus on recent work that is contributing to a better understanding of the key meiotic events involved in meiotic recombination and DNA repair in Arabidopsis thaliana.

MOLECULAR GENETIC STUDIES OF HOPS

Branka Javornik
University of Ljubljana, Biotechnical Faculty, Jamnikarjeva 101, 1000 Ljubljana, Slovenia

Hop growing has a tradition of more than 100 years in Slovenia, and there is significant agricultural production oriented towards export. The Slovenian Institute for Hop Research and Brewing (SIHRB) has released eleven hop varieties, four of which occupy the majority of hop fields in Slovenia. Such a varietal structure emphasizes the importance of "regional" hop breeding and the adaptation of hop varieties to specific growing conditions. Hop genetic studies have been set up in order to assist the hop breeding program at the SIHRB. The main approach of genetic research is rational use of the genetic diversity available from the hop germplasm collection, and gene incorporation or introgression by means of hop genome mapping, marker-assisted selection and/or cloning. Major effort is directed towards improving hop quality (resin content) and resistance to biotic stresses (Verticillium wilt and hop Damson aphid). We present a review on our research work on the development of the hop molecular markers, on the assessment of genetic variability and genome mapping and analysis of QTL markers.

MOLEKULARNO GENETSKE RAZISKAVE HMELJA

Branka Javornik
Univerza v Ljubljani, Biotehnična fakulteta, Jamnikarjeva 101, 1000 Ljubljana, Slovenija

V članku so predstavljene molekularno genetske raziskave hmelja, ki so bile opravljene z namenom prispevati k učinkovitejšemu žlahtnjenju novih sort hmelja. Kratko bodo opisane metode molekulskih markerjev razvite za proučevanje hmelja ter rezultati vrednotenja genske variabilnosti kolekcije hmeljnih genotipov in akcesij v genski banki. Predstavljeni bodo rezultati kartiranja genoma hmelja za natančnejšo opredelitev genskih dejavnikov, ki pogojujejo visoko vsebnost alfa kislin ter odpornost na hmeljevo uvelost.

SLOVENE AUTOCHTHONOUS COLLECTION OF CRISP LETTUCE (*LACTUCA SATIVA* L.): MORPHOLOGICAL AND MOLECULAR VARIABILITY, *BREMIA* RESISTANCE

Šuštar-Vozlič J.[1], Ugrinović K.[1], Maras M.[1], Javornik B.[2], Lebeda A.[3], Petrželová I.[3], Meglič V.[1]
[1] Agricultural Institute of Slovenia, Crop and Seed Science Department, Hacquetova 17, 1000 Ljubljana, Slovenia
[2] Biotechnical Faculty, Agronomy Department, Jamnikarjeva 101, 1000 Ljubljana, Slovenia
[3] Palacky University, Faculty of Science, Department of Botany, Šlechtitelu 11, 783 71 Olomouc-Holice, Czech Republic

Numerous autochthonous varieties have been developed during centuries of lettuce cultivation in Slovenia. In the Slovene Plant Gene bank (SPGB) - Gene Bank of Agricultural Plants at the Agricultural Institute of Slovenia 170 accessions are included that were obtained from various parts of Slovenia in the nineties of the last century. The majority of them are of crisp type. The most well known variety is 'Ljubljanska ledenka', some of the varieties derived from it are included also in the Common Catalogue of Varieties of the European Union under synonyms 'Laibacher Eis' 2, 3 and 4. With the aim to assess the genetic variability of Slovene lettuce collection a total of 138 accessions were analysed using AFLP markers. Seventeen accessions of 'Ljubljanska ledenka' from other gene banks in the world were included as well. Six primer combinations were applied. The results obtained by cluster analysis revealed close relationships among the accessions studied. A set of accessions was evaluated also for morphological and phenological parameters using UPOV descriptors for lettuce (TG13/8) in order to determine the origin of 'Ljubljanska ledenka'. Thirty eight parameters were evaluated in three successive years and the results were in accordance with the results of molecular analysis. Thirty five accessions were screened for resistance to the lettuce downy mildew (*Bremia lactucae* Regel.). The majority of accessions were susceptible to all of the 12 races tested. However, in some accessions resistance and/or incomplete resistance to the specific races was recorded. This is showing that at least in some genotypes the gene(s) of race-specificity can be expected. In the future a breeding programme should be designed in order to improve resistance against this important pathogen in the Slovene lettuce germplasm.

MORFOLOŠKA IN MOLEKULSKA RAZNOLIKOST SLOVENSKE AVTOHTONE SOLATE (*LACTUCA SATIVA* L.) TER NJENA ODPORNOST NA SOLATNO PLESEN

Šuštar-Vozlič J.[1], Ugrinović K.[1], Maras M.[1], Javornik B.[2], Lebeda A.[3], Petrželová I.[3], Meglič V.[1]
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V stoletjih pridelovanja so se v Sloveniji razvile številne avtohtone sorte solate. V Slovenski rastlinski genski banki – genski banki kmetijskih rastlin (SRGB) hranimo skupno 170 genskih virov solate, ki so bili pridobljeni z zbiranjem v devetdesetih letih prejšnjega stoletja. Večina virov je uvrščenih v tip krhkolistnih solat. Najbolj poznana med njimi je 'Ljubljanska ledenka'. Nekatere sorte, ki izvirajo iz nje, so vključene tudi v Skupni katalog sort Evropske unije kot 'Laibacher Eis' 2, 3 in 4. Za proučevanje genetske raznolikosti zbirke slovenske avtohtone solate smo v raziskavo z AFLP markerji vključili 138 genskih virov. V to raziskavo smo vključili tudi 17 genskih virov 'Ljubljanske ledenke', ki smo jih dobili od drugih genskih bank po svetu. Uporabili smo kombinacije šestih začetnih oligonukleotidov. Rezultati klaster analize so pokazali, da so posamezni genski viri ozko sorodni. Z namenom proučevanja izvora sorte 'Ljubljanska ledenka' smo del genskih virov ovrednotili tudi morfološko in fenološko s pomočjo UPOV deskriptorjev za solato (TG13/8). Tri leta smo ocenjevali in vrednotili 38 parametrov. Petintrideset genskih virov iz SRGB smo testirali tudi na odpornost proti solatni plesni (*Bremia lactucae* Regel.). Ugotovljeno je bilo, da je bila večina genskih virov solate, vključene v testiranje, občutljiva na vseh 12 ras patogena, ki smo jih uporabili pri testiranju. Vendar so bili kljub temu nekateri viri odporni in/ali delno odporni na specifične rase, kar kaže na to, da gene za odpornost lahko pričakujemo vsaj pri nekaterih virih. Za povečanje odpornosti slovenskih virov solate proti tej pomembni bolezni bi bilo v prihodnje potrebno zasnovati poseben žlahtniteljski program.

BIOLOGICAL DIVERSITY OF *GRAPEVINE FANLEAF VIRUS*

Maruša Pompe-Novak[1], Ion Gutiérrez-Aguirre[1], Jana Vojvoda[1], Marjanca Blas[1], Irma Tomažič[2], Zora Korošec-Koruza[3], Emmanuelle Vigne[4], Marc Fuchs[5], Maja Ravnikar[1] and Nataša Petrovič[1]
[1] National Institute of Biology, Večna pot 111, 1000 Ljubljana, Slovenia
[2] University of Nova Gorica, Vipavska 13, 5000 Nova Gorica, Slovenia
[3] University of Ljubljana, Biotechnical Faculty, Jamnikarjeva 101, 1000 Ljubljana, Slovenia
[4] National Institute of Agronomic Research and Louis Pasteur University, 28 rue de Herrlisheim, 68021 Colmar, France
[5] Cornell University, New York State Agricultural Experiment Station, Geneva, NY 14456, USA.

Grapevine fanleaf virus (GFLV) is responsible for fanleaf degradation disease that is spread all over the world and causes important economic losses in grapevines (*Vitis* L.). Classical way of limiting of the spread of the disease is testing and usage of healthy planting material. New strategies, such as cross-protection or transgenic grapevine production are emerging as suitable candidates for virus control. To study the biological diversity of GFLV isolates we selected vines from several vineyards based on the results of ELISA test for several viruses. We characterized their RNA2-encoded genes 2A, 2B and 2C by IC-RT-PCR-RFLP. The number of restrictotypes varied with the host plant and target gene. The RNA2 ORF sequence was determined for the nine GFLV isolates by IC-RT-PCR, cloning and sequencing. Sequence analysis confirmed mixed infection and the occurrence of a recombination event on the 2A gene. No clear association was apparent between symptomatology and restrictotypes.

BIOLOŠKA RAZNOVRSTNOST VIRUSA PAHLJAČAVOSTI LISTOV VINSKE TRTE

Maruša Pompe-Novak[1], Ion Gutiérrez-Aguirre[1], Jana Vojvoda[1], Marjanca Blas[1], Irma Tomažič[2], Zora Korošec-Koruza[3], Emmanuelle Vigne[4], Marc Fuchs[5], Maja Ravnikar[1] in Nataša Petrovič[1]
[1] Nacionalni inštitut za biologijo, Večna pot 111, 1000 Ljubljana, Slovenija
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Virus pahljačavosti listov vinske trte (GFLV) povzroča bolezen imenovano kužno izrojevanje vinske trte, ki je razširjena po vsem svetu in na vinski trti (*Vitis* L.) povzroča veliko ekonomsko škodo. Klasični način omejevanja širjenja virusa temelji na uporabi neokuženega sadilnega materiala in zatiranju ogorčic, novejši možnosti pa sta navzkrižna zaščita in uporaba gensko spremenjene vinske trte. Trse za študij biološke raznolikosti GFLV smo izbrali na podlagi rezultatov testiranja prisotnosti različnih virusov z ELISA testom. Z metodo IC-RT-PCR-RFLP smo analizirali vse tri gene (2A, 2B in 2C), ki jih kodira RNA2. Število različnih restriktotipov se je razlikovalo pri posameznih genih in v posameznih rastlinah. Z metodo IC-RT-PCR in določanjem nukleotidnega zaporedja smo v 9 izolatih določili nukleotidno zaporedje celotnega bralnega okvirja RNA2. Z analizami dobljenih nukleotidnih zaporedij smo potrdili mešane okužbe in rekombinacijo v genu 2A. Med posameznimi restriktotipi in izražanjem bolezenskih znamenj nismo našli povezave.

THE DEVELOPMENT OF A SRY ASSAY USEFUL FOR SEX DETERMINATION IN FORENSIC STR MULTIPLEX KITS

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Sex determination is fundamental to forensic investigation, especially in sexual assault cases, and also to prenatal diagnosis, where it is assumed that an unborn male child could inherit gene disorder. In forensic science gender determination is based on the amelogenin sex test, which is included in commercially available human identification PCR kits. Although this method has been thought reliable, it is now known that the frequency of deletion of the Amel-Y gene varies from 0,0002 % to 0,08 %. It therefore follows that the result of the amelogenin gene amplification would indicate that the male suspect is actually identified as female. As a result, it is now recommended that gender determination be based also on other sex determining markers. One of them is the sex-determining region Y (SRY). The SRY gene is located on the Y-chromosome and triggers the events that convert an embryo into a male. We designed a new pair of primers in the SRY gene resulting in 96 based pair amplification product occurring in male samples only. The amplification of the SRY gene with our new pair of primers was successful even when the new pair of primers was added to other primers in the AmpFlSTR SGM Plus Amplification (Applied Biosystems) kit and in PowerPlex 16 (Promega) kit. The new pair of primers is highly specific for the SRY gene, since a very low concentration of such primers is sufficient for positive amplification.

RAZVOJ TESTA SRY ZA DOLOČANJE SPOLA V FORENZIČNIH KOMPLETIH STR

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Določanje spola igra pomembno vlogo pri forenzičnih preiskavah, predvsem pri kaznivih dejanjih zoper spolno nedotakljivost, prav tako pa tudi pri prenatalni diagnostiki, v tistih primerih, ko je možnost da le moški potomec podeduje gensko obolenje. V bioloških forenzičnih vzorcih ugotavljanje spola s komercialnimi identifikacijskimi kompleti STR še vedno temelji na pomnoževanju dela znotraj introna 1 v amelogeninskem genu, čeprav so raziskave dokazale, da se v povprečju pri moških pojavljajo delecije v tem delu amelogeninskega gena v 0,0002 % do 0,08 %. Zaradi česar bi pri omenjenih moških rezultati pomnoževanja amelogeninskega gena pokazali, da gre v bistvu za žensko osebo. Zaradi tega so začeli raziskovalci uporabljati tudi druge genske označevalce za ugotavljanje spola, eden od njih je tudi gen SRY, ki je odgovoren za razvoj moških spolnih znakov. V raziskavo smo vpeljali nov par začetnih oligonukleotidov znotraj gena SRY, katerega končni amplifikacijski produkt je dolg le 96 baznih parov in se pomnoži le pri moških osebah. Pomnoževanja dela ena SRY z novim parom začetnih oligonukleotidov je bilo uspešno tudi ob prisotnosti ostalih začetnih oligonukleotidov, ki so del obeh komercialnih kompletov, ki smo jih uporabili v raziskavi (AmpFlSTR SGM Plus Amplification (Applied Biosystems) in PowerPlex 16 (Promega)). Nov par začetnih oligonukleotidov je visoko specifičen, saj smo uspešno pomnožili del gena tudi pri uporabi zelo nizkih koncentracij omenjenih začetnih oligonukleotidov.

REVEALING OF POPULATION STRUCTURE IN SELECTED JELLYFISH SPECIES USING GENETIC MARKERS

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In the last few years unusual massive blooms of scyphozoan jellyfishes were recorded in the Northern Adriatic Sea causing serious damages to the local fisheries. Populations of *Rhizostoma pulmo, Aurelia aurita* and *Pelagia noctiluca* were sampled to understand genetic structure of these jellyfishes. Populations of *R. pulmo* were sampled in the Gulf of Trieste and in the Black Sea. Indigenous populations of *A. aurita* were sampled from the Mljet lake and the Baltic Sea. Furthermore, some morphological characteristics were measured during several samplings in order to better understand the sampled populations. *Pelagia noctiluca* was collected during massive bloom in the middle Adriatic and around Malta. Protocols for the field sampling and the isolation of high molecular DNA were optimized. The mitochondrial cytochrome C oxidase (subunit I) was successfully PCR amplified in all three species using universal primers LCO1490 and HCO2198 (Folmer *et al.*, 1994; Dawson 2004). The length of amplified PCR product was 700 bp. Phylogenetic relationships were conducted using Minimum evolution (ME), Maximum parsimony (MP) and Neighbor joining (NJ) using MEGA 3.1 software (Kumar *et al.*, 2004). Among samples of *A. aurita* clear distinction between samples from Mljet lake and Baltic Sea were found. Samples of *R. pulmo* from the Black Sea form own group well supported by bootstrap values (97%), but with less supported separation with samples from the Gulf of Trieste. *P. noctiluca* from the middle Adriatic Sea and around Malta formed the same group. Further studies on genetic structure using nuclear markers such as microsatellites are in progress.

RAZISKOVANJE GENETSKE STRUKTURE IZBRANIH MEDUZNIH VRST Z GENETSKIMI MARKERJI

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V zadnjih letih se v obalnih območjih severnega Jadrana neobičajno množično pojavljajo klobučnjaške meduze (Scyphozoa), ki imajo velik vpliv na plankton in lahko povzročajo lokalnemu ribištvu precejšnjo škodo. Populacije morskih klobukov (*Rhizostoma pulmo*), uhatih klobučnjakov *(Aurelia aurita*) in mesečik (*Pelagia noctiluca*) so bile vzorčene za raziskovanje genetske strukture teh izbranih vrst meduz. Morski klobuki so bili vzorčeni v Črnem morju in v Tržaškem zalivu. Uhati klobučnjaki so bili vzorčeni v Mljetskem jezeru in v Baltskem morju. Da bi bolje opredelili vzorčne populacije, smo ob vzorčenju izmerili nekatere morfološke značilnosti. Mesečinka je bila vzorčena med množičnim pojavljanjem v srednjem Jadranu in v obalnem območju Malte. Optimizirali smo protokol za vzorčenje tkiva in izolacijo visokomolekularne DNA. Z reakcijo PCR smo v vseh treh izbranih vrstah meduz s parom začetnih oligonukleotidov LCO1490 in HCO2198 (Folmer *et al.*, 1994; Dawson 2004) uspešno namnožili 700 baznih parov dolg odsek mitohondrijske citokrom C oksidaze (podenota I). Filogenetske odnose med haplotipi smo rekonstruirali z metodo minimalne evolucije (ME), metodo največje varčnosti (MP) in sosedsko pridružitveno metodo (NJ) v programu MEGA 3.1 (Kumar *et al.*, 2004). Med vzorci uhatih klobučnjakov je opazna razlika med vzorci iz Mljetskega jezera in vzorci iz Baltskega morja. Vzorci morskega klobuka iz Črnega morja tvorijo enotno skupino, ki je dobro podprta z metodo vezanja (97%), ločitev z vzorci iz Tržaškega zaliva je slabše podprta. Mesečinke iz obalnega območja Malte in srednjega Sredozemskega morja tvorijo enotno skupino. V teku je študij genetske strukture populacij z jedrnimi markerji, kot so mikrosateliti.

References / Viri: -Dawson M N (2004) Hydrobiologia 530/531:249-260; -Folmer O, Black M, Hoeh W, Lutz R and Vrijenhoek R (1994) Molecular Marine Biology and Biotechnology 3(5):294-299; -Kumar S, Tamura K and Nei M (2004) Briefings in Bioinformatics 5:150-163

GENETIC DIVERSITY BASED ON PEDIGREE ANALYSIS OF SLOVENIAN SHEEP BREEDS

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Sheep breeding has long tradition in Slovenia. Four indigenous sheep breeds were included in the genetic diversity study based on shared ancestry among animals: the Bela Krajina Pramenka (BP), the Istrian Pramenka (IP), the Bovec sheep (BS), and the Jezersko-Solčava sheep (JS). Populations are of different size. Pedigree information included between 1020 animal for BP and 9251 animals for JS. Kinship and inbreeding coefficients, family size, effective number of founders (EF), and effective number of ancestors (EA) as diversity measures were calculated for reference populations (RP) which consisted of animals born in years 2001-2005. Ratio between ewes and rams as parents of RP was the widest in IP (10.46) and more favourable in BP (6.91). Family size for pairs was similar across breeds, between 1.06 and 1.17 progeny with standard deviation between 0.22 and 0.47. Rams had on average from 4.78 (BS) to 8.53 offspring (JS) with very skewed distributions for family size. Large variation for family size of rams showed the unbalanced usage of sires. All breeds had quite incomplete pedigree since all herdbooks are still open for registration of new animals. Equivalent number of known generations for animals of RP was between 1.38 in BP rams and 3.25 in BS rams. Average kinship coefficient and inbreeding coefficient were biased downward due to incomplete pedigree. The EA in RP was from 19.2 in males and 35.5 in females of BP to 107.7 in males and 84.1 in females of BS. The EF was slightly higher than EA, as expected. The contribution of ancestors to gene pool is more uniform in BS and JS. The IP and BP are endangered populations, while JS and BS have better status.

GENETSKA PESTROST NA OSNOVI POREKLA PRI SLOVENSKIH PASMAH OVC

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Ovčereja ima v Sloveniji dolgo tradicijo. Štiri avtohtone pasme ovc smo vključili v študijo genetske pestrosti, ki je temeljila na poznanem sorodstvu med živalmi: belokranjska pramenka (BP), istrska pramenka (IP), bovška ovca (BS) ter jezersko-solčavska ovca (JS). Populacije so različno velike. Poreklo je vključevalo med 1020 živali pri BP in 9251 živali pri JS. Koeficienta sorodstva in inbridinga, velikost družin ter efektivno število osnovalcev (EF) in prednikov (EA) smo, kot merila pestrosti, izračunali za referenčno populacijo (RP), ki so jo predstavljale živali rojene v letih 2001-2005. Razmerje med ovcami in ovni, ki so starši RP, je bilo najširše pri IP (10,46) in najugodnejše pri BP (6,91). Velikost družin po parih je bila med pasmami podobna, med 1,06 in 1,17 potomcev s standardnim odklonom med 0,22 in 0,47. Ovni so imeli v povprečju od 4,78 (BS) do 8,53 potomcev (JS) z zelo nesimetrično porazdelitvijo za velikost družin. Velika variacija velikosti družin pri ovnih kaže na neuravnoteženo rabo samcev. Vse vključene pasme so imele precej nepopolno poreklo, kar je posledica odprtih rodovniških knjig za registracijo novih živali. Ekvivalent števila znanih generacij za živali RP je bil med 1,38 pri ovnih BP in 3,25 pri ovnih BS. Povprečni koeficient sorodstva in koeficient inbridinga sta verjetno precej podcenjena zaradi nepopolnega porekla. EA za RP je znašal od 19,2 pri samcih in 35,5 pri samicah BP do 107,7 pri samcih in 84,1 pri samicah BS. EF je bil pričakovano nekoliko večji kot EA. Pasmi IP in BP sta ogroženi populaciji, medtem ko sta JS in BS v boljšem položaju.

MUTAGENESIS
MUTAGENEZA

ON THE CAUSES AND NATURE OF HPRT MUTATION IN HUMAN T-CELLS IN VIVO

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Mutation analysis can provide information on gene-environment interaction at the cell and molecular level. We have studied the frequency and spectrum of in vivo mutation at the hypoxanthine-phosphoribosyl transferase (*HPRT*) locus in T-lymphocytes in the peripheral blood of healthy subject and untreated cancer patients. While occupational exposures show little effect on the frequency of mutation in healthy people, age, smoking and diet have significant influences on the frequency of mutation. Increasing age and smoking dose is associated with increasing HPRT mutant frequency (MF), while high consumption of dietary factors such as green leafy vegetables, citrus fruits and berries is associated with lower MF. Comparisons of the molecular spectrum of mutation between different populations show overall similarities suggesting strong effects of endogenous factors, probably associated with normal metabolism. However, some remarkable differences in the spectrum of mutation have also been observed, possibly indicating general environmental influences. Using modelling methods, we have studied the correlation between the mutable and non-mutated amino acid residues on one hand, and sequence conservation and predicted phenotypic effects on the other hand. Our results demonstrate that most of the mutations are explainable by the predicted effect on protein structure and function. They are also consistent with sequence conservation. However, some theoretically mutable regions of the HPRT show few or no mutations, suggesting the possibility that they are protected from mutagenesis.

DECREASED EFFICIENCY OF BASE EXCISION REPAIR IN TERMINALLY DIFFERENTIATED MUSCLE CELLS

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Base Excision Repair (BER) is the main repair mechanism of oxidative DNA damage. BER is initiated by a DNA glycosylase that removes the damaged base leaving an apurinic/apyrimidinic (AP) site. BER proceeds through two alternative pathways: short-patch (SP-) and long-patch (LP-) BER. DNA polymerase beta is responsible for the repair synthesis in the SP-BER while DNA polymerase delta/epsilon and replication auxiliary proteins are involved in LP-BER. BER capacity was compared in proliferating (P) and terminally differentiated (TD) cells. Murine satellite muscle cells differentiate to myotubes (TD) upon growth in appropriate medium condition. Microarray analysis showed a reduction in the expression levels of several BER genes and, in particular, in LP-BER genes in TD compared to P cells. BER capacity and kinetics were analysed by incubating total cell extracts from the two cellular types with plasmids containing an AP site. We observed a delay in BER kinetics in TD as compared with P cells. By a fine dissection of the BER steps we identified a defect in the ligation step in TD cells. A decreased level of DNA Ligase I was detected in TD cells by a functional assay. A higher intracellular ROS concentration and 8-oxoguanine level was reported in TD cells compared to their precursors. Interestingly, LP-BER has been specifically involved in the repair of oxidized AP sites induced by ROS. TD muscle cells must rely upon a specific strategy to preserve the integrity of the genes that must be expressed from endogenous and exogenous DNA damage.

ALTERNATIVE TEST METHODS IN GENOTOXICITY AND CARCINOGENICITY

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The current EU political environment urgently calls for the use of alternative test methods and testing strategies. The new chemicals policy REACH requires the reassessment of thousands of chemicals, while the 7th Amendment of the Cosmetics Directive foresees the complete ban on animal testing for cosmetics ingredients. Taking into account the *in vivo* methods mostly in use in regulatory toxicology, the European Centre for the Validation of Alternative Methods (ECVAM) has established the key area genotoxicity/carcinogenicity. The area focuses on the activities related to the promotion and the validation of alternative methods to refine, reduce and replace animal experiments in the area of genotoxicity and carcinogenicity. Currently, ECVAM is coordinating an international validation study on the Cell Transformation Assay (CTA). This is the first ECVAM validation study which involves laboratories from Japan, US and Europe and focuses on both SHE and Balb/c 3T3 assays. In the area of genotoxicity, ECVAM has carried out a retrospective validation of the Micronucleus Test (MNT) *in vitro*, based on existing data. The report on the retrospective validation of *in vitro* MNT has been submitted to the ECVAM Scientific Advisory Committee (ESAC) for peer review and an ESAC statement on the validity of the test is foreseen for November 2006. The high false positive rate (low specificity) of the established *in vitro* tests still represents a bottleneck in the assessment of genotoxicity. In fact, this leads to an increased number of *in vivo* genotoxicity tests, which could be avoided if current testing would be more predictive. In order to address this issue, a two days workshop was held at ECVAM in April 2006. The participants were invited to review data from the current available systems, to review potential modifications to existing protocols and cell systems, and to review the performance of some new test systems that show promise of improved specificity. Considerable *in vivo* testing is still required for confirmation of the genotoxic prediction *in vitro*. Therefore, it became clear that it is crucial to address issues related to the reduction and refinement of genotoxicity tests. The collection of relevant data might be considered as a basis for possible amendments of guidelines to reduce animal consumption.

AUTOCRINE AND PARACRINE EFFECTS OF DOWNREGULATION OF LYSOSOMAL PROTEASE GENES IN NORMAL AND NEOPLASTIC CELLS - REVIEW

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Lysosomal proteases- cathepsins, take part in various cellular processes, such as partial or extensive degradation of protein substrates, trafficking and recycling of relevant biological molecules, such as receptors and growth factors, post-translation modifications of secretory proteins and antigen presentation in immuno-competent cells. A broad pH optima and selective specificity of these enzymes allows them to participate in critical processes relevant for cancer progression, such as apoptosis, angiogenesis, invasion and drug resistance. Recent experiments, using various methods for knocking out and silencing of cathepsins genes in normal and tumour cells, have revealed some unexpected and interesting functions of these enzymes, which may also affect the gene regulation in the same and/or surrounding cells. A review of recent findings and our results in glioma and carcinoma cells lines and the conclusions on gene expression and regulation of lysosomal cysteine cathepsins B and L will be presented.

AUTOKRINI IN PARAKRINI UČINKI ZNIŽANJA IZRAŽANJA GENOV LIZOSOMSKIH PROTEAZ V NORMALNIH IN NEOPLASTIČNIH CELICAH - PREGLED

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Lizosomske proteaze- katepsini, sodelujejo v različnih celičnih procesih, kot je delna ali popolna razgradnja proteinskih substratov, potovanje in obnova pomembnih bioloških molekul, kot so receptorji in rastni faktorji, post-translacijske spremembe izločenih molekul in antigenska prezentacija v imunokompetentnih celicah. Širok pH optimum in selektivna specifičnost teh encimov jim omogočata sodelovanje v kritičnih procesih, ki so relevantni za napredovanje raka, kot je apoptoza, angiogeneza, invazija in odpornost proti drogam. Nedavni poskusi z uporabo izbijanja in utišanja genov v normalnih in tumorskih celicah, so pokazali na nepričakovane in zanimive funkcije teh encimov, ki lahko vplivajo na gensko regulacijo v istih in/ali sosednjih celicah. Podan bo pregled nedavnih raziskav in naših rezultatov v gliomskih in karcinomskih celicah ter zaključki o genskem izražanju in regulaciji lizosomskih katepsinov B in L.

ANTIOXIDANT AND FREE RADICAL SCAVENGING ACTIVITIES OF SUMAC (*RHUS CORIARIA*) AND IDENTIFICATION OF GALLIC ACID AS ITS ACTIVE PRINCIPLE

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It is known, that certain spices are rich in antioxidants. Sumac (*Rhus coriaria*) is widely consumed in Middle-Eastern countries. We tested its DNA-protective effects in a human intervention trial. Eight participants consumed 3 g of sumac for 3 days. We found strong protective effects in single cell gel electrophoresis assays (SCGE) with endonuclease III (ENDO III), formamidopyrimidine glycosylase (FPG) and hydrogen peroxide in human peripheral lymphocytes. H_2O_2-induced DNA-migration was reduced by 30%, oxidized pyrimidines 36% and oxidized purines 41% respectively after the intervention. Subsequent *in vitro* experiments indicated that gallic acid (GA) is the active principle of sumac. GA is also contained in certain plants (mango, rhubarb, strawberries). In a subsequent trial, 8 participants consumed GA (0,2 mg/kg BW/d) for 3 days and strong protective effects were observed with this phenolic compound, which is very rapidly absorbed in the GI tract. The reduction of DNA migration induced by H_2O_2 was 40%, ENDO III 58%, FPG 52%. Comparisons show that GA is 50 times more protective than the vitamins C and E. The protective effects of sumac and GA were also investigated in animal experiments. 8 male rats per group were fed 3 days with sumac (0,02 g/kg BW/d) and GA (0,2 mg/kg BW/d). After irradiation in a ^{60}Co source (7,74Gy/1min), the animals were killed immediately and protective effects were seen in lymphocytes, brain, liver, colon and lung. Taken together our finding indicate that GA is a "super-antioxidant", which protects against ROS-induced DNA-damage.

MECHANISMS OF GENOTOXIC AND MUTAGENIC EFFECTS OF HORMONES

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Epidemiological and experimental investigations indicated that sexual steroids contribute to the processes of carcinogenesis. Apart from the role of sexual steroids as tumor promoters, these hormones can also act in the processes of tumor initiation. The metabolic reactions of oestrogens, especially at elevated tissue concentrations, may become the predominant biochemical activity overshadowing their hormonal effects. Namely, metabolic conversion during the redox cycling of oestrogens leads to the condition of oxidative stress which causes damage to cellular macromolecules (proteins, lipids and DNA). This may cause genetic instability accompanied by covalent DNA modifications, chromosomal lesions and aberrations. The existence of at least three different groups of DNA adducts was determined by ^{32}P-postlabelling chromatographic analysis: a) nucleotides covalently bound to reactive derivatives of hormones, b) oestrogen-induced endogenous DNA adducts possibly caused by derivatives of lipids or lipid peroxides, and c) nucleotides covalently damaged by reactive oxygen species (ROS). Individual and tissue susceptibility to changes of genetic material also depends on efficiency of enzymatic and non-enzymatic mechanisms of resistance to oxidative stress. On the other hand, mutagenic effects of non-steroidal hormones and hormon-like substances are not thoroughly investigated. There are some experimental findings that phenolic moieties of catecholamines and thyroid hormones can be involved in redox cycling and generation of ROS. Therefore, it seems that the basic molecular mechanism both of sexual steroids and some nonsteroidal hormones imply the creation of oxydative stress and subsequent DNA damage caused by ROS and possibly by reactive hormone derivatives created during their redox cycling.

GENOMIC TECHNOLOGIES I
GENOMSKE TEHNOLOGIJE I

MICROARRAY TECHNOLOGY AND ITS USE IN STUDYING AVIAN INNATE IMMUNITY

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The innate immune system represents a protective mechanism from infectious challenge that does not depend on specific antigen recognition. Elements of the innate immune system, including macrophages, heterophils, dendritic cells, and natural killer cells are involved in destroying intracellular and extracellular pathogens and in providing instructions for the initialization of the proper acquired immune response. The microarray has become a powerful tool for the study of immune system function. Although a variety of large-scale commercial arrays are available for humans and other mammalian species, there are few such tools available for agricultural species such as the avian. Our laboratory has developed a cDNA microarray, which is being used to characterize changes in the transcriptome of cells of the avian innate immune system when they are exposed to various avian pathogens. This array consists of 4,959 elements that were obtained from EST libraries of stimulated avian macrophages and supplemented by genes of interest identified by the chicken genome project. The array contains elements for several innate immune pathways, including 42 genes involved in the Toll-like receptor (TLR) pathway and 22 avian interferon/antiviral response pathway genes. In addition, the array contains 29 cell surface marker genes for monocytes, macrophages, heterophils, dendritic cells, and T-cells. The elements are spotted in triplicate on the slide, giving 14,877 total spots per slide. Through several collaborative efforts this array has been used to monitor gene expression in a number of immunologically significant tissues (air sac, lung, spleen (pre- and post-hatch), thymus, liver). Transcription patterns have also been examined in several specific cell types (peripheral blood-derived monocytes and heterophils, intestinal epithelial lymphocytes, and two avian macrophage cell lines). Furthermore, transcriptional responses have been elucidated to bacteria (*Salmonella, E. coli, Mycoplasma*), viruses (influenza), parasites (*Eimeria*), cell components (LPS), and immune modulators (interferon-γ).

DNA MICROARRAY TECHNOLOGY: APPLICATION TO ECOTOXICOLOGY

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Molecular diagnostic technologies play a significant role in the practice of medicine, public health, pharmaceutical industry and more recently in the environmental field. DNA Microarray is one of these significant technologies which has progressed rapidly in the hands of biological researchers for assessing gene expression analysis. Microarrays are providing insights into areas such as toxicology, pharmacology and tumorigenesis. The application to Ecotoxicology is still at an early stage but already many applications have been published. Molecular biomarkers offer the possibilities, of early detection of environmental stressor, inferred mechanisms of action and to improve the monitoring of environmental stressors. We are currently performing DNA Microarray analysis to identify new biomarkers for the detection of chemical stressors in environment. The budding yeast *Saccharomyces cerevisiae* is one of the organism used as model since the genome has been sequenced, the mutants are characterized as well as metabolic pathways and more recently the protein interactions. The presentation will be an overview of the technology and will show the gene expression profile of *S. cerevisiae* exposed to two class of chemicals which can affect human and environment health.

Reference: Teresa Lettieri. "Recent applications of DNA Microarray technology to Toxicology and Ecotoxicology" Environ Health Perspect 114:4-9 (2006).

TOWARDS BETTER UNDERSTANDING OF PLANT-PATHOGEN/PESTS INTERACTIONS - EXPRESSION PROFILING AS A TOOL IN SYSTEMS BIOLOGY

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Plants, being sessile organisms, are constantly facing a wide range of different pathogens and pests, and have developed a whole array of defence strategies to combat them. On the other hand, the pathogenicity systems in microorganisms and insects are evolving in parallel, making the equilibrium balance between compatible and incompatible interactions very fragile. Many viruses, bacteria, fungi, nematodes and insects are able to overcome plant defence barrier thus becoming effective pathogens and pests. In agriculture, a wide range of chemical pesticides are applied to enhance the defence and keep crop plants healthy. Understanding the mechanisms of compatible as well as incompatible interactions is critical for the development of effective alternative to chemical treatment. We have used systemic non-targeted approach to study those processes. As transcriptomics is currently the technically most developed level in systems biology, expression profiling techniques (DNA microarrays and qPCR) were applied. Additionally, we are starting to integrate obtained data also other research levels, like proteomics. Results from experiments in potato - virus PVY, potato – Colorado potato beetle and grapevine – phytoplasma interactions will be presented and discussed.

ANALIZA INTERAKCIJ MED RASTLINO IN PATOGENOM OZ.ŠKODLJIVCEM – EKSPRESIJSKO PROFILIRANJE KOT ORODJE SISTEMSKE BIOLOGIJE

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Rastline so, kot sesilni organizmi, pod konstantnim pritiskom različnih patogenov in škodljivcev in so zato razvile nabor obrambnih strategij za boj proti njim. Podobno so se na strani mikroorganizmov vzporedno razvijale tudi strategije napada. Tako je ravnovesje med tem ali je interakcija dveh organizmov kompatibilna ali nekompatibilna zelo nestabilno. Veliko virusev, bakterij, gliv, nematodov in žuželk je sposobnih obiti obrambni mehanizem rastline in s tem postati učinkoviti škodljivci. V poljedelstvu se tako uporablja številne kemične preparate in s tem ojača obrambo rastline. Le podrobno razumevanje mehanizmov kompatibilne in nekompatibilne interakcije bo omogočilo razvoj učinkovite alternative kemičnim preparatom. Za proučevanje teh procesov smo uporabili sistemski netarčni pristop. Ker je transkriptomika trenutno tehnično najbolj izdelan nivo proučevanja v sistemski biologiji, smo uporabili tehnike ekspresijskega profiliranja (DNA mikromreže, qPCR). V teku pa je tudi priprava sistemov za integracijo tega nivoja proučevanja z drugimi, npr. proteomiko. Predstavljeni bodo rezultati proučevanja interakcij med krompirjem in virusom PVY, krompirjem in koloradskim hroščem ter vinsko trto in fitoplazmami.

APPLICATION POSSIBILITIES FOR MULTIPLEX LIGATION DEPENDENT PROBE AMPLIFICATION (MLPA)

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Introduction: Multiplex ligation-dependent probe amplification is a molecular genetic method for exact determination of locus copy number present in an analyzed nucleic acid sample. Commercially available probe kits can be used to analyze loci copy number changes associated with different cancers, syndromes or monogenetic diseases. This study present results of molecular karyotyping with MLPA and the use of custom made MLPA probes for analysis of a selected DNA sequences. **Methods:** Genomic DNA was extracted from embryonic tissues, peripheral venous blood and paraffin tissue sections. MLPA analysis was performed using commercially available kits from MRC-Holland, Netherlands. In addition, long oligonucleotides were purchased and used as MLPA probes for the analysis. The results were detected with the capillary electrophoresis and with agarose gel electrophoresis. Confirmatory methods included karyotyping, comparative genomic hybridization and different PCR based methods. **Results:** Several major chromosomal abnormalities (trisomies, translocations) was observed in genomic DNA extracted from embryonic tissues from spontaneously aborted pregnancies. The frequency of the HLA-A29 allele was determined in a sample of blood donors. The presence of human papilloma virus type 16 was detected in DNA from tissue sections from cervical cancer samples. **Conclusions:** MLPA is a robust and highly reproducible molecular genetic method. It can be used for detection of sequence variations ranging between chromosomal abnormalities and single nucleotide polymorphisms. The analysis can be performed quantitatively and/or used for end-point detection assays. As a high-throughput method it is also very flexible and can be easily modified to accomplish a specific task by using custom made long oligonucletides as MLPA probes.

APLIKACIJSKE MOŽNOSTI METODE POMNOŽEVANJA OD LIGACIJE ODVISNIH SOND (MLPA)

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Uvod: Pomnoževanje od ligacije odvisnih sond (MLPA) je molekularna tehnika namenjena točnemu določanju števila lokusov v analiziranem vzorcu nukleinske kisline. Dosegljivi komercialni kiti sond MLPA omogočajo določanje razlik v številu kopij lokusov, ki so pomembni pri različnih vrstah raka, sindromih oz. pri monogenetskih boleznih. Študija predstavlja rezultate molekularne kariotipizacije z uporabo metode MLPA in izbrane primere uporabe MLPA sond lastne izdelav. **Metode:** Genomsko DNA uporabljeno pri analizi smo izolirali iz različnih virov. Kite za MLPA analizo je dobavilo podjetje MRC-Holland (Nizozemska). Za MLPA sonde smo uporabili tudi običajne dolge začetne oligonukleotide. Detekcijo rezultatov smo opravili s pomočjo kapilarne elektroforeze oz. agarozne gelske elektroforeze. Kontrolne metode so vključevale citogenetsko analizo, primerjalno genomsko hibridizacijo in različne tehnike verižne reakcije s polimerazo. **Rezultati:** V vzorcu genomskih DNA izoliranih iz embrionalnih tkiv po spontanih prekinitvah nosečnosti so bile prisotne različne pomembne kromosomske aberacije, ki so vključevale trisomije in translokacije. Pri vzorcu krvodajalcev smo določili frekvenca alela HLA-A29. V vzorcih tkiv s karcinomom vratu maternice smo dokazali prisotnost humanega papiloma virusa HPV tip 16. **Zaključki:** Pomnoževanje od ligacije odvisnih sond (MLPA) je robustna in reproducibilna molekularno genetska metoda. Uporabna je za analizo nukleotidnih zaporedij, ki vključujejo kromosomske anomalije in enobazne polimorfizme. Metoda se lahko uporablja za kvantitativno analizo ali kot analiza končnih rezultatov. Omogoča hkratno analizo večjega števila vzorcev in s pomočjo začetnih oligonukleotidov izdelanih po meri kot MLPA sond je metodo mogoče enostavno prilagoditi za najrazličnejše naloge.

COMPARATIVE GENETIC AND SEQUENCE ANALYSES OF ASPARAGUS BACS REVEAL NO MICROSYNTENY WITH ONION OR RICE

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Asparagus and onion are members of a monophyletic group within the monocot order Asparagales and have relatively large nuclear genomes compared to rice, member of the Poales order. Comparative genomic analyses have revealed a high degree of synteny among the grasses; but it is not tested yet if synteny extends to other major monocot groups. Previously, no evidence for synteny at the recombinational level between onion and rice was reported, but microsynteny could still exist across some genomic regions. We sequenced nine asparagus BACs, (705.279 bp), to determine physically linked sequences and locate their most similar positions in the onion and rice genomes. Five asparagus BACs were assembled using three low copy onion cDNAs that mapped to three different onion chromosomes and four were selected using asparagus molecular markers tightly linked to the sex-determining M locus on chromosome 5 of asparagus. Some BACs assemblies, showed significant similarities ($e < -20$) to expressed sequences on different rice chromosomes, but no evidence for microsynteny between asparagus and rice across these regions was revealed. Genic-like sequences from these BACs were used to find highly similar ESTs of onion, which were mapped either by SNP or RFLP approach. They mapped to different onion chromosomes, thus no relationship was observed between physical linkages in asparagus and genetic linkages in onion. Results further indicate that synteny among grass genomes does not extend to a sister order in the monocots and that asparagus may not be an appropriate smaller-genome model for plants in the Asparagales with enormous nuclear genomes.

PRIMERJALNA GENETSKA IN SEKVENČNA ANALIZA UMETNIH BAKTERIJSKIH KROMOSOMOV (BAC) ŠPARGLJA V PRIMERJAVI S ČEBULO IN RIŽEM POTRJUJE NEOHRANJENO ZAPOREDJE GENOV NA MIKRO NIVOJU

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Špargelj in čebula, enokaličnici, sta predstavnika monofiletske skupine znotraj reda špargljevk z relativno velikima genomoma, ki sta 2,7- in 36-krat večja od riževega. Številne primerjalne genomske analize so dokazale visoko stopnjo ohranjenega zaporedja genov pri travah, ni pa znano, če je to zaporedje ohranjeno tudi v primerjavi z ostalimi pomembnejšimi predstavniki enokaličnic. Na nivoju genetskih rekombinacij niso dokazali ohranjenega zaporedja genov na makro nivoju med čebulo in rižem, lahko pa pričakujemo, da je na mikro nivoju pri določenih regijah zaporedje še ohranjeno. Nukleotidno zaporedje smo določili devetim bakterijskim umetnim kromsomom (BAC) šparglja (705.279 bp) z namenom, da bi odkrili fizično povezana zaporedja in s pomočjo primerjalne analize določili njihova najbolj podobna mesta v genomu čebule in riža. Pet BAC-ov smo izbrali s pomočjo treh čebulnih cDNA z različnih kromosomov, ostali štirje pa so bili izbrani s pomočjo špargljevih markerjev, tesno vezanih z lokusom M. Pri nekaterih BAC regijah so bile ugotovljene podobnosti ($e < -20$) z izraženimi zaporedji riža, ki pa so se nahajala na različnih kromosomih. Tako za te regije nismo potrdili ohranjenega zaporedja genov na mikro nivoju med špargljem in rižem. Genom podobna zaporedja BAC-ov smo uporabili za iskanje visoko podobnih EST zaporedij čebule, ki smo jih kartirali s pomočjo SNP ali RFLP markerjev. Zaporedja smo kartirali na različne kromosome čebule in tako tudi tu nismo ugotovili povezave med fizično vezanimi zaporedji šparglja z genetsko povezanimi regijami čebule. Rezultati nadalje kažejo, da genomski podatki, razviti pri travah, niso prenosljivi na sestrski red znotraj enokaličnic in da špargelj ni primeren modelni organizem za čebulo.

RECENT DEVELOPMENTS IN REAL-TIME PCR - THE EPPENDORF MASTERCYCLER EP *REALPLEX*

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Real-time PCR has developed from a sophisticated application for technology enthusiasts into a reliable routine application for molecular biology labs. With the concept of the Mastercycler® ep *realplex,* Eppendorf accommodates this development and offers a fast and reliable device for daily use. Long-lived LEDs, robust channel photomultipliers and a proven thermocycler technology form the basis for an easy to use and flexible real-time PCR device. In addition to this, automated reaction setup using the epMotion liquid handling workstations provides a maximum of accuracy and reproducibility, making qPCR even applicable and cost effective for routine applications.

BIOINFORMATICS
BIOINFORMATIKA

HOW DID *SACCHAROMYCES* YEASTS EVOLVE TO BECOME GOOD BREWERS?

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Brewing and wine production are one of the oldest technologies and their products have for several millennia been an almost indispensable part of our daily life. The central biological agents of beer and wine fermentation are yeasts belonging to the genus *Saccharomyces*, which are able to accumulate ethanol even in the presence of oxygen. The recent advances in yeast comparative genomics and bioinformatics have helped to elucidate when, why and how the remarkable property to produce ethanol in high concentrations originated and developed during the evolutionary history. The end of the Cretaceous age provided excess of fruits and therefore larger amounts of fermentable substrates became available for many microbial communities. Apparently, the ability of fast ethanol accumulation and ethanol tolerance could then be crucial for inhibiting the growth of competing organisms, while later the accumulated ethanol could be "digested" by yeast in peace. We think that *Saccharomyces* owe their competitiveness to a combination of several properties, including fast growth, efficient glucose repression, good ability to produce and consume ethanol and very high tolerance towards several environmental stresses, such as high ethanol concentration and almost total absence of oxygen. These properties are unevenly distributed among different modern yeasts, but uniquely combined, specialized to perfection, and regulated and coordinated through an efficient network only in *S. cerevisiae* and its closest relatives. It could be that only this last aspect, the efficient regulation, is the most unique invention of the *Saccharomyces* yeasts, providing also a crucial competitive "advantage" in the breweries and wineries.

COMPUTATIONAL PHENOMICS

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A standard definition of a phenotype associates it to the organism's morphological, biochemical or physiological properties. From an engineering perspective, a phenotype describes the state of the organism at a distinct time: if the genotype and the environment are inputs, then the phenotype is the output of the system under study. Observations of relations between environmental/genetic changes and corresponding phenotypes provide ground for studies in functional genomics. Yet, if phenotypes are limited to a certain manifestation and report only on the effects of specific type (e.g., growth, sporulation, etc.), they are insufficient for genome-wide studies pertinent to system's biology. We argue that for systems biology classical phenotypes should be complemented with surrogates that can encode the state of the entire organism and provide increased resolution. As a candidate for such a phenotype we propose a global gene expression profiles and illustrate its use in the analysis of epistasis. Because phenotypes of this kind are in a sense raw and may only be used in association with adequate computational tools that may include preprocessing, phenotype characterization and feature construction, we refer to the methods that use them as computational phenomics.

RAČUNSKA FENOMIKA

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Phenotip organizma navadno povzema njegove morfološke, biokemične ali fizioške lastnosti oz. stanje. S perspektive inženirstva fenotip označuje stanje sistema ali stanje njegovega dela ob določenem času; če sta genotip in okolje vhoda, je fenotip izhod sistema, ki ga preučujemo. Funkcijska genomika tako na primer preučuje povezave med genotipom/okoljem in fenotipom, ter iz njih sklepa na funkcijo genov. A ker klasični fenotipi popisujejo samo določen, navadno omejen vidik stanja organizma (npr. rast, tvorjenje spor, ipd.), ti niso primerni za sistemske študije. V sistemski biologiji je zato potrebno ob teh fenotipih opazovati tudi nadomestne fenotipe, ki bi lahko popisali celotno stanje organizma in ob kvantitavnem zapisu fenotipa izboljšali resolucijo opazovanj. Kot kandidat za tak fenotip predlagamo genski transkripcijski profil, katerega uporabo bomo ilustrirali na analizi epistaze. Ker so tovrstni fenotipi kvantitativni in predstavljajo zbirko meritev, opravljenih na organizmu, so za njihovo predobdelavo in analizo ter uporabo potrebne računske metode. Ustrezno področje zato imenujemo računska fenomika.

COMBINATION OF MUTANT AND EXPRESSION DATA TO PREDICT THE MOLECULAR MECHANISM OF ACTION OF PERTURBATIONS TO YEAST CELLS

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Different types of '-omics' data can be used to analyze the cellular physiology on a global scale. Whole transcriptome analysis has proved invaluable in assigning the function of genes however data on gene expression is often insufficient to predict the molecular events on the whole scale from the genome to the phenome. Therefore, additional global phenotypic analyses should be used to provide missing data to understand the cellular physiology on a systems level. In systems biology, whose aim is quantitative in contrast to only qualitative analysis of biological phenomena, it is not enough to solely map the interactions between the genes and proteins involved in a process, but it is necessary also to provide deterministic rules of how the interactions govern the process under investigation. Only such analysis can generate predictions on how a perturbation would affect a certain cellular process. We have been using the combination of whole-genome gene expression data and chemical genomics or synthetic effects data to determine the molecular mechanism of action of small molecules or proteins, respectively, in a yeast cell. In conjunction, we have proposed deterministic rules linking specific combinations of gene expression and mutant data to molecular events, and developed bioinformatics tools to analyze the data, which paves the way for high-throughput analysis. Results on perturbations from rapamycin treatment and a neurotoxin expression will be presented.

KOMBINIRANJE PODATKOV O RASTI MUTANT IN O IZRAŽANJU GENOV ZA NAPOVEDOVANJE MOLEKULSKIH MEHANIZMOV DELOVANJA PERTURBACIJ NA CELICE KVASOVKE

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Za analizo celičnih procesov na celostni ravni lahko uporabljamo različne tipe podatkov, pridobljene z genomskimi eksperimentalnimi pristopi. Transkriptomske analize so postale nepogrešljivo orodje za določitev funkcije genov, vendar so podatki o izražanju genov pogosto nezadostni za napovedovanje molekulskih dogajanj v celotnem spektru od genoma do fenoma. Za razumevanje procesov na sistemski ravni so dodatno potrebni še celostni podatki o fenotipu. V sistemski biologiji, katere cilj je kvantitativna in ne le kvalitativna analiza bioloških pojavov, ni dovolj samo kartiranje povezav med geni in proteini, ki so udeleženi v preučevanem procesu, pač pa je potrebno tudi poznavanje pravil, kako te povezave vplivajo na proces. Le z uporabe takšne analize je moč napovedovati, kako določena perturbacija vpliva na celične procese. V našem laboratoriju uporabljamo kombinacijo podatkov o izražanju genov na ravni celotnega transkriptoma ter podatkov o vplivu majhnih molekul ali proteinov na hitrost rasti vseh viabilnih delecijskih mutant kvasovke *Saccharomyces cerevisiae*, kar nam služi za napovedovanje molekulskega mehanizma delovanja perturbacije na celice. Predlagali smo deterministična pravila, ki povezujejo značilne kombinacije sprememb v izražanju genov in rasti posameznih mutant z dogodki v celici na molekulski ravni. Razvili smo orodja bioinformatike za analizo tovrstnih podatkov, s čimer je omogočena hitra in standardizirana analiza podatkov. V predavanju bodo kot primer predstavljeni rezultati analize delovanja rapamicina oziroma nevrotoksina na celice kvasovke.

TEORETICAL ASPECTS - STATISTICAL ANALYSIS FROM CROSSBREEDING SCHEMES

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The model allowing genetic evaluation of purebred animals using measurements on purebred animals and crossbred relatives is implemented. Such aproach is suitable in species where production is mainly based on crossbred animals. Therefore, selection might be focused on improvement of crossbreds. Data is obtained in two purebred lines A and B and their cross(es) AB (and BA). List of traits can vary among populations. Measurements among populations are treated as different traits, while they are frequently obtained under different conditions. Thus, different statistical models are appropriate. While traits in purebred populations are recorded on testing stations under controled environment, traits in crossbreds are collected under production conditions. The greater the difference among rearing systems the smallest is the correlation among traits from different populations. For A and B, analyses are based on animal model. In order to reduce the number of equations, reduced animal model is utilized for AB or BA. Otherwise, the model may contain any fixed or random effect including random regression. The procedure allow heterogeniety of genetic (co)variances between populations. In addition, the genetic (co)variances in crossbred population may be different when used as sire or dam line. It allows correlations among traits measured in purebred populations and their crossbreds. Besides additive genetic effect, dominance is implemented as well. The models are supported in statistical package VCE 5. Thus, they can be combined with all other options in the software. Extension to more complex crossbred systems is intended but models must be verified. The procedure is implemented only for static crossbreeding systems.

TEORETIČNA IZHODIŠČA - STATISTIČNA ANALIZA PODATKOV IZ KRIŽANJ

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Namen predstavitve je razvoj modela, ki omogoča genetsko vrednotenje čistopasemskih živali na osnovi meritev opravljenih na živalih in njihovih sorodnikih križancih. Tak pristop je primeren pri speciesih, pri katerih prireja temelji na živalih križancih. S tem lahko selekcijo usmerimo predvsem v izboljšanje križancev. Podatke pridobimo na dveh čistopasemskih linijah A in B in križancih AB (in BA). Seznam lastnosti se lahko med populacijami razlikuje. Meritve v posameznih populacijah so upoštevane kot različne lastnosti, saj jih pogosto merimo v različnih pogojih in tako zanje praviloma uporabljamo različne statistične modele. Podatki za čistopasemske živali so pridobljeni v kontroliranih pogojih testnih postaj, podatki križancev pa pogosto v pogojih reje. Večje so razlike med sistemoma reje, manjše so korelacije med lastnostmi pridobljenimi v različnih populacijah. Pri populacijah A in B za analizo uporabimo model živali. Da pa bi zmanjšali število emačb in s tem velikost sistema pri križancih uporabimo reduciran model živali. Postopek dovoljuje heterogenost genetskih komponent (ko)varianc med populacijami. Model dopušča korelacije med meritvami merjenimi pri čistih pasmah in njihovih križanjih. Nadalje omogoča različne genetske korelacije glede na to, ali nastopa pasma kot očetovska ali maternalna linija. Poleg aditivnih genetskih komponent modeli omogočajo vključevanje dominance. Opisani modeli so vključeni v statistični paket VCE 5 in so tako lahko kombinirani z drugimi opcijami. Tako je omogočeno izvrednotenje komponent (ko)variance in napoved genetskih vrednosti. Predvidena je razširitev na bolj sestavljene sisteme križanja, a je še nujna preveritev modelov. Zaenkrat je aplikacija omejena na nekuntinuirane sheme križanja.

GENETIC EVALUATION FOR LITTER SIZE IN SWINE BY JOINT PUREBRED AND CROSSBRED DATA

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The aim of investigation was genetic evaluation of purebred animals for litter size using records of purebred and crossbred relatives from crossbreeding scheme. Data was obtained for two breeds Slovenian Landrace (line 11, SL) and Large White (LW), and their crosses SL (dam) x LW (sire) from three Slovenian pig farms. There were between 97308 and 146609 litters recorded per farm since 1989. Because populations on farms were not genetically connected, separate analyses were performed for each farm. Heterogeneous genetic (co)variances among populations and the other covariance components were estimated by the REML method using the VCE-5 package. Litter size in purebred and crossbred populations was treated as different traits with different models. The repeatability model was utilised, the fixed effects differed for gilts and sows. Common litter and permanent environment were included as trivial random effects. Animal model with direct additive genetic effect was used for purebreds, while reduced animal model was applied for crossbreds. Common litter effect accounted for 0.2% to 1.7% of variation in litter size, while permanent environment explained between 4.3 and 7.9% of variation for purebred animals and between 10.7% and 12.1% for crossbreds. Heritabilities reached values between 10.2% and 11.9% in purebreds and between 8.8% and 13.5% in crossbreds. Genetic correlations between SL and SLxLW were from 0.89 to 0.99, while between LW and SLxLW, they were lower and ranged from 0.84 to 0.92. Results recommend utilisation of crossbred records in the genetic evaluation of purebred parents.

GENETSKO VREDNOTENJE VELIKOSTI GNEZDA NA PODATKIH ČISTOPASEMSKIH IN HIBRIDNIH PRAŠIČEV

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Namen raziskave je bil genetsko vrednotenje čistopasemskih živali za velikost gnezda za uporabo meritev pri čistopasemskih in hibridnih sorodnikih iz sheme križanja. Uporabili smo podatke dveh pasem slovenske landrace (linije 11, SL) in large white (LW) ter njihovih križank SL (mati) x LW (oče) s treh slovenskih farm prašičev. Od leta 1989 je bilo na farmah med 97308 in 146609 prasitev. Ker populacije med farmami niso genetsko povezane, smo opravili ločene analize. Heterogene genetske (ko)variance in preostale komponente kovariance smo ocenili po metodi REML s paketom VCE-5. Velikost gnezda za čistopasemske in hibridne populacije smo obravnavali kot različne lastnosti z različnimi modeli. Uporabili smo ponovljivostni model, pri mladicah in starih svinjah so se razlikovali sistematski vplivi. Skupno okolje v gnezdu in permanentno okolje sta bila vključena kot navadna naključna vpliva. Za čistopasemske živali smo se poslužili modela živali z vključenim direktnim aditivnim genetskim vplivom, medtem ko smo za hibridne živali uporabili reducirani model živali. Vpliv skupnega okolja v gnezdu je predstavljal med 0,2 % in 1,7 % variabilnosti za velikost gnezda, permanentno okolje pa med 4,3 % in 7,9 % pri čistopasemskih ter med 10,7 % in 12,1 % variabilnosti pri hibridih. Heritabiliteta je dosegla vrednosti med 10,2 % in 11,9 % pri čistopasemskih ter med 8,8 % in 13,5 % pri hibridih. Genetske korelacije so bile med SL in SLxLW od 0,89 do 0,99, medtem ko so bile med LW in SLxLW nekoliko nižje, med 0,84 in 0,92. Rezultati kažejo, da je vključitev meritev na hibridih v genetsko vrednotenje čistopasemskih staršev priporočljiva.

HERITABILITY ESTIMATES FOR MILK TRAITS WITH REGRESSION MODELS IN DAIRY SHEEP IN SLOVENIA

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Heritability estimates for daily milk yield (DMY), fat (FC) and protein (PC) contents for longitudinal records in dairy sheep were obtained using regression models. In the period 1994-2002, 38983 test-day records of 3068 ewes were collected. Covariance components were estimated with restricted maximum likelihood (REML) using models with fixed regression (TDM), fixed regression on intervals (MTM) and random regression (RRM) using Legendre polynomials. In random part, models contained additive genetic effect, common flock environment and permanent environment of the ewe. Additionally, TDM and RRM contained a permanent environment effect within lactation. Heritabilities from TDM were 0.11 for DMY, 0.08 for FC and 0.10 for PC. From MTM, the heritability for DMY increased from 0.15 in the first to 0.23 in the fifth month of lactation. Afterwards, it decreased to 0.10 in the eighth month. Heritability for FC increased from 0.10 in the first to 0.18 in the eighth month of lactation. For PC, the heritability oscillated within 0.19 in the first and 0.23 in the third and seventh month of lactation. Heritabilities from RRM were lower than from MTM, but tendencies were similar. An increase of heritability for DMY from 0.11 at the beginning to 0.17 in the middle of lactation (around 167th day) was noticed. Later, heritability was decreasing toward 0.08 at the end of lactation. Heritabilities for FC (0.08 - 0.13) and PC (0.16 - 0.28) were increasing during lactation. RRM enables to observe changes in heritabilities and genetic values at any point along lactation trajectory.

OCENE HERITABILITET ZA LASTNOSTI MLEČNOSTI S POMOČJO REGRESIJSKIH MODELOV PRI MLEČNIH OVCAH V SLOVENIJI

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Ocenjevali smo heritabilitete za dnevno količino mleka (DKM) in vsebnosti maščobe (VM) ter beljakovin (VB) na dan kontrole pri mlečnih ovcah s pomočjo regresijskih modelov. Analizirali smo 38983 meritev zbranih v letih od 1994 do 2002 od 3068 ovc. Komponente (ko)varianc smo ocenili z metodo omejene največje zanesljivosti (REML) s pomočjo modelov s sistematsko regresijo (TDM), s sistematsko regresijo z intervali (MTM) in z naključno regresijo (RRM) z uporabo Legendrovih polinomov. Modeli so v naključnem delu vsebovali aditivni genetski vpliv, skupno okolje v tropu in permanentno okolje živali. Modela TDM in RRM sta dodatno vsebovala še permanentno okolje znotraj laktacije. Heritabilitete iz TDM modela so bile 0,11 za DKM, 0,08 za VM in 0,10 za VB. Pri MTM modelu se je heritabiliteta za DKM povečala od 0,15 v prvem do 0,23 v petem mesecu laktacije, nakar se je do osmega meseca zmanjšala na 0,10. Za VM se je heritabiliteta povečala od 0,10 v prvem do 0,18 v osmem mesecu laktacije. Heritabiliteta za VB je nihala med 0,19 v prvem in 0,28 v tretjem in sedmem mesecu laktacije. Heritabilitete pri RRM modelu so bile manjše kot pri MTM modelu, trendi pa podobni. Za DKM se je heritabiliteta povečevala od 0,11 na začetku do 0,17 na sredini laktacije (okrog 167. dne), nakar se je do konca laktacije zmanjšala na 0,08. Tekom laktacije sta se heritabiliteti za VM (0,08 - 0,13) in VB (0,16 - 0,28) povečevali. Model RRM omogoča spreminjanje heritabilitet in genetskih vrednosti v vsaki točki tekom laktacije.

GOLDEN CHROMOSOME
ZLATI KROMOSOM

DHPLC BASED METHOD USING MONONUCLEOTIDE REPEATS AND PENTAPLEX PCR FOR RAPID AND ACCURATE ANALYSIS OF MICROSATELLITE INSTABILITY IN COLORECTAL CANCER

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Microsatellites are tandemly repeated sequences of DNA distributed throughout the genome. Microsatellite instability (MSI) is a phenomenon characterized by small deletions or insertions within microsatellites in tumour DNA compared to matching normal DNA. MSI analysis is very important tool for detection of hereditary non-polyposis colorectal cancer and MSI high sporadic primary colorectal tumours with specific clinicopathological features. Set of two mononucleotide and three dinucleotide microsatellite markers was proposed in 1997 to provide uniform criteria for MSI analysis. In 2002 the guidelines were revised and an exclusive use of mononucleotide markers was proposed. The purpose of our study was to develop a new method for MSI analysis with use of denaturing high performance liquid chromatography (DHPLC) and mononucleotide microsatelite markers in order to simplify the screening for MSI high tumours. We analysed 595 colorectal tumours and 145 normal samples. Five microsatellite markers BAT-25, BAT-26, NR-21, NR-22, and NR-27 were amplified in a single multiplex PCR reaction and analysed using DHPLC and capillary electrophoresis. Here we report a new DHPLC based method for MSI analysis. Analysis and cross-examination of results obtained from 96 samples using DHPLC and capillary electrophoresis showed the same sensitivity and specificity of both methods for detection of MSI-H tumours. Using our new method we have shown that tested markers are quasimonomorphic in Slovenian population with frequencies of polymorphisms 0,07%, 1,4%, 2,1%, 1,4%, and 1,4% for BAT-25, BAT-26, NR-21, NR-22, and NR-27 respectively. We identified 43 (7,2%) new MSI-H tumours. Overall, we developed a high-throughput, robust, accurate and cost-effective method for detection of MSI-H tumours using DHPLC.

METODA NA OSNOVI TEHNOLOGIJE DHPLC IN UPORABE MONONUKLEOTIDNIH TANDEMSKIH PONOVITEV TER MULTIPLE PCR ZA HITRO IN ZANESLJIVO ANALIZO MIKROSATELITNE NESTABILNOSTI PRI KOLOREKTALNEM RAKU

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Mikrosateliti so tandemsko ponavljajoča zaporedja DNA razporejena po celotnem genomu. Mikrosatelitna nestabilnost (MSI) je fenomen, ki ga označujejo manjše delecije in vstavitve na področju mikrosatelitov tumorske DNA v primerjavi z DNA zdravega tkiva. Analiza MSI je pomembno orodje za zaznavo dednega nepolipoznega kolorektalnega raka in sporadičnih visoko mikrosatelitno nestabilnih tumorjev s specifičnimi kliničnimi in patološkimi značilnostmi. V letu 1997 je bila predlagana paleta dveh mononukleotidnih in treh dinukleotidnih mikrosatelitnih označevalcev z namenom poenotenja analize MSI, ki so jo v letu 2002 spremenili in predlagali uporabo izključno mononukleotidnih mikrosatelitnih označevalcev. Z razvojem metode za analizo MSI na osnovi tehnologije denaturacijske visoko ločljivostne tekočinske kromatografije (DHPLC) in uporabo mononukleotidnih mikrosatelitnih označevalcev želimo poenostaviti analizo za presejanje visoko mikrosatelitno nestabilnih tumorjev. Analizirali smo 595 kolorektalnih tumorjev in 145 vzorcev zdravega tkiva. Pet mikrosatelitnih označevalcev BAT-25, BAT-26, NR-21, NR-22, in NR-27 smo pomnožili z multiplo PCR reakcijo, ter jih analizirali s pomočjo DHPLC in kapilarne elektroforeze. Z uporabo tehnologije DHPLC smo razvili novo metodo za analizo mikrosatelitne nestabilnosti. Navzkrižno preverjanje rezultatov 96 vzorcev analiziranih z DHPLC in kapilarno elektroforezo je pokazalo enako občutljivost in specifičnost obeh metod. Z uporabo nove metode smo pokazali, da so uporabljeni mikrosatelitni označevalci v slovenski populaciji skoraj monomorfni (frekvence polimorfizmov: BAT-25 0,07%, BAT-26 1,4%, NR-21 2,1%, NR-22 1,4% in NR-27 1,4%) in odkrili 43 (7,2%) novih visoko mikrosatelitno nestabilnih tumorjev. S pridobljenimi rezultati smo uspeli potrditi, da je nova metoda, ki smo jo razvili z uporabo tehnologije DHPLC, visoko zmogljiva, robustna, zanesljiva in cenovno ugodna.

MOLECULAR CHARACTERIZATION OF ERYTHROPOIETIC PROTOPORPHYRIA (EPP) IN SLOVENIA – IDENTIFICATION OF NOVEL MUTATIONS IN THE FERROCHELATASE GENE

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Erythropoietic protoporophyria (EPP) is a photodermatosis due to accumulation of protoporphyrin (PP) IX in the red blood cells, caused by complete or partial deficiency of the enzyme ferrochelatase (FECH) responsible for the incorporation of the ferrous ion into PP, the last step in the synthesis of hem. The increased amount of PP in erythrocytes, blood serum and skin triggers severs photosensitivity reactions. The mode of inheritance is complex, autosomal dominant with low clinical penetrance (in most cases) or autosomal recesive. The *FECH* gene is located on chromosome 18q21.3 with 11 exons and spans ~45 kbp. 51 blood samples were obtained for analysis, 14 of them from verified EPP patients. DNA, extracted from blood samples and amplified in PCR for genetic analysis of *FECH* gene, was analysed by denaturating high pressure liquid chromatography (DHPLC) using mutation detection method and sequence analysis was performed by ABI PRISM 310 Genetic Analyzer. Preliminary results showed 14 different nucleotide substitutions and deletions, some of them described as polymorphism strongly related with EPP: (i) IVS1-23C/T linked to the low expressed *FECH* allele described by Gouya et al, 1999, 287G/A (Q96R) and 921G/A (P307P) described by Wiman et al, 2003; 10 of them not yet described, as example: IVS3-45_46delCT, 1168delC, IVS5-3c/t, and others. IVS3-45_46delCT could have effect on the splice site modulator IVS3-48T/C described by Wiman et al, splice site could be affected by IVS5-3c/t polymorphism, protein could be affected more severly by 1168delC. All found changes should be investigated further.

MOLEKULARNA KARAKTERIZACIJA ERITROPOETSKE PROTOPORFIRIJE V SLOVENIJI – ODKRITJE NOVIH SPREMEMB V GENU ZA FEROKELATAZO

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Za eritropoetsko protoporfirijo (EPP) je značilno vnetje na koži bolnikov ob izpostavitvi le-te žarkom UV. Zaradi nezadostnega delovanja encima ferokelataze (FECH) je vgrajevanje železa v protoporfirinski obroč, zadnjega koraka v biosintezi hema, nezadostno. Odvečni protoporfirin (PP) IX se kopiči v eritrocitih, krvnem serumu in koži ter kot močan fotosenzibilizator povroča vnetje. Način dedovanja obolenja EPP je kompleksen, v večini primerov avtosomno dominanten z nizko penetranco, ali pa avtosomno recesiven. Gen *FECH* je zapisan na ~ 45 kb dolgem odseku kromosoma 18, na mestu q21.3 in ima 11 eksonov. Od 51-ih vzorcev krvi bolnikov in njihovih svojcev smo podrobneje preučili tistih 14, pri katerih je bilo obolenje EPP potrjeno. Izolirana genomska DNA je služila kot vzorčna DNA za pomnoževanje gena *FECH* z reakcijo verižne polimerizacije, posamezne pomnožene eksone smo nadalje analizirali z denaturirajočo visokotlačno tekočinsko kromatografijo (DHPLC) za prisotnost sprememb, ki smo jih kasneje potrdili s sekvenčno reakcijo. Odkrili smo 13 različnih nukleotidnih sprememb (zamenjav in izbrisov). Nekateri od polimorfizmov so bili predhodno že opisani kot polimorfizmi močno povezani z obolenjem EPP, in sicer: (i) IVS1-23C/T povezan z nižjim izražanjem alela *FECH* (Gouya in sod, 1999), 287G/A (Q96R), 921G/A (P307P) (Wiman et al, 2003); ostale odkrite spremembe še niso bile opisane: IVS3-45_46delCT, 1168del C , IVS5-3c/t, 798(G/C) in drugi polimorfizmi. IVS3-45_46delCT bi lahko vplival na modulatorsko mesto izrezovanja intronov IVS3-48T>C (Wiman et al, 2003), na izrezovanje intronov bi lahko imel vpliv IVS5-3c/t, potrebno je potrditi vpliv 1168delC in drugih odkritih polimorfizmov na protein FECH.

TEMPERATURE DEPENDENT COLICIN K SYNTHESIS

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Temperature is an important environmental signal to which bacteria respond by altering gene expression at the level of transcription and/or posttranscriptionally. Colicin K (Cka) is a toxic pore-forming exoprotein synthesized by *E. coli* active against strains of the same or related species. We have found that besides ppGpp and the LexA protein, temperature is also a strong signal for *cka* expression as expression is optimal at 37 °C. To elucidate the mechanism underlying temperature-dependent Cka expression studies at the level of the native protein, *cka* mRNA and ß galactosidase activity of a number of *cka-lacZ* fusions were performed. To emphasize the ecological significance of our results, the temperature dependence of synthesis of four other colicins, E4, E7, N and colicin A was also studied. Our results revealed that temperature dependent regulation of *cka* expression is imposed at the level of transcription and that it also has a profound effect on the other tested colicins with the exception of colicin A. To elucidate the mechanism involved in temperature dependent regulation, we determined whether DNA itself serves as a thermosensor or alternatively, if a sensor protein responding to temperature change is involved. Using *cka-gfp* fusions with mutations in LexA binding sequences and computer modelling we also determined the role of the LexA repressor in temperature dependent cka expression.

S TEMPERATURO URAVNANA SINTEZA KOLICINA K

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Temperatura je pomemben okoljski signal na katerega se bakterije odzovejo s spremembo izražanja genov na stopnji transkripcije in/ali stopnji postranskripcije. Kolicin K (Cka) je toksičen protein, ki tvori pore v celični membrani bakterij. Sintentizirajo ga sevi bakterij *E. coli*. Toksično delovanje toksina je zelo specifično, saj deluje na bakterije iste vrste. Ugotovili smo, da je poleg ppGpp in proteina LexA, temperatura močan signal za izražanje *cka,* optimalno izražanje gena *cka* je pri 37 °C. Za razjasnitev mehanizma s temperaturo uravnanega izražanja *cka* so bile izvedene raziskave na nivoju nativnega proteina, *cka* mRNA ter ß-galaktozidazne aktivnosti fuzij promotorske regije *cka-lacZ*. Dokazali smo, da temperatura uravnava izražanje gena *cka* na stopnji transkripcije. Proučili smo tudi vpliv temperature na sintezo kolicinov E1, E7, N in A. Potrdili smo, da je, z izjemo kolicina A, tudi sinteza drugih testiranih kolicinov uravnana s temperaturo. Da bi razjasnili molekularni mehanizem od temperature odvisnega uravnavanja izražanja, smo raziskali dve možnosti: (1) DNA deluje kot senzor, (2) vpletenost regulatornih proteinov. Z uporabo *cka-gfp* fuzij mutiranih v LexA vezavnem zaporedju ter z računalniškim modeliranjem smo potrdili vpliv represorja LexA na s temperaturo uravnano sintezo Cka.

CYTOGENETICAL AND MORPHOLOGICAL STUDIES OF NOVEL GREAT HEADED GARLIC (*ALLIUM* SP.) ACCESSIONS

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The genus *Allium* consists of about 700 species spread throughout the Northern hemisphere. Garlic (*A. sativum* L.), onion (*A. cepa* L.) and in Europe leek (*A. porrum*) are the major cultivated Allium species. In addition, "Great headed garlic" (GHG) together with leek, kurrat and pearl onion are also commercially grown. GHG is utilized as a garlic substitute, having the appearance of a robust garlic plant, with similar bulb structure. Known forms are hexaploid (2n= 6x=48) and seed-sterile therefore only clonal propagation is possible. GHG is believed to belong taxonomically to a heterogeneous *A. ampeloprasum* complex. Some recent data suggest that the taxonomic position of GHG needs to be reevaluated. Cytogenetically, this complex group is extremely diverse, consisting of several cytotypes (2x-3x-4x-5x and 6x). A new form of GHG was collected from local growers and included in a breeding program, during the 1990s in the province of Mendoza (Argentina). In contrast to other known forms of GHG, it was found that this novel form is fertile. The aim of our studies was to describe the basic morphological and cytogenetic characteristics of this valuable novel accession. Results revealed that all investigated accessions produced fertile seeds, with germination rate around 30%. Genome size analysis and chromosome counting of the GHG accessions, made in comparison to hexaploid forms, revealed that the accessions were octoploid, with a genome size around 121 pg. Further studies are underway to reveal genetic constitution of these unusual accessions, which are according to their karyotype most likely of allopolyploid origin.

CITOGENETSKE IN MORFOLOŠKE RAZISKAVE NOVIH AKCESIJ POLETNEGA LUKA (ALLIUM SP.)

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Rod *Allium* zajema približno 700 vrst, ki so razširjene po celi severni polobli. Med njimi sta glavni gojeni vrsti česen (*A. sativum* L.) in čebula (*A. cepa* L.), v Evropi pa tudi por (Allium porrum). V skupini sorodni poru se prideluje tudi "poletni luk" (slovenski izraz je potrebno še premisliti, angleški izraz je Great headed garlic - GHG ali elephant garlic), kurat in biserni luk (pearl onion). GHG, ki je videti kot orjaška rastlina česna s podobno strukturo čebulice, se uporablja kot njegov nadomestek. Je heksaploid (2n= 6x=48) in ima sterilna semena, zato ga množijo le klonsko. Taksonomsko ga uvrščamo v heterogeno skupino *A. ampeloprasum,* ki pa jo je treba kot kaže dodatno proučiti. Citogenetsko je to izredno raznolika skupina kjer najdemo di-, tri-, tetra-, penta- in heksaploidne rastline. Leta 1990 so v provinci Mendoza (Argentina) odkrili novo obliko GHG, ki je za razliko od ostalih, fertilna. Cilj naših raziskav je bil opisati njegove osnovne morfološke in citogenetske značilnosti. Rezultati so pokazali, da so imeli vsi preiskovani vzorci fertilna semena z okoli 30% kalivostjo. Štetje kromosomov in analize velikosti genoma so pokazali, da so bili vzorci oktaploidni z velikostjo genoma približno 121 pg. Kariološke raziskave nakazujejo alopoliploidni izvor njihovega genoma, z raziskavami, ki so trenutno v teku pa želimo pojasniti njegov izvor.

GENETIC VARIATIONS OF THE HORSE KAPPA CASEIN GENE (*CSN3*) AND COMPARATIVE GENOMICS APPROACH TO STUDY CONSERVED REGIONS

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Kappa casein is milk protein that determines the size and specific function of the milk micelles and its clevage by chymosine is resonsible for milk coagulation. We identified two single nucleotide polymorphisms (SNPs) in exon 1 and two in exon 4 of the horse kappa casein gene and genotyped them in three horse breeds. SNPs in exon 4 cause a.a. change in the mature product and may render chemical/functional properties of the protein. We identified 15 SNPs in *CSN3* gene promoter in six horse breeds and investigated them for involvment in putative transcription factor binding sites. Using horse primers we determined promoter and exon 4 nucleotide squence in donkey and zebra. Alignment of the promoter sequence in nine species (sheep, goat, cow, zebra, donkey, horse, human, chimp, macaque) revealed high conservation and placed them in three distinct groups. Two SNPs within zebras exon 4 were discoverd, bouth causing a.a. substitutions. Alignment of exon 4 sequence between donkey, zebra and horse revealed almost perfect conservation exept three substituted nucleotides, one of them causing a.a. substitution within chimosin sensitive region of the kappa casein protein.

GENETSKA VARIABILNOST KAPA KAZEINSKEGA GENA (*CSN3*) PRI KONJU IN PRISTOP PRIMERJALNE GENOMIKE ZA ŠTUDIJ OHRANJENIH PODROČJI

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Kapa kazein je mlečni protein, ki določa velikost in specifično funkcijo mlečnih micel, njegova razgradnja s kimozinom pa je odgovorna za koagulacijo mleka. Izvedli smo genetsko analizo dveh nukleotidnih zamenjav v eksonu 1 in dveh v eksonu 4 kapa kazeinskega gena in jih genotipzirali pri treh pasmah konj. Zamenjavi nukleotidov v eksonu 4 povzročita zamenjavo aminokislin v končnem produktu, kar pa lahko spremeni kemične/funkcionalne lastnosti proteina. V promotorju gena *CSN3* smo pri šestih pasmah konjev identificirali 15 nukleotidnih zamenjav ter proučili njihovo udeleženost v potencialna vezna mesta za transkripcijske faktorje. Z uporabo začetnih oligonukleotidov za konja, smo določili nukleotidno zaporedje promotorja in eksona 4 pri oslu in zebri. S primerjavo promotorske sekvence pri devetih vrstah (ovca, koza, krava, zebra, osel, konj, človek, šimpanz, makak) smo ugotovili visoko ohranjenost in jih razvrstili v tri skupine. Našli smo tudi dve nukleotidni zamenjavi v eksonu 4 zebre, ki povzročita zamenjavo aminokisline. S poravnavo zaporedja eksona 4 med oslom, zebro in konjem, smo ugotovili skoraj popolno ujemanje z izjemo treh nukleotidov. Pri enem od njih pride posledično do zamenjave aminokisline v na kimozin občutljivem področju proteina.

GENOTOXICITY OF ORGANOPHOSPHOROUS PESTICIDES CORRELATES WITH THE INDUCTION OF DNA DAMAGE RESPONSIVE GENES

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Organophosphorous compounds (OPs) are the most commonly used pesticides worldwide. Their neurotoxic effect are relatively well explored, however, very little is known about the chronic effects of OPs on non-target human cells and the possible secondary mechanisms of OPs' activity. Our aim was to investigate whether low concentrations of OP pesticides can cause DNA damage in human liver cells and affect expression of genes known to be involved in the DNA damage response. Genotoxicity of three model OP pesticides – parathion-methyl (PT), paraoxon-methyl (PO) and dimefox (DF) was evaluated using the bacterial Ames reverse mutation assay and by the comet assay in human hepatoma HepG2 cells. All three OPs were negative in the bacterial Ames assay. In HepG2 cells exposed to sub-cytotoxic concentrations (0.01 – 100 μg/mL) of PT and PO increased the level of DNA strand breaks was detected only at the highest concentration (100 μg/mL), while DF did not induce DNA damage. Using RT-PCR, we found that exposure of HepG2 cells to PT and PO, caused an increase in the expression of genes that are involved in the response to genotoxic stress: P53, MDM2, GADD45 and P21, while DF upregulated only the expression of P53. Upregulation of MDM2, GADD45 and P21 correlated with the ability of OPs to induce genotoxic effects indicating that analysis of the expression of DNA damage responsive genes is a promising method for evaluating genotoxicity. Based on these data we conclude that PT and PO have genotoxic potential, while DF is probably not genotoxic.

GENOTOKSIČNOST ORGANOFOSFATNIH PESTICIDOV KORELIRA Z INDUKCIJO IZRAŽANJA GENOV, KI SE ODZOVEJO NA POŠKODBE DNA

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Organofosfatni pesticidi (OP) so najbolj široko uporabljani pesticidi današnjega časa. Njihovi nevrotoksični učinki so razmeroma dobro raziskani, vendar je zelo malo znanega o kroničnih učinkih OP na netarčna človeška tkiva in o možnih sekundarnih mehanizmih delovanja OP. Naš cilj je raziskati, ali nizke koncentracije OP lahko povzročijo poškodbe DNA v netarčnih človeških celicah in ali vplivajo na izražanje izbranih genov, za katere je znano, da sodelujejo v odzivu na poškodbe DNA. S pomočjo bakterijskega Ames testa in s komet testom na človeških HepG2 celicah smo ugotavljali genotoksičnost treh OP pesticidov – metil-paration (PT), metil-paraoksona (PO) in dimefoksa (DF). V Ames-ovem testu so bili vsi trije OP negativni. Pri HepG2 celicah izpostavljenih ne-citotoksičnim koncentracijam (0,01 – 100 μg/mL) sta PO in PT povzročila povečanje števila prelomov DNA pri najvišji koncentraciji (100 μg/mL), medtem, ko DF ni povzročil poškodb DNA. S kvantitativnim PCR v realnem času (RT-PCR) smo ugotovili, da je izpostavljenost HepG2 celic PT in PO povzročila povečano izražanje genov, ki sodelujejo pri odgovoru na genotoksični stres: P53, MDM-2, GADD45 in P21, medtem, ko je DF povečal le izražanje gena P53. Povečano izražanje genov MDM2, GADD45 in P21 se je ujemalo s sposobnostjo povzročanja genotoksičnih učinkov, kar kaže, da je analiza izražanja genov obetavna metoda za ocenjevanje genotoksičnosti. Na osnovi dobljenih rezultatov lahko sklepamo, da PT in PO imata genotoksični potencial, DF pa ne deluje genotoksično.

MICROSATELLITE MARKER FOR HOMOZYGOSITY TESTING OF *MIMULUS AURANTIACUS*

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Haploids are sporophytic plants with gametophytic chromosome number originating from a female (gynogenesis) or male (androgenesis) gametic cell. Haploid induction through *in vitro* culture techniques lead to regeneration of haploid and/or diploid regenerants. The latter could be spontaneously arising homozygous diploids (doubled haploids) which are directly useful in breeding. Aditionally, diploid regenerants could be also heterozygous regenerants from sphorophytic cells or heterozygous zygotic embryos, both unwanted for haploid induction purposes. This is the first report about the discovery of a very efficient method for homozygosity determination of *M.aurantiacus* regenerants using microsatellite marker. Microsatellite markers previously isolated from two *Mimulus* species failed to amplify DNA fragments in *M.aurantiacus* genotypes. Degenerated primers designed for the microsatellite locus located in the intron of the top6B gene (subunit B of the topoisomerase gene) isolated from hop (*Humulus lupulus* L.) were able to amplify alleles in the analysed plants. PAGE profiles revealed high level of polymorphism between four *M.aurantiacus* genotypes and two *Mimulus* species. Three of the genotypes proved to be heterozygous for the analysed locus. Sequence analysis of amplified bands defined allelic forms and enabled us to design the locus specific primers. These amplified all analysed *M.aurantiacus* genotypes and also five other *Mimulus* species. Developed primer pair represents necessary tool for *Mimulus* haploid induction research and applications. Early homozygosity determination enables successful selection of doubled haploids from unwanted heterozyogotes which saves time and funds needed for phenotypic evaluations. It is an unambiguous demonstration of homozygosity of regenerants and could be also used for interspecific hybrid determination.

TESTIRANJE HOMOZIGOTNOSTI PRI VRSTI *MIMULUS AURANTIACUS* S POMOČJO MICROSATELITNEGA MARKERJA

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Haploidi so sporofitne rastline z gametofitnim številom kromosomov. Običajno jih izzovemo iz monoploidnih celic moškega ali ženskega gametofita z *in vitro* postopki, saj se v naravi redko pojavljajo. Poznani sta dve osnovni poti nastanka haploidnega osebka in sicer po poti androgeneze (t.j. kulture anter oz. mikrospor) in po poti ginogeneze (t.j. kulture semenskih zasnov, plodnic, celih cvetov, socvetij). V obeh primerih se iz inokuliranih struktur lahko razvijejo haploidni in/ali diploidni regeneranti. Slednji so lahko homozigotni podvojeni haploidi ali heterozigoti izvirajoči iz somatskih celic oz. zigotnih embrijev. Namnoževanje mikrosatelitskih lokusov izoliranih iz dveh vrst rodu *Mimulus* pri treh genotipih vrste *Mimulus aurantiacus* ni bilo uspešno. Bolj uspešno je bilo namnoževanje mikrosatelitskega lokusa iz introna top6B gena (B podenota topoizomeraznega gena) izoliranega iz hmelja (*Humulus lupulus* L.). Rezultati PAGE elektroforeze so pokazali polimorfizem namnoženih fragmentov med genotipi vrste *M.aurantiacus* in med dvema vrstama rodu *Mimulus*. S sekveniranjem namnoženih fragmentov so bile izločene nespecifične namnožitve in izdelani lokusno specifični začetni oligonukleotidi s katerimi je mogoče namnoževati genotipe vrste *M.aurantiacus* in pet vrst rodu *Mimulus*, ki se uporabljajo pri žlahtnjenju vrste *M.aurantiacus* s pomočjo medvrstnih križanj. Izmed štirih analiziranih genotipov vrste *M.aurantiacus* so bili trije heterozigotni za izdelani mikrosatelitski lokus, kar omogoča testiranje homozigotnosti diploidnih regenerantov. Zgodnje preverjanje izvora regenerantov bistveno pospeši in poceni raziskave in uporabnost indukcije haploidov. S pomočjo odkritega markerja je mogoče nedvoumno dokazati izvor diploidnih regenerantov in preveriti medvrstne križance.

GENETIC CHARACTERIZATION OF BUMBLEBEES (HYMENOPTERA: APIDAE) IN SLOVENIA

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About 300 bumblebee species (Apidae: Bombus) are known world-wide. They are divided into 38 subgenera and species from which 16 subgenera can be found also in Europe. Based on previous studies using morphological characterisations 31 bumblebees species belonging to nine subgenera can be found in Slovenia. In this study mitochondrial DNA (mtDNA) sequences and microsatellite analysis for assessment of genetic population structure and diversity of bumblebees in Slovenia were used. Approximately 500 bumblebees from 102 different localities in Slovenia were included. 21 different bumblebee-species were identified by morphology using different keys. Sequencing of 450-bp fragment of mtDNA COI confirmed 21 different species and also showed that all sequences obtained from the same species were monomorphic, with exceptions of species *B. lucorum, B. pascuorum and B. terrestris* where different numbers of transitions were found. Sequences have been compared with 333 sequences of bumblebee mitochondrial COI fragments deposited in the GenBank. The sequence similarity with deposited sequences was high, however, deletions, insertions and transitions were found in almost all species. In addition six microsatellite loci were used to investigate genetic differentiation for six of the most widespread species over Slovenia: *B. hortorum, B. humilis, B. lapidarius, B. lucorum, B. pascuorum* and *B. terrestris*. All microsatellite loci displayed high levels of polymorphism in all analyzed species. The total number of alleles detected per locus ranged from 21 to 27 and calculated heterozygosities ranged from 0.67 to 0.85 and showed high variability within species. The 10 species previously described in Slovenia but not found in the current study might still be present in nature in very low numbers. However, they could already be extinct because of destruction of their natural habitats.

GENETSKA KARAKTERIZACIJA ČMRLJEV (HYMENOPTERA: APIDAE) V SLOVENIJI

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Na svetu je pozanih približno 300 vrst čmrljev (Apidae: Bombus). Razdeljene so v 38 podvrst, od katerih jih je v Evropi prisotnih 16. Na podlagi prejšnjih študij, ki so temeljile le na morfološki karakterizaciji, je v Sloveniji prisotnih 31 vrst čmrljev, ki so razdeljeni v 9 podvrst. V tej študiji smo poleg morfoloških znakov preiskovali tudi sekvenčno zaporedje odseka mitohondrijske DNK (mtDNK) in mikrosatelitne markerje, za ocenitev genetske strukture populacije in raznolikosti čmrljev v Sloveniji. V analizo je bilo vključenih približno 500 čmrljev iz 102 lokacij po Sloveniji. S pomočji opisov in različnih indentifikacijskih ključev, je bilo določenih 21 različnih vrst čmrljev. Tudi sekvenčna analiza 450-bp dolgega odseka mtDNK je potrdila 21 različnih vrst in poleg tega odkrila, da so vse dobljene sekvence istih vrst enake, razen pri vrstah *B. lucorum, B. pascuorum in B. terrestris,* kjer so bile najdene točkaste mutacije. Sekvence smo primerjali tudi s 333-imi različnimi sekvencami, ki so shranjene v bazi GenBank. Sekvence so si bile med sabo zelo podobne, našli smo le posamezne mutacije. V mikrosatelitno analizo smo vključili šest mikrosatelitnih lokusov in šest najbolj razširjenih vrst čmrljev v Sloveniji: *B. hortorum, B. humilis, B. lapidarius, B. lucorum, B. pascuorum* in *B. terrestris*. Vsi mikrosatelitni lokusi so bili zelo polimorfni, pri vseh analiziranih vrstah. Skupno število najdenih alelov na polimorfen lokus je bilo med 21 in 27 in izračunana heterozigotnost je bila od 0.67 do 0.85, kar kaže na veliko znotrajvrstno variabilnost. 10 preostalih vrst, ki so bile opisane v Sloveniji, v tej študiji niso bile vključene, ker jih nismo našli. Njihovo število v naravi je lahko zelo majhno, lahko pa so tudi že izumrli, predvsem zaradi uničevanja njihovega naravnega okolja.

MOLECULAR CHARACTERIZATION OF CHICKEN IMMUNOCOMPETENT CELL RESPONSE TO *MYCOPLASMA SYNOVIAE* HAEMAGGLUTININS

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Mycoplasma synoviae (MS) is the causative agent of chronic respiratory disease and infectious synovitis in chickens and turkeys. Only scarce data is available on the interaction of MS and its antigens with chicken immunocompetent cells. Our research included chicken macrophages infected with MS or exposed to MS antigens. From MS surface antigens, VlhA haemagglutinin, a major immunomodulatory membrane protein, was selected for more exhaustive research. MS infection, exposure to MS membrane antigens or exposure to VlhA, showed induced expression of genes for proinflammatory cytokines and nitric oxide synthesis in chicken macrophages, as well as induced secretion of listed molecules. Using a 5k avian innate immunity microarray, a variety of chicken macrophage chemokine and cytokine genes involved in the avian inflammatory response as well as genes involved in other aspects of innate immune response, were found to respond to MS infection or exposure to VlhA. Microarray results of selected genes were confirmed with qRT-PCR.

MOLEKULARNA KARAKTERIZACIJA ODZIVA KOKOŠJIH IMUNSKO ZMOŽNIH CELIC NA PRISOTNOST HEMAGLUTININOV IZOLIRANIH IZ BAKTERIJE *MYCOPLASMA SYNOVIAE*

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Mycoplasma synoviae (MS) je povzročitelj kronične respiratorne bolezni in infekcioznega sinovitisa pri kokoših in puranih. Na voljo so le skopi podatki o interakciji MS in njenih antigenov s kokošjimi imunsko zmožnimi celicami. V sklopu naših raziskav smo kokošje makrofagi okužili z MS ali izpostavili MS antigenom. Izmed površinskih antigenov MS smo za izčrpnejše raziskave izbrali VlhA, ki je poglavitni membranski imunomodulatorni protein MS. Okužba z MS, izpostavitev membranskim antigenom MS ali izpostavitev proteinu VlhA so povzročili porast izražanja genov za sintezo proinflamatornih citokinov in dušikovega oksida, skupaj s povečano sekrecijo naštetih molekul. Z uporabo 5k mikromreže za ptičjo prirojeno imunost smo ob okužbi kokošjih makrofagov z MS ali njihovi izpostavitvi VlhA, opazili odziv vrste kemokinskih in citokinskih genov vpletenih v ptičjem vnetnem odgovoru ter genov značilnih za druge aspekte prirojenega imunskega odgovora. Po analizi z mikromrežo so se rezultati odziva izbranih genov potrdili z uporabo qRT-PCR.

CHARACTERIZATION OF SLOVENE COMMON BEAN GENETIC RESOURCES BY MOLECULAR, BIOCHEMICAL AND MORPHOLOGICAL MARKERS

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Common bean (*Phaseolus vulgaris* L.) is a widely distributed crop, representing a major protein input in the population diet. Two distinct gene pools of cultivated beans in the Andes and in Middle America have been described. The Agricultural Institute of Slovenia holds 995 bean accessions collected from various parts of Slovenia. In a study reported here, the genetic variation and relationships among 139 accessions were evaluated by AFLP markers. In the UPGMA dendrogram, Slovene accessions clustered near Andean (Group 1) and Mesoamerican control genotypes (Group 2). A set of 42 accessions clustered into Sub-group A within Group 1, that is specific to the area examined or represents additional variation already existing in the New World. The distribution of the accessions according to the type of phaseolin seed protein was observed. The accessions of Group 1 had Andean "C" and "T" type, whereas the accessions of Group 2 showed Mesoamerican "S" type. The majority of Sub-group A accessions showed new variation of "C" phaseolin ("CS"). Similar proportion of "C" phaseolin type has been identified in the Mediterranean area and Chile, indicating the possible origin of Slovene bean accessions. Morphological traits were found inadequate to accurately place the accessions into their proper gene pool, and there was overlap between the groups for the traits scored. This is in agreement with other reports that gene exchange between Andean and Mesoamerican germplasm has played a major role in the evolution of additional variation in primary and secondary centers of diversification of this species.

KARAKTERIZACIJA SLOVENSKIH GENSKIH VIROV NAVADNEGA FIŽOLA Z MOLEKULSKIMI, BIOKEMIJSKIMI IN MORFOLOŠKIMI MARKERJI

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Navadni fižol (*Phaseolus vulgaris* L.) je pomembna kmetijska kultura, ki v mnogih predelih sveta predstavlja glavni vir proteinov v prehrani. Obstoječa genska sklada navadnega fižola sta nastala na področju Andov in Srednje Amerike. Kmetijski inštitut Slovenije hrani 995 akcesij fižola, zbranih na področju celotne Slovenije. V raziskavo genetske raznolikosti in sorodstvenih odnosov z AFLP markerji je bilo vključenih 139 akcesij. Na UPGMA dendrogramu so se slovenske akcesije porazdelile poleg andskih (Skupina 1) in srednjeameriških kontrolnih genotipov (Skupina 2). Znotraj andske Skupine 1 je del akcesij (42) tvoril Podskupino A, ki predstavlja dednino, svojstveno proučevanemu geografskemu področju, ali pa vneseno dodatno raznolikost s področja Južne Amerike. Grupiranje akcesij na osnovi tipa fazeolina, založne beljakovine v semenu, je bilo skladno z rezultati klastrske analize. Pri akcesijah iz Skupine 1 smo identificirali andska tipa "C" in "T", pri akcesijah iz Skupine 2 pa srednjeameriški "S" tip. Pri večini akcesij iz Podskupine A smo identificirali nov tip fazeolina "CS". Tako velik delež genskih virov s "C" tipom so doslej zasledili le v sredozemskih deželah in v Čilu, kar nakazuje možen izvor slovenskega fižola. Morfološki markerji so se pokazali kot nezadostni pri določanju genetske sorodnosti in porekla akcesij fižola, saj se iste morfološke lastnosti izražajo pri akcesijah iz obeh genskih skladov. Ti rezultati in izsledki tujih raziskav nakazujejo, da je izmenjava genetskega materiala med andskimi in srednjeameriškimi genotipi odigrala ključno vlogo pri nastanku dodatnih variant znotraj primarnih in sekundarnih centrov genetske raznolikosti navadnega fižola.

TRANSFECTION EFFICIENCY OF ELECTRICALLY-ASSISTED GENE DELIVERY TO TUMORS IS TUMOR TYPE AND TIME DEPENDENT

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Effective delivery of plasmid DNA by electroporation into cells of tumor tissue is still a major obstacle in successful electrogene therapy. In order to improve transfection efficiencies *in vivo* we determined optimal time interval between DNA injection and electroporation. Additionally, some possible physiological factors affecting DNA distribution and consequently transfection efficiency, such as the cell density as well as proteoglycans and collagen content of tumors were evaluated. Four tumor models (B16F1, EAT, SA-1, LPB) and two plasmid DNAs encoding luciferase or GFP were used. Plasmids were intratumorally injected at different time intervals before electroporation (8 square wave electric pulses, 600 V/cm, 5 ms, 1 Hz). Luciferase activity was measured by luminometer. GFP expression was estimated in frozen tumor sections using fluorescence microscope. The histological analysis for cell density, proteoglycans and collagen content was performed on sections cut from formalin fixed and paraffin embedded tissue using light microscopy. Transfection efficiencies were compared between the tumor models and correlated with histological properties.

UČINKOVITOST VNOSA GENOV V TUMORJE S POMOČJO ELEKTROPORACIJE *IN VIVO* JE ODVISNA OD TIPA TUMORJA IN ČASOVNEGA INTERVALA

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Učinkovitost vnosa plazmidne DNA v tumorje z elektroporacijo predstavlja oviro pri protitumorski uspešnosti elektrogenske terapije. Namen našega dela je bil izboljšati učinkovitost transfekcije v tumorjih na osnovi določitve optimalnega časovnega intervala med vnosom plazmidne DNA v tumorje in elektroporacijo. Raziskali smo tudi strukturne lastnosti tumorjev (gostoto celic ter vsebnost proteoglikanov in kolagena), ki bi lahko vplivale na razporeditev injicirane DNA v tumorju in s tem na učinkovitost transfekcije. V raziskavo smo vključili štiri tumorske modele (B16F1, EAT, SA-1, LPB). V tumorje smo injicirali plazmidno DNA z vključenim genom za luciferazo ali GFP ter jih nato izpostavili 8 električnim pulzom (600 V/cm, 5 ms, 1 Hz). Pri tem smo spreminjali časovni interval med injiciranjem plazmidne DNA ter elektroporacijo. Aktivnost luciferaze smo izmerili z luminometrom, ekspresijo GFP pa smo določili na zmrzlih rezih s fluorescentnim mikroskopom. Za histološko analizo tumorskih rezin smo tumorsko tkivo fiksirali v formalinu ter ga vklopili v parafin. Z uporabo svetlobnega mikroskopa smo določili gostoto celic ter vsebnost proteoglikanov in kolagena. Primerjali smo učinkovitost transfekcije med tumorskimi modeli ter jo analizirali v povezavi s histološkimi lastnostmi tumorjev. Rezultati raziskave so pokazali, da sta učinkovitost transfekcije ter optimalni časovni interval med injiciranjem DNA in elektroporacijo odvisna od tipa tumorja; najvišja je bila pri B16F1 tumorjih. Optimalni časovni interval pri B16F1 in EAT je krajši (5 – 15 min) v primerjavi z LPB in SA-1 tumorji (do 1 ure). Statistična analiza je pokazala, da med učinkovitostjo transfekcije ter celično gostoto kot tudi vsebnostjo proteoglikanov in kolagena obstaja negativna korelacija. V zaključek, učinkovitost transfekcije je odvisna od časovnega intervala med injiciranjem DNA in elektroporacijo kot tudi celične gostote ter zgradbe ekstracelularnega matriksa tumorjev.

DNA MICROARRAYS AND HEREDITARY EYE DISEASES

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Arrayed Primer Extension (APEX) is a genotyping method. It is based on hybridization of probe and target nucleic acid and subsequent extension of probe with DNA polimerase. Microarray is composed of 25 base probe oligonucleotides, which are covalently linked at their 5' to the support, which leaves 3' free for DNA synthesis. Each probe on such microarray represents a specific base that is being identified and anneals directly in front of that base at its 5' end. DNA which is being genotyped is amplified with PCR, fragmented and denatured. The extension of probe proceeds with the use of ddNTPs. Because each ddNTP is labeled with different fluorescent dye each subsequent nucleotide complementary to target can be determined. With APEX we intend to shorten time and increase flexibility when detecting known mutations. We used APEX to create ABCR400 chip for detection of 382 know variants of ABCR gene. Screening included 28 Slovenian patients with diagnosed Stargardt disease. We found 27 mutations in 19 patient of which 13 were different. The most frequent of 27 mutation were G1961E, R681X in Q1412X. ABCR400 chip was more than 98% effective in detecting variants included on chip. The chip alone determined 54% of all possible disease-associated ABCR alleles in random cohort of Stargardt disease patients. The main advantage of APEX is speed, flexibility and low cost. However method allows for discovery of new mutation only at particular positions and is unable to detect large rearrangements.

DNA ČIPI IN DEDNE OČESNE BOLEZNI

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Podaljševanje oligonukleotidov na čipu (Arrayed Primer Extension – APEX) je genotipizacijska metoda. Temelji na hibridizaciji tarče in sonde in naknadnega podaljševanja sonde s polimerazo DNK. Čip je sestavljen iz oligonukleotidnih sond dolgih 25 baz, ki so na nosilec vezane preko 5' konca, kar pušča 3' konec prost za sintezo DNA. Vsaka oligonukleotidna sonda predstavlja bazo, ki jo želimo identificirati in leži na 5' koncu pred nezano bazo v tarči. DNA v kateri želimo določiti spremembe pomnožimo z verižno reakcijo s polimerazo, jo fragmentiramo in denaturiramo. Podaljševanje sonde sledi po hibridizaciji tako, da uporabimo dideoksinukleotide (ddNTPje). Vsak ddNTP je ozančen z drugačnim barvilom, kar omogoča določitev nukleotida, ki je ko-mplementaren prvemu tarčnemu nukleotidu. Z metodo želimo skrajšati čas in povečati fleksibilnost pri detekciji že znanih mutacij v genu ABCR. Na podlagi metode smo izdelali čip ABCR400 za določevanje 382 znanih variant gena ABCR, ki so odgovorne za nastanek Stargadtove bolezni. V raziskavo je bilo vključenih 28 Slovenskih bolnikov s Stargardovo boleznijo. Pri 19 od 28 bolnikov smo potrdili 27 mutacij, od teh je bilo 13 različnih. Najpogostejše mutacije so bile G1961E, R681X in Q1412X. Zanesljivost metode smo ocenili na več kot 98% pri variantah vključenih na čip. Pri naključno izbrani skupini bolnikov s Sta-rgardtovim sindromom pa smo s čipom uspeli zaznali 54% vseh možnih alelov povezanih s Stargardovo boleznijo. Prednosti metode so predvsem hitrost testiranja dednine na že znane mutacije, flaksibilnost pri razvoju in nizka cena. Metoda omogoča zaznavanje de novo sprememb samo na specifičnih pozicijah in ni zmožna zaznati velikih sprememb.

THE CONSTRUCTION OF BOVINE-HUMAN SYNTENY MAP USING AVAILABLE MAPPED BOVINE MARKERS

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The term synteny refers to two regions of two genomes that show considerable sequence similarity and rough conservation of the gene order in those regions, and thus are likely to be related by common descent. Practical use of synteny maps is limited mostly by marker. Thus, in our study, three databases (MARC; Itoh *et. al,* 2005; Everts-van der Wind *et al.,* 2005) of markers with known location on *Bos taurus* genome were integrated. Different methods were used for individual database construction. Marker sequences were compared to the human genome (build 35). The best matches to the more investigated human genome were used as guides for identification of 213 synteny blocks containing 6023 markers. Further 208 synteny blocks contain just one marker. These singletons are most likely microrearrangements or false positives from similarity search. Genetic and physical maps can be easily integrated using human genome as a guideline which is of special importance for unsequenced genomes. Synteny maps can improve gene search, gene annotation, enable faster assemblance of shot gun genome sequencing project and reconstruction of phylogeny.

IZDELAVA KARTE SINTENIJE MED GOVEDOM IN ČLOVEKOM NA OSNOVI RAZPOLOŽLJIVIH OZNAČEVALCEV NA GENOMU GOVEDA

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Izraz sintenija označuje vsaj dva dela genomov, za katera je očitna podobnost nukleotidnih zaporedij in v grobem ohranjen vrstni red genov, kar kaže na skupen izvor. Uporabnost kart sintenije je odvisna predvsem od gostote označevalcev. V naši raziskavi smo zato združili tri večje podatkovne zbirke (MARC; Itoh in sod., 2005; Everts-van der Wind in sod., 2005) označevalcev, katerih pozicija na genomu goveda (*Bos taurus*) je bila določena z različnimi metodami. Označevalcem smo določili najbolj podobna zaporedja v genomu človeka (verzija 35). Bolj raziskani človeški genom smo uporabili kot vodilo za določitev 213 blokov sintenije med primerjanima genomoma, ki vsebujejo 6023 označevalcev. Dodatnih 208 blokov sintenije, ki vsebujejo po en označevalec, nismo upoštevali, ker gre najverjetneje za primere mikroprerazporeditev oziroma za lažne pozitivne rezultate pri iskanju najbolj podobnih zaporedij. Genetske in fizične karte se z uporabo človeškega genoma kot vodila enostavno integrirajo v karto sintenije. Taka integracija ima uporabno vrednost zlasti pri slabše raziskanih genomih. Karte sintenije lahko močno pospešijo iskanje genov pri organizmih, ki nimajo znanega celotnega nukleotidnega zaporedja genoma, olajšajo anotacijo, pospešijo delo pri sestavljanju zaporedij iz »shot gun« genomskih projektov in omogočajo rekonstrukcijo filogenije.

PHYSIOLOGICAL AND MICROARRAY ANALYSES OF CHOLESTEROL HOMEOSTASIS IN THE POLYGENIC MOUSE MODEL OF OBESITY

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World health organization reports that obesity related diseases have become the primary cause of death in developed countries while the developing world follows the same trend. Many of these obesity-related diseases are associated with the increased level of plasma cholesterol. We study a unique animal model for elucidating the genetic basis of obesity that has been developed by several generation of selective breeding for high (F line) and low (L line) body fat content. One quantitative trait locus (QTL) on chromosome 15 (isolated in a congenic line U) has been previously shown to affect the RNA expression levels of some cholesterol biosynthesis genes. Here we first confirmed by the real time PCR that HMG-CoA reductase gene is indeed differentially expressed between the F and the congenic line U and that this QTL has significant effects on various fat pad weights as well as other obesity-related traits. In addition, we tested for RNA expression differences of all the genes involved in the cholesterol synthesis pathway by using the "sterol talk" custom microarray. Current status of this analysis as well as the phenotype screen analysis (plasma, liver lipid profile, physical activity, oxydative capacity of striated muscle) will be presented and discussed. The combined genetic mapping, physiological and transcriptome analyses should help us to identify causal genes responsible for the obesity QTL effects as well as disentangle the complexity of gene-gene interactions involved in the control of cholesterol homeostasis in our animal model.

FIZIOLOŠKE ANALIZE IN ANALIZE MIKROMREŽ HOMEOSTAZE HOLESTEROLA PRI POLIGENEM MODELU MIŠI ZA DEBELOST

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Svetovna zdravstvena organizacija poroča, da so obolenja v povezavi z debelostjo postala primarni vzrok smrti v deželah razvitega sveta, podobne smernice pa so prisotne tudi v razvijajočem svetu. Številne bolezni v povezavi z debelostjo so povezane s povišanim nivojem holesterola v krvni plazmi. Pri pojasnjevanju genetskih osnov debelosti proučujemo edinstven živalski model, ki je bil razvit na podlagi selekcioniranja na višji (F linija) in nižji (L linija) delež telesnih maščob. Predhodne raziskave kvantitativnega lokusa za debelost na kromosomu 15 (izoliran v kongeni liniji U) so pokazale, da ima le-ta vpliv na izražanje nekaterih genov v biosintetski poti holesterola. V naši raziskavi smo s PCR analizo v realnem času potrdili, da se gen za HMG-CoA reduktazo različno izraža pri mišji liniji F in U, poleg tega pa ima omenjeni kvantitativni lokus tudi vpliv na relativni delež telesnih maščob, kot tudi na ostale fenotipske lastnosti, ki so povezane z debelostjo. Razlike v izražanju RNA vseh genov, ki so vključeni v biosintetsko pot holesterola, smo analizirali s pomočjo "sterol talk" mikromrež. Rezultati analize mikromrež bodo predstavljeni v povezavi s fenotipskimi lastnostmi (plazma, jetrni lipidi, fizična aktivnost, oksidativna kapaciteta prečno progastih mišic). Genetsko kartiranje, fiziološke analize in analize transkriptoma bodo prispevale k identifikaciji vzročnih genov za debelost, kot tudi pojasnili medsebojne vplive genov, ki so vključeni v homeostazo holesterola pri našem živalskem modelu.

SPORADIC AND FAMILIAL CJD IN SLOVENIA: MOLECULAR CLASSIFICATION AND CHARACTERISATION

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Prion diseases (PrDs) are progressive fatal diseases characterised by the accumulation of a disease-associated, pathological form of prion protein (PrPSc) in the central nervous system. The most frequent human PrD is Creutzfeldt-Jakob disease (CJD) and the majority of cases are sporadic disorders without specified aetiology. Disease-specific mutations in the prion protein gene (*PRNP*) and the codon 129 polymorphism have been proven to influence the phenotype of PrDs. Determination of 14-3-3, amyloid-β ($A\beta_{1-42}$) and phosphorylated tau (Tau) proteins in the cerebrospinal fluid (CSF) are becoming important factors in molecular classification of PrDs. In 10 year period 22 from 39 suspected cases have been confirmed as sporadic Creutzfeldt-Jakob disease (sCJD), because the analysis of the whole gene revealed no mutations, typical for familiar form of CJD. With single stranded conformational polymorphism analysis and sequencing we determined that in 11 cases codon 129 of the *PRNP* gene was homozygous (1 VV and 10 MM) and in one heterozygous. 14-3-3 protein was verified in 11 of 12 tested CSF samples from the sCJD cases and higher levels of Tau (>1300pg/ml) and threshold levels of $A\beta_{1-42}$ (100-400pg/ml) detected. Molecular genetics and immunohistochemical results have been in accordance with results from neuropathological analysis of the brain. The number of Slovenian sCJD cases during the last two decades years has shown an increase in incidence, probably due to improved surveillance and research. Confirmation and characterisation of the CJD is complex and done on clinical, neuropathological and molecular level. Biomarker analysis of CSF combined with genetic screening of the *PRNP* gene show a potential for sensitive and specific differential diagnosis of CJD. Nevertheless, the only way of establishing a definite diagnosis of Creutzfeldt-Jakob disease is a post mortem examination of the brain.

SPORADIČNE IN DRUŽINSKE OBLIKE CJD V SLOVENIJI: MOLEKULARNA KLASIFIKACIJA IN KARAKTERIZACIJA

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Prionske bolezni so progresivne, smrtne bolezni, ki jih opredeljuje kopičenje bolezenske oblike prionskega proteina (PrPSc) v centralnem živčnem sistemu. Najpogostejše obolenje je sporadična oblika Creutzfeldt-Jakobove bolezni (CJB), brez značilne etiologije. Ugotovljeno je, da na fenotip bolezni vplivajo specifične mutacije v genu *PRNP* in polimorfizem kodona 129. K molekularni klasifikaciji bolezni pa spada tudi analiza dokazovanje proteinov 14-3-3, amiloid-β ($A\beta_{1-42}$) in fosforiliranega tau (Tau) v cerebrospinalni tekočini(CSF). V desetih letih je bilo v Sloveniji 39 primerov suma na CJB. Z analizo celotnega gena smo pri 22 primerih potrdili sporadično obliko CJB (sCJB), saj nismo zasledili mutacij v genu *PRNP*, kar je značilnost družinske oblike te bolezni. Z enoverižno konformacijsko analizo in potrditvijo s sekveniranjem smo v 11 primerih določili homozigotnost kodona 129 (10 MM in 1VV) v enem pa heterozigotnost (M/V). Pri 11 od 12 potrjenih primerov sCJB smo dokazali protein 14-3-3 v likvorju. V primerih potrjene diagnoze sCJB je bila ugotovljena tudi povišana vrednost Tau (>1300 pg/ml) in vrednost $A\beta_{1-42}$, v ali blizu meje (100-400 pg/ml), ki ločuje med CJB in drugimi nevrološkimi obolenji. Molekularno-genetski in imunohistokemijskimi rezultati so bili v skladu z rezultati, dobljenimi z nevropatološkimi analizami možganskega tkiva. Število slovenskih primerov sCJB v zadnjih 20ih letih narašča, kar je posledica boljšega nadzora in večje obveščenosti o tej bolezni. Karakterizacija in potrditev te bolezni je zahtevna in na več nivojih- kliničnem, nevropatološkem in molekularnem. Kombinacija uporabe omenjenih biomarkerjev pri preiskovanju CSF in genske analize, bi v prihodnosti lahko postala ogrodje občutljive in specifične diferencialne diagnoze CJB, vendar pa je še vedno edina možnost zagotove diagnoze analiza možganov po smrti pacienta.

BIOTECHNOLOGY
BIOTEHNOLOGIJA

STUDYING HUMAN PATHOGENS IN ANIMAL MODELS: FINE TUNING A HUMANIZED MOUSE

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Animal models are essential tools for studying the dynamics of host pathogen interaction *in vivo*. A mouse model of the human coronavirus HCoV-229E infectious disease has been generated by additive gene transfer of aminopeptidase N (APN)/CD13, the human receptor for virus-cell interaction into mice. Primary embryonic cells from transgenic animals were susceptible to HCoV-299E, indicating the functionality of the transgene receptor. However, expression of the human receptor was not sufficient to confer virus susceptibility *in vivo*. Crossing huAPN transgenic mice with IFN-unresponsive Stat1-/- mice resulted in markedly enhanced virus replication *in vitro,* but, again, the virus failed to infect immunocompromised huAPN-transgenic mice. Finally, adaptation of the human virus to murine cells led to successful infection of the humanized and immuno-compromised mice. Virus was not only deteced in large amounts in different tissues, but also the lung histopathology of transgenic mice was consistent with active virus replication. Our humanized mice allow studies into HCoV-229E pathogenesis, tropism, replication and spread in immunocompromised host. Crossing the huAPN-transgenic mice to mutants solely deficient in IFN type I and IFN type II will further elucidate the effects of IFNs on HCoV-229E infections and also mimic the situation in humans more accurately.

ŠTUDIJ ČLOVEŠKIH PATOGENOV Z ŽIVALSKIMI MODELI: NATANČNO PRIREJENA HUMANIZIRANA MIŠ

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Živalski modeli predstavljajo osnovno orodje za študij dinamike interakcij med patogeni in gostitelji in vivo. Mišji model za nalezljivo bolezen človeka, ki jo povzroča koronavirus HCoV-229E smo pripravili z vstavljanjem genskega konstrukta aminopeptidaza N (APN)/CD13, ki predstavlja človeški receptor za interakcijo med virusom in gostiteljsko calico, v genom miši. Primarne embrionalne celice transgenih miši so bile dovzetne za infekcijo z HCoV-299E, kar kaže na funkcionalnost transgenega receptorja. Izražanje človeškega receptorja pa ni bilo zadostno za demonstracijo dovzetnosti za virus in vivo. S križanjem huAPN transgenih miši z IFN-neodzivnimi Stat1-/- mišmi smo dosegli bistveno boljšo replikacijom virusa in vitro, vendar virus spet ni bil sposoben infekcijo imunsko kompromitiranih huAPN transgenih miši. Končno je adaptacija človeškega virusa na celic miši vodila do uspešne infekcije humaniziranih in imunsko kompromitiranih miši. Virusa nismo odkrili samo v velikih količinah v različnih tkivih, temveč so bolezenski znaki v pljučih okuženih transgenih miši tudi odražali posledice aktivne replikacije virusa. Naše humanizirane miši omogočajo študij HCoV-229E patogeneze, tkivno sprcifičnost, replikacijo in širtev v imunsko kompromitiranem gostitelju. Križanje huAPN transgenih miši z mutantami, ki so deficientne le za IFN tipa I in IFN tipa II bo nadalje osvetlilo učinek interferonov na infekcijo s HCoV-229E in bolj natančno ponazorilo situacijo ob infekciji s HCoV-229E pri človeku.

MOLECULAR MECHANISMS INVOLVED IN THE REGULATION OF LACTOPROTEIN GENE EXPRESSION

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Mammary gland is a highly specialized organ in mammals designed to produce milk in order to supply appropriate nutrition and immunological protection of the young during its early period of life. Regulation of gene expression in the mammary gland depends on complex hormonal stimulation of the with prolactin as a major player. Molecular dissection of regulatory sequences of the lactoprotein genes offers possibility to discover common mechanisms involved in coordinated gene expression in the mammary gland through different stages of lactation. Genomic approaches have been used for identification of QTL regions and candidate genes with major effect on milk production. A few examples for these strategies will be presented. Promoter studies were used for detection of common regulatory elements, responsible for tissue specific and timely co-ordinated expression of lactoprotein genes in mammary gland in different mammalian species. In addition, the allele specific polymorphisms within the 3'-UTR sequences were found to be involved in the regulation of transcript stability and consequently in the fine regulation of gene expression. In vitro systems have been used for the study of promoter- and 3'-UTR regulatory elements. Differential mRNA splicing is also a frequently observed phenomenon in lactoprotein gene expression. Our results suggest that at least a part of minor protein products observed in protein fraction of milk may be caused by differencial RNA splicing which is a consequence of the presence of weak exons in lactoprotein genes. Deeper understanding of regulatory events at molecular level will allow identification of molecular markers suitable for marker assisted selection in dairy species.

MOLEKULSKI MEHANIZMI, KI VPLIVAJO NA URAVNAVANJE IZRAŽANJA LAKTOPROTEINSKIH GENOV

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Mlečna žleza je visoko specializiran organ sesalcev, namenjen proizvodnji mleka za zagotavljanje ustrezne prehrane in imunološke zaščite mladiča v zgodnjem obdobju življenja. Uravnavanje izražanja genov v mlečni žlezi je odvisno od kompleksne hormonalne stimulacije, kjer igra glavno vlogo prolaktin. Molekularna analiza regulatornih zaporedij laktoproteinskih genov omogoča odkritje splošnih mehanizmov, ki so vpleteni v koordinirano izražanje genov v različnih fazah laktacije. Genomski pristop se pogosto uporablja za odkrivanje QTL regij in kandidatnih genov z velikim učinkom na proizvodnjo mleka. Predstavljeni bodo nekateri primeri uspešne identifikacije kandidatnih genov, ki vplivajo na mlečnost. Študij promoterskih zaporedij omogoča odkritje splošnih kontrolnih elementov, ki so odgovorni za tkivno specifično in časovno koordinirano izražanje laktoproteinskih genov v različnih fazah laktacije pri različnih vrstah sesalcev. Polimorfizmi v 3'-neprevedenih regijah so vključeni v uravnavanje stabilnosti prepisov in posledično v fino regulacijo genske ekspresije. Učinek promoterskih in 3'-UTR elementov smo proučevali z in *vitro sistemi*. Alternativno procesiranje mRNA je tudi pogost mehanizem, ki ga zasledimo pri laktoproteinskih genih. Naši rezultati nakazujejo, da je vsaj del proteinskih komponent mleka posledica alternativnega procesiranja mRNA, kar je posledica prisotnosti šibkih eksonov v laktoproteinskih genih. Boljše razumevanje mehanizmov, ki na molekulski ravni uravnavajo izražanje genov, bo omogočilo tudi uporabo molekulskih markerjev za sodobne selekcijske postopke.

TRANSGENIC RABBITS AS MODELS FOR PRODUCING BIOLOGICALLY ACTIVE HUMAN RECOMBINANT PROTEINS

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Transgenic rabbits are excellent bioreactors for the production of recombinant proteins in their milk both on an experimental and commercial scale. Transgenic rabbits offer an attractive alternative to dairy species because of their litter size, short generation interval and high milk protein content. We are producing transgenic rabbits by pronuclear microinjection. They express different active human recombinant proteins in the milk. The human clotting factor VIII (hFVIII-MT-I) gene construct under the mammary gland specific mWAP promoter was expressed in a tissue specific manner. The activity of the recombinant protein ranged from 5 to 8% of that found in normal human plasma. Gram negative sepsis, an lipopolysaccharide mediated disease, is a major cause of mortality in hospitals. To test our hypothesis that TNAP (tissue non specific alkaline phosphatase) treatment might be favorable in the prevention of Gram negative sepsis, transgenic rabbits expressing TNAP in their milk under control of WAP promoter were created. High TNAP activity was detected in the milk of the #932 founder while in the milk of line #949 the activity was lower but significant. Phenylketonuria is a genetic disorder that is characterized by an inability of the body to utilize phenylalanine causing mental retardation of patients which can be avoided by low Phe diet. In our transgenic rabbits which express low-Phe kappa-casein the endogenous Phe codons were replaced in the recombinant kappa-casein. Casein micelle sizes were reduced in the higher of the two expressing lines showing that recombinant kappa-casein is functional.
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TRANSGENI KUNCI KOT MODEL ZA PROIZVODNJO BIOLOŠKO AKTIVNIH REKOMBINANTNIH ČLOVEŠKIH PROTEINOV

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Transgeni kunci so odlični bioreaktorji za proizvodnjo rekombinantnih proteinov v mleku tako na poskusni kot na komercialni ravni. Transgeni kunci so privlačna alternativa za vrste, ki jih navadno uporabljamo za proizvodnjo mleka, ker imajo velika gnezda, kratek generacijski interval in visoko vsebnost beljakovin v mleku. Genski konstrukt s človeškim faktorjem VIII za strjevanje krvi (hFVIII-MT-I) pod kontrolo za mlečno žlezo specifičnega promotorja mWAP smo uspeli izraziti kivno specifično. Aktivnost rekombinantnega proteina je dosegala 5 do 8% aktivnosti, ki jo najdemo v normalni človeški plazmi. Gram negativna sepsa, je bolezen, ki jo povzročajo lipopolisaharidi, in je najpogostejši vzrok smrti v bolnišnicah. Da bi preverili našo hipotezo, da povečana raven TNAP (tkivno nespecifična alkalna fosfataza) lahko ugodno vpliva na preprečevanje Gram negativne sepse, smo pripravili transgene kunce, ki v mleku proizvajajo TNAP pod kontrolo WAP promotorja. Visoko aktivnost TNAP smo odkrili v mleku linije #932, linija #949 pa je kazala nižjo a značilno ekspresijo TNAP. Fenilketonurija je genetska bolezen, ki povzroča motnjo pri presnovi fenilalanina, kar vodi do duševne zaostalosti pacientov, kar pa jo lahko omilimo s prehrano, ki vsebuje malo Phe. Pri naših transgenih kuncih smo nadomestili kodone za Phe v genu za kappa kazein z drugimi kodoni in ugotovili zmanjšano velikost micel pri liniji kuncev, ki je izražala velike količine rekombinantnega kappa kazeina, kar kaže na funkcionalnost rekombinantnega kapa kazeina.
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GENETIC VACCINES FOR B-CELL LYMPHOMAS

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Immunization by delivery of DNA gene constructs encoding specific antigens and/or molecules with immunomodulatory of immunoadjuvant activities is a relatively simple and economically affordable technology that allows stimulation of the immune system, to obtain both antibody and T cell responses. While in many cases, anti-tumour immune responses based on antibodies would be sufficient to eliminate tumour cells, in many others activation of citotoxic T cells would also be required. Manipulation of the antigen gene construct by using, for instance, tissue specific promoters, specific cellular localization signals, cell-type targeting moieties, etc., allows to target gene expression to defined tissues or cell populations and to achieve B and T cell responses to relevant tumour antigens. We have explored the use of DNA vaccines to induce anti-tumour responses in a murine B-cell lymphoma model. The target antigen was represented by the idiotype, a clonal marker of the membrane immunoglobulin expressed by the malignant B-cell that represents a suitable tumour associated antigen. Engineered immunogenic Id gene constructs were used to obtain anti-idiotypic immune responses. Vaccination was performed by gene-gun delivery of naked DNA, by infection with a recombinant Adeno Associated Virus (rAAV) encoding the immunogenic Id gene, or by a combination of both. Contrary to protein immunisation, DNA vaccination results in presentation of properly folded Ids and low antigenic doses, which leads to an anti-Id antibody response directed exclusively to idiotypic determinants of the immunising VL/VH combination. Not surprisingly, we found that the mechanism of tumour protection was entirely antibody dependent, since mice vaccinated with chimeric constructs that do not induce anti-Id antibodies, were not protected, in spite of exposure to all tumour-Id derived $CD4^+$ and $CD8^+$ T-cell epitopes.

INCREASED PRODUCTIVITY OF RECIPIENT COMMERCIAL MICRO-ORGANISMS AFTER THE INSERTION OF MODIFIED *PFK*A GENE FROM *ASPERGILLUS NIGER*

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Recently spontaneous posttranslational modification of 6-phosphofructo-1-kinase (PFK1), a key regulatory enzyme of glycolysis in *Aspergillus niger* was described. After proteolytic cleavage and phosphorylation by PKA, remaining PFK1 fragment became resistant to citrate inhibition, while various positive effectors increased its activity to a greater extend. Kinetic characteristics of the shorter PFK1 fragment enable undisturbed metabolic flux through glycolysis which leads to increased levels of tricarboxylic acids (enhanced anaplerosis). Reinforced anaplerotic reactions are necessary for increasing the productivity in various microorganisms. An attempt was made to force *Aspergilli* cells to synthesise active PFK1 fragment directly from a mutated truncated *pfk*A gene, in order to avoid complex posttranslational modification. A number of truncated *pfk*A genes of different length were tested to find the one that coded for an enzyme that retained activity after phosphorylation. Additionally some site specific mutations were introduced into the gene to avoid the need for phosphorylation, therefore highly active PFK1 enzyme was formed directly from the mt-*pfk*A gene. Mutated truncated mt-*pfk*A genes were inserted into *A. niger* and *A. terreus* cells. In *A. niger* transformants carrying several copies of mt-*pfk*A gene increased rate of citric acid overflow and alpha-amylase production was detected in respect to the parental strain, while in *A. terreus* increased yields and specific productivity of itaconic acid was recorded.

POVEČANJE PRODUKTIVNOSTI KOMERCIALNIH MIKROORGANIZMOV PO VNOSU SPREMENJENEGA *PFK*A GENA GLIVE *ASPERGILLUS NIGER*

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Nedavno smo pri glivi *Aspergillus niger* opisali posttranslacijsko modifikacijo encima 6-fosfofrukto-1-kinaze (PFK1), ključnega regulatornega encima glikolize. Po razcepu proteinske molekule s proteazami in fosforilacijo proteinskega ostanka s PKA encimom, postane aktivni fragment rezistenten na inhibicijo s citratom, medtem ko različni pozitivni efektorji povečujejo njegovo aktivnost bolj kot nativni encim. Kinetične karakteristike krajšega PFK1 fragmenta omogočajo nemoten metabolni pretok preko glikolize, kar pripelje do povečanja nivoja intermediatov cikla trikarboksilnih kislin (ojačana anapleroza). Ravno pospešene anaplerotske reakcije so odgovorne za povečano produktivnost pri različnih mikroorganizmih. V ta namen smo poskusili celice glive *A. niger* prisiliti v sintezo aktivnega, kratkega fragmenta PFK1 neposredno iz mutiranega, skrajšanega *pfk*A gena, predvsem zato, da se izognemo zapletenemu posttranslacijskemu procesu. Pripravili smo serijo skrajšanih t-*pfk*A genov, da smo določili primerno dolgega, ki sintetizira aktivni fragment. Z mestno specifičnimi mutacijami smo spremenili zaporedje nukleotidov, tako da po sintezi kratkega fragmenta za aktivacijo ni bila več potrebna njegova fosforilacija s PKA encimom. Transformante tako visoko aktiven encim sintetizirajo neposredno iz mt-*pfk*A gena. Mutirani skrajšani mt-*pfk*A gen smo vnesli v celice glive *A. niger* in *A.terreus*. Pri transformantah glive *A.niger*, z vgrajenim večjim številom kopij mt-*pfk*A gena, zaznamo povečano hitrost izločanja citronske kisline in pospešeno produkcijo alfa-amilaz, medtem ko pri glivi *A. terreus* dosežemo z vnosom modificiranega *pfk*A gena povečano produkcijo itakonske kisline.

ALTERNATIVES METHODS TO ANIMAL USE AND 3RS IN VETERINAY TOXICOLOGY

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3Rs Russel and Burch concept, to Refine, Reduce and Replace animal use in research, is well known in toxicology. The advantages of alternatives are ethical, e.g. *in vivo* painful Draize Test, economical, less expensive than *in vivo*, and practical, for the evaluation of mechanistic aspects related to different animal species, included humans. With total respect to Russel and Burch, the 3Rs could be extented using the term of Recycling, where the bank of the cells is directly from the slaughterhouse: it is a sort of "from knife to life" with the application of veterinary toxicological studies with a species – specific approach, recovering materials that normally is rejected. Veterinary toxicology with alternatives means studies on absorption, biotransformation, metabolism and toxicity of xenobiotics (drugs, contaminants, natural toxins, etc.) with species-specific animal cells. The use of biomaterials, like bioplymers, as specific support, can improve the tridimensional growth of different cells, also in co-culture. The comparison of different toxicological in vitro models (e.g. the comparison of RTG-2 cells from fish normally used in ecotoxicological studies with swine granulosa cells with different aromatase activity) and the interaction with disciplines like proteomic, food safety and nanoscience are fundamental for an innovative *in vitro* veterinary toxicological research. Finally is important to implement the application of alternatives models in Veterinary Toxicology for Education in the University, teaching with 3Rs, in order to stimulate students toward learning, critical thinking and training close to-practice without animal use, with economic and ethical advantages and decrease of environmental impact.

PHARMACOGENOMICS
FARMAKOGENOMIKA

INTEGRATION OF GENOME-WIDE DATA TO INFER GENETIC NETWORKS

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To comprehend biology as a system, one needs to analyze the structure and dynamics of cell components as modules rather than isolated part. Progress in technological devices, analytical methods and biological models are required to decipher molecular networks and eventually analyze the cell as a system. Clustering analysis of gene expression profiles allows the analysis of "correlation" between genes and biological conditions. However it is yet restrictive as it does not reveal the causality of regulatory relationships. Besides it is very difficult to infer molecular networks from expression profiling only, as the only accessible information is the steady-state concentration of mRNA. This information is necessary but not sufficient to characterize the structure of transcriptional network and analyze its dynamic and functional properties. Modeling of transcriptional networks should take into account information such as RNA concentrations, *cis*-acting sequences, transcriptional activity and so forth, since each variable carries unique biological information. However due to limitation in accurate and highly parallel measuring technologies, these data are not routinely accessible. We have developed innovative bioarrays to measure with sufficient accuracy, parallelism and throughput relevant data to infer transcriptional networks. For instance, we are manufacturing DNA array containing promoter regions (human) to perform ChIP on chip analysis in order to localize for a given transcription factor, all putative binding sites onto the genome. One can then return to DNA arrays to confirm hypothesis generated from ChIP on chip data. We are also developing cell microarrays to characterize, genome wide, upstream regulators for a given gene on one hand and transcriptional activity on the other hand. Technological breakthroughs in micro and nanotechnologies to generate comprehensive and relevant data are thus as critical as innovation in analytical methods for deciphering transcriptional regulatory networks and developing system biology.

PHARMACOGENOMIC APPROACHES IN NOVEL DRUG DISCOVERY

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The identification of a promising molecular target for drug action is a first step in a contemporary drug development process. The overall objective of our studies is to delineate the apoptotic process initiated by the B cell antigen receptor (BCR) as it is essential for life and death decision, and further to find a promising drug target, manipulation of which would allow the interference with various B lymphocyte activities including cell activation, proliferation, anergy and deletion. We have addressed this issue by applying, in parallel reverse and direct pharmacogenomic strategies. The first strategy is based on our previous results demonstrating involvement of serine proteases in B cell apoptosis, and moreover in the latest stages of DNA fragmentation In the reverse pharmacogenomic approach we have used newly synthesized inhibitors of serine proteases, which were tested on B cells aiming to discover a desirable effect, the inhibition of intranucleosomal apoptotic DNA laddering. Subsequently, we are in search of protein and gene target. In a direct genomic approach we have addressed the question of weather transcriptional changes underly the BCR-initiated distinct biological responses. Mature and immature primary B lymphocytes were BCR-trriggered and transcriptional changes monitored by cDNA microarrays. Our results provide evidence that the BCR-operated transcriptional changes play a crucial role in the control of B-cell function and that differentially expressed genes will lead to the identification of molecules involved in BCR-driven apoptosis, providing the understanding of its regulation and a foundation for a more rational approach to drug design.

FARMAKOGENOMIKA V PROCESU RAZVOJA NOVIH ZDRAVIL

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Sodoben pristop v razvoju novih zdravil vključuje identifikacijo tarčne molekule. Namen naših raziskav je doprinesti k razumevanju molekularnih mehanizmov celične smrti limfocitov B po zamreženju antigenskega receptorja (BCR), predvsem pa najti perspektivno molekularno tarčo, manipulacija katere bi nam omogočala usmerjanje spreminjanje aktivnosti celične aktivacije, proliferacije, anergije in celične smrti. Pri raziskovalnem delu smo uporabili komplementarna pristopa reverzne in direktne farmakogenomske analize. Odločitev za reverzni farmakogenomski pristop sloni na naših predhodnih rezultatih, ki dokazujejo vlogo serinskih proteaz v procesu apoptoze limfocitov B, in sicer v pozni fazi oligonukleosomske cepitve DNA. Limfocite B smo inkubirali v prisotnosti novo sintetiziranih inhibitorjev serinskih proteaz in opazovali želeno inhibicijo internukleosomske DNA razgradnje. Cilj tega pristopa je identifikacija ključnega proteina in gena. Z direktnim genomskim pristopom smo želeli odgovoriti na vprašanje ali so BCR-inducirani specifični biološki odgovori zrelih in nezrelih primarnih limfocitov B povezani s transkripcijskimi spremembami, kar smo spremljali kot diferencilno ekspresijo genov z uporabo cDNA mikromrež. Naši rezultati potrjujejo, da vrši BCR-inducirana ekspresija genov kontrolo nad funkcijo limfocitov B, iz česar sklepamo, da bo identifikacija ključnih molekul omogočila ne le razumevanje mehanizmov BCR-inducirane apoptoze pač pa omogočila racionalen pristop k načrtovanju novih zdravil.

THE CHROMOKINESIN KID SPECIFICALLY SAFEGUARDS EARLY STAGE EMBYONIC CLEAVAGES

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The chromokinesin Kid/Kinesin-10 is a plus end-directed microtubule (MT)-based motor that binds both MTs and chromosomes. Antibody microinjection and RNAi-mediated depletion of Kid in cultured mammalian cells have revealed that Kid plays important roles throughout mitosis, including chromosome alignment at metaphase plate, spindle morphogenesis, and proper chromosome dynamics during anaphase. To explore further the physiologic function of Kid, we disrupted the *Kid* gene in mice. Genotype analysis of more than 250 live-born mice from *Kid*+/- intercrosses yielded a *Kid*+/+: +/-: -/- ratio of ~2:4:1, suggesting that about 50% of *Kid*-/- embryos die before birth. Live-borne *Kid*-/- mice were normal in appearance, and healthy into adulthood. Both male and female *Kid*-/- mice were fertile but *Kid*+/- x *Kid*-/- crosses also yielded *Kid*+/- and *Kid*-/- offspring with a skewed ratio of approximately 2:1. Moreover, even at E9.5, the number of *Kid*-/- embryos was about 50% fewer expected. To further examine when half of the *Kid*-/- embryos die, we obtained 1-cell stage embryos, cultured in vitro, and analyzed these at the 2-, 4-, and 8-cell stages. At these stages, *Kid*-/- embryos were found at the expected mendelian frequency. However, about 20% ~ 60% of these embryos showed abnormal nuclear morphology including severe fragmentation of nuclei, micronuclei, and irregular shaped nuclei, presumably resulting from the mitotic failure. These results suggest that although *Kid* is dispensable for somatic mitosis and meiosis, *Kid* plays a critical role during early embryonic cleavage stages.

INFLUENCE OF 118 A>G POLYMORPHISM IN THE OPRM1 GENE ON THE TREATMENT EFFICIENCY WITH TRANSDERMAL FENTANYL

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Introduction Mu opioid receptor 1 (OPRM1) is a primary site for opioid analgesics. Variations in the gene for OPRM1 can change their analgesic efficiency. **Objectives** To determine if 118 A>G mutation in healthy Slovenian population is in Hardy Weinberg equlibrium. To determine the presence of 118 A>G mutation in patients treated with transdermal fentanyl. To assess the influence of the mutation on the efficiency of treatment. It should lower the analgesic efficiencyl and cause more pronounced adverse effect. **Methods** After obtaining written informed consent 135 healthy volunteers and 24 patients were included in the prospective study. In patients with malignant and nonmalignant chronic pain the efficiency of analgesia and severity of adverse effects were evaluated in two month period. DNA from blood of the patients and healthy volunteer was isolated using QIAamp® DNA Mini Kit (QIAGEN) and 118 A>G mutation determined using ABI Prism® 7700 Sequence Detection System (Applied Biosystem). **Results** DNA analisys of healthy volunteers showed that distribution of the mutation in Slovenian population is in Hardy Weinberg equilibrium and that it is equal in both groups. Alele frequency is high (13%). Mutation was determined in 8 patients. Among them 6 patients had severe adverse effects and inefficent analgesia, therefore in 4 treatment with fentanyl was discontinued, in 2 the dose of fentanyl had to be raised. **Conclusion** The preliminary results of the study show that mutation 118 A>G decreases the efficiency of transdermal fentanyl. Insufficient pain relief and more pronounced adverse effects lead to discontinuation of treatment.

VPLIV POLIMORFIZMA 118A>G V GENU ZA OPRM1 NA UČINKOVITOST ZDRAVLJENJA BOLEČINE S FENTANILOM V TRANSDERMALNEM OBLIŽU

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Uvod Mu opioidni receptor 1 (OPRM1) je primarno mesto delovanja opioidnih analgetikov, zato lahko variacije v genu za OPRM1 spremenijo njihov analgetični učinek. **Namen** Določiti pogostnost mutacije 118 A>G v zdravi slovenski populaciji in preveriti, ali je v Hardy Weinbergovem ravnotežju.Določiti prisotnost mutacije 118 A>G pri bolnikih, ki se zdravijo s fentanilom v transdermalnem obližu, in primerjati njeno porazdelitev s skupino zdravih preiskovancev. Oceniti vpliv te mutacije na učinkovitost zdravljenja. Pri bolnikih z mutacijo naj bi bil fentanil manj analgetično učinkovit, neželeni učinki pa izrazitejši. **Metode** Po pridobitvi pisnega soglasja je bilo v prospektivno raziskavo vključenih 135 zdravih prostovoljcev in 24 bolnikov. Pri bolnikih z maligno in kronično nemaligno bolečino smo v obdobju dveh mesecev spremljali učinkovitost analgezije in prisotnost neželenih učinkov. DNA iz krvi bolnikov in zdravih preiskovancev je bila izolirana z uporabo QIAamp® DNA Mini Kita (QIAGEN) in mutacija 118A>G določena z uporabo sistema ABI Prism® 7700 Sequence Detection System (Applied Biosystems). **Rezultati** Analiza DNA zdravih prostovoljcev je pokazala, da je mutacija 118 A>G v slovenski populaciji v Hardy Weinbergovem ravnotežju in se enako porazdeljuje v skupini bolnikov in zdravih preiskovancev. Alelna frekvenca je visoka (13 %). Mutacija je bila določena pri 8 bolnikih. 6 bolnikov je imelo izrazite neželene učinke in neučinkovito analgezijo, zato smo pri 4 opustili zdravljenje s fentanilom, pri 2 je bilo potrebno odmerek fentanila zvišati. **Zaključki** Preliminarni rezulati raziskave kažejo, da mutacija 118 A>G zmanjša učinkovitost fentanila. Olajšanje bolečine je nezadostno, neželeni učinki so izrazitejši, zato bolniki opustijo zdravljenje z obližem.

GILBERT'S SYNDROME IN SLOVENIAN POPULATION – A NEW CASE OF A $(TA)_8$ ALLELE IN THE *UGT1A1* GENE PROMOTER IN CAUCASIANS

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Gilbert's syndrome is a mild hereditary unconjugated hyperbilirubinemia caused by mutations in the bilirubin UDP-glucuronosyltransferase gene (*UGT1A1*). The causative mutation in Caucasians is almost exclusively a (TA) dinucleotide insertion in the TATA-box of the *UGT1A1* promoter. Affected individuals are homozygous for the variant promoter and have 7 TA repeats instead of 6 TA repeats. The aim of the present study was to determine the frequency of *UGT1A1* $(TA)_n$ gene promoter polymorphism in healthy Slovenian population and to investigate the association of genotypes with total serum bilirubin levels. A single- strand conformation polymorphism analysis was developed and used to genotype 236 healthy subjects. The frequencies of genotypes were as follows: $(TA)_{6/6}$ (38.1%), $(TA)_{6/7}$ (47.9%), $(TA)_{7/7}$ (13.6 %). There was a statistically significant association of genotypes with serum bilirubin levels ($P < 0.001$). Subjects with genotype $(TA)_{7/7}$ had the highest and subjects with genotype $(TA)_{6/6}$ had the lowest total serum bilirubin levels. One individual in the group had the rare genotype $(TA)_{7/8}$ (0.4 %). Analysis of his family showed the following genotypes: $(TA)_{6/8}$ in his father and sister and $(TA)_{7/8}$ in his two brothers. In conclusion, frequency of *UGT1A1* $(TA)_n$ gene promoter polymorphism genotypes was determined for the first time in Slovenian population and is similar to frequencies observed in other Caucasian populations. The extremly rare $(TA)_8$ allele in Caucasians was found also in Slovenians.

GILBERTOV SINDROM V SLOVENSKI POPULACIJI – NOV PRIMER ALELA $(TA)_8$ V PROMOTORJU GENA ZA UGT1A1 PRI KAVKAZIJCIH

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Gilbertov sindrom je dedna motnja, za katero je značilna blaga, nekonjugirana hiperbilirubinemija. Nastane kot posledica mutacij v genu za bilirubin UDP-glukuronil transferazo (UGT1A1). Mutacije se med rasami razlikujejo, pri Kavkazijcih pa je za Gilbertov sindrom skoraj izključno odgovorna vstavitev dinukleotida TA v zaporedje TATA v promotorju gena za UGT1A1. Normalno je prisotnih šest TA ponovitev, med bolnike pa prištevamo homozigote s sedmimi TA ponovitvami. Namen naše študije je bil ugotoviti pogostost polimorfizma $(TA)_n$ v promotorju gena za UGT1A1 v zdravi slovenski populaciji in dokazati povezanost genotipov s koncentracijo celotnega bilirubina v serumu. Razvili smo metodo konformacijskih polimorfizmov enoverižnih DNA, s katero smo genotipizirali 236 zdravih prostovoljcev. Frekvence genotipov so bile: $(TA)_{6/6}$ (38,1%), $(TA)_{6/7}$ (47,9%), $(TA)_{7/7}$ (13,6 %). Dokazali smo statistično značilno povezavo genotipov s serumskimi koncentracijami bilirubina. Preiskovanci z genotipom $(TA)_{7/7}$ so imeli najvišje, preiskovanci z genotipom $(TA)_{6/6}$ pa najnižje koncentracije celotnega bilirubina v serumu. Pri enem od preiskovancev smo ugotovili prisotnost redkega genotipa $(TA)_{7/8}$. V nadaljnji analizi smo genotipizirali člane njegove družine in dokazali genotip $(TA)_{6/8}$ pri sestri in očetu in genotip $(TA)_{7/8}$ pri dveh bratih. V naši študiji smo prvič določili pogostost polimorfizma $(TA)_n$ v promotorju gena za UGT1A1 v slovenski populaciji. Frekvence so podobne kot pri ostalih kavkazijskih populacijah. Ugotovili smo tudi prisotnost alela z osmimi ponovitvami TA - $(TA)_8$, ki je pri Kavkazijcih izjemno redek.

GENOMIC TECHNOLOGIES II
GENOMSKE TEHNOLOGIJE II

MAPPING AND CLONING OF DISEASE GENES - PAST AND PRESENT

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Mapping and cloning of disease genes for inherited diseases has been largely carried out during the last 15 years and has led to identification of genes involved in many genetic diseases. This activity has been facilitated by the development of maps of microsatellites and by technological improvements. By this approach our group has contributed to the mapping of several disease loci and to the identification of corresponding genes. During the last few years, the Human Genome Project has generated tremendous resources and technologies that can be used to elucidate the etiology of common diseases. Only a small fraction of genes thought to play a role in common diseases has been so far identified, and there is a growing realization that the primary resource needed to identify these genes are large collections of samples from human populations that include extensive clinical information. Contrary to the usual gene discovery study design, the participants in population-based projects are not selected by the fact that they have a specific disease, but via a random selection process. The unbiased nature of the recruitment process provides a more accurate measure of the disease risk provided by a particular genetic variant. Longitudinal population-based cohort studies often involve frequent environmental and health assessments helping to understand the interplay of genes and environment. Data from research activities performed to identify monogenic as well as complex diseases in isolated populations will be presented and discussed.

MINING GENE EXPRESSION, PROTEOMICS AND HAPLOTYPES FOR COMPLEX TRAIT GENES

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The use of QTL analysis has proven successful in detecting hundreds of loci for heritable quantitative traits. Through the combination of molecular tools, some of these QTL have been cloned and the causal genes established. However, the nature of complex genetic diseases means that identifying the causal genes remains a non-trivial matter. Often QTL are large, containing hundreds of genes, so different tools and approaches are needed to arrive at a manageable number of probable candidate genes for further testing. Combining several approaches, one can generate small candidate gene lists for each QTL by searching for differential amounts of either mRNA using microarrays or protein abundance using mass spectrometry and by mining publicly available sequence and SNPs, firstly to reduce regions of the QTL that are inferred to be identical by descent and secondly to identify candidate genes with protein coding differences that may indicate functional differences.

ELECTROGENE THERAPY OF CANCER

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Advances in molecular biology techniques, which lead to high scale sequencing of genomes enable us to identify potential targets for gene therapy of cancer. However, to successfully apply gene therapy into clinical protocols several obstacles have to be overcome, one of them being successful gene delivery method. In electrogene therapy, electroporation is used to enhance delivery of plasmid DNA, oligonucleotides or siRNA molecules into cells. Electroporation is a physical method for delivery of various molecules into the cells by transiently increasing permeability of cell membrane using application of controlled external electrical field to the cells. The transfection efficiency of this methodology *in vivo* is still low compared to the viral vectors. However, due to the lack of immunogenicity of the method, easiness of the preparation of large quantities of endotoxin free plasmid DNA, control and reproducibility of the method and the development of electric pulse generators approved for the clinical use, electrically-assisted gene delivery holds a great potential for the clinical application (electrogene therapy). Until now, most of the work has been done on the optimization of electrical parameters, on the timing of plasmid DNA injection with respect to application of electric pulses, as well as on modulation of extracellular matrix for successful gene delivery. Furthermore, electrogene therapy using a variety of therapeutic genes, mostly encoding cytokines, but also antiangiogenic factors, suicidal and apoptosis inducing genes has been tested in preclinical studies with very good antitumour effectiveness on variety of different tumour models in a preclinical level. First clinical trials on electrogene therapy are also in progress.

ELEKTROGENSKA TERAPIJA RAKA

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Napredek na področju tehnik molekularne biologije, ki je vodil v sekveniranje genoma, je omogočil tudi določitev novih potencialnih tarč za gensko terapijo raka. Vendarle, pa je za uspeh terapije, še vedno največja prepreka učinkovito dostavljanje genskega materiala v tarčne celice. Pri elektrogenski terapiji uporabljamo elektroporacijo za povečan vnos plazmidne DNA, oligonukleotidov ali siRNA molekul v celice. Elektroporacija je postopek, pri katerem z uporabo definiranega zunanjega električnega polja začasno povečamo prepustnost celične membrane in omogočimo vnos različnih vrst molekul v celice.V *in vivo* pogojih je žal uspešnost transfekcije z elektroporacijo še vedno nizka v primerjavi z virusnimi vektorji. Kljub temu pa ima električno posredovan vnos DNA v tarčne celice veliko možnosti za klinično uporabo (elektrogenska terapija), kajti metoda ne sproži imunskega odziva, priprava velikih količin plazmidne DNA proste endotoksinov je nezahtevna, postopek izolacije plazmidne DNA je kontroliran in ponovljiv. Poleg tega pa je v klinični uporabi tudi že generator električnih pulzov. Do sedaj so bile raziskave usmerjene predvsem v optimizacijo in izboljšanje učinkovitosti električno posredovane transfekcije. Testirali so se različni parametri električnih pulzov, čas med vbrizganjem plazmidne DNA in aplikacijo električnih pulzov, ter vpliv razgradnje ekstracelularnega matriksa na uspešnost transfekcije. Poleg tega, pa je bilo narejenih tudi nekaj raziskav o elektrogenski terapiji z uporabo terapevtskih genov kot so geni, ki nosijo zapis za citokine, angiogene faktorje in tumorske supresorske proteine. Pri vseh pristopih na je bila dokazana protitumorska učinkovitost na različnih tumorskih modelih na predkliničnem nivoju. Potekajo pa tudi že prve klinične študije elektrogenske terapije.

CAN MOLECULAR MECHANISMS BE REVEALED BY LARGE-SCALE EXPRESSION PROFILING

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Background: Large-scale transcription profiling of cell models and model organisms has been used to identify new molecular components involved in fat cell development. However, detailed characterization of the sequences of the identified gene products has not been performed and global mechanisms have not been addressed. We asked to which extent can molecular processes be revealed by expression profiling and functional annotation of genes differentially expressed during fat cell development. **Results:** Mouse microarrays with >27.000 elements were developed and transcription profiles of 3T3-L1 cells were monitored during differentiation. 780 differentially expressed ESTs were subjected to in-depth bioinformatics analyses. The analysis of 3'UTR sequences from 395 ESTs showed that 71% of the differentially expressed genes could be regulated by miRNAs. Molecular atlas of fat cell development was then constructed by *de novo* functional annotation on a sequence segment/domain-wise basis of 659 protein sequences, and subsequent mapping onto known pathways, possible cellular roles and subcellular localizations. We found that key enzymes in 27 out of 36 investigated metabolic pathways were regulated at the transcriptional level, typically at the rate limiting steps in these pathways. We also found that co-expressed genes rarely shared consensus transcription factor binding sites, and were typically not clustered in adjacent chromosomal regions, but were instead rather widely dispersed throughout the genome. **Conclusion:** This study shows that large-scale transcription profiling in conjunction with sophisticated bioinformatics analyses can provide not only a list of novel players in a particular setting, but also a global view on biological processes and molecular networks.

GENE DISCOVERY IN INFLAMMATORY BOWEL DISEASES- LESSONS FOR COMPLEX DISEASES?

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The human inflammatory bowel diseases (IBD), usually classified into Crohn's disease (CD) and ulcerative colitis (UC), are typical examples of complex diseases where several genes and environmental factors are involved. Linkage analysis revealed several IBD susceptibility chromosomal regions, nine of which have been replicated in independent studies. As of today, fine mapping of chromosome 16p12-q13 candidate region and mutation analysis confirmed NOD2/CARD15 as the first discovered IBD gene. We used bioinformatics approaches including literature data mining tools, microarray expression data analysis and pathway analysis for candidate gene selection. We conducted case-control disease association study including linkage disequilibrium (LD) approaches to identify single-nucleotide polymorphisms (SNPs) and haplotypes within selected candidate genes contributing to IBD and predicting treatment response in IBD patients. In addition we have developed new approach for high-throughput identification of functional SNPs in noncoding regulatory regions of selected candidate genes using allele specific expression in lymphoblastoid cell lines and segregation analysis in reference CEPH families. Candidate genes showing most significant association with IBD include genes involved in cellular (CARD4, 7p14) and cell surface *TLR4* (9q32-q33) bacteria recognition, genes maintaining epithelial integrity (DLG5 (10q23), multidrug transporter (MDR1/ABCB1, 7q) and organic cation transporter *OCTN/SLC22A4* (5q31). Common polymorphisms as well as unique mutations in coding and non-coding regions were identified in IBD associated genes. ABCB1/MDR1 genes showed a complex pattern of IBD susceptibility and IBD protective haplotypes. These results confirmed several genes contribute to IBD. The identification of novel IBD genes will lead in better understanding disease pathophysiology, discovery of new therapeutic targets and in better disease management.

ODKRIVANJE GENOV ZA KRONIČNE VNETNE ČREVESNE BOLEZNI - MODEL ZA GENETSKE ŠTUDIJE KOMPLEKSNIH BOLEZNI?

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Kronične vnetne črevesne bolezni (KVČB) so tipični primer kompleksnih multifaktorskih bolezni, ki nastanejo kot posledica delovanja faktorjev okolja in večih genov. Študije genetske vezave so odkrila vsaj 9 kandidatnih področij, kjer se nahajajo geni, ki povečujejo dovzetnost za KVČB. V naših študijah smo uporabili orodja bioinformatike za podatkovno rudarjenje v znanstveni literature in analizo podatkov pridobljenih z ekspresijskimi biočipi za izbor kandidatnih genov. Izvedli smo asociacijsko študijo bolniki-kontrole ter s pomočjo izračunov genetskega neravnotežja in statističnih testov analizirali polimorfizme enega nukleotida in haplotipe v kandidatnih genih. Razvili smo tudi novo metodologijo za odkrivanje funkcijskih SNP-ov v nekodirajočih področjih izbranih kandidatnih genov. Analizirani kandidatni geni, ki so izkazali največjo statistično povezanost s KVČB vključujejo gene odgovorne za prepoznavanje bakterij znotraj celice (CARD4, 7p14) in na površini celične membrane (TLR4, 9q32-q33), nadalje gene odgovorne za vzdrževanje integritete črevesnegs epitelija (DLG5, 10q23), gen za multiplo rezistenco na ksenobiotike (MDR1/ABCB1, 7q) ter gen za organski kationski transporter (OCTN/SLC22A4, 5q31). Polimorfizme povezane s KVČB smo našli tako v kodirajočem kot nekodirajočem delu kandidatnih genov. Povezavo z boleznijo so izkazali tako funkcijski polimorfizmi z visoko alelno frekvenco, ki jih najdemo tudi v zdravi populaciji kot tudi redke mutacije prisotne izključno pri bolnikih. V genu ABCB1/MDR1 smo odkrili haplotipe, ki so bili povezani z večjo dovzetnostjo za bolezen kot tudi haplotipe povezane z zvečano dpornostjo na bolezen. Naši rezultati potrjujejo vlogo večih genov pri nastanku KVČB. Odkritje novih genov povezanih s KVČB bo omogočilo odkritje molekularnih tarč za načrtovanje novih bioloških zdravil kot tudi izbiro ustrezne individualizirane terapije prilagojene na posameznikovo genetsko zasnovo.

THE USE OF SUBTELOMERIC FISH, MLPA AND CGH TO INVESTIGATE CHROMOSOMAL REARRANGEMENTS ASSOCIATED WITH MENTAL RETARDATION

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Due to the development of molecular and cytogenetic techniques, it is now possible to identify cryptic rearrangements involving the ends of chromosomes which are a common cause of idiopathic mental retardation (IMR). The screening method generally used for detection of subtelomeric rearrangements is multiprobe telomere fluorescent in situ hybridization (T-FISH). Hundred patients (0-19-years old) with MR and dysmorphic features were screened using specific T-FISH probes. Multiplex ligation-dependent probe amplification (MLPA) and comparative genomic hybridization (CGH) were used for the confirmation of the results. T-FISH revealed 11 subtelomeric abnormalities in 10 patients (10%, 95% CI 5.0 – 17.5 %). Four of these had only a deletion of subtelomere 2q, which was apparently a normal variant. Among true aberrations (6%, 95% CI 2.5 – 12.5 %) we found two de novo subtelomeric deletions and four unbalanced subtelomeric rearrangements (one de novo). All clinically significant subtelomeric rearrangements were confirmed by MLPA. CGH was used to investigate the whole genome of patients in whom a subtelomeric anomaly was found, confirming some, but not all subtelomeric rearrangements. Our study estimated the frequency of subtelomeric abnormalities in patients with mental retardation and/or developmental disabilities and determined the feasibility of using these three methods in clinical testing. We concluded that T-FISH and MLPA are both very useful and interchangeable methods for detection of unbalanced chromosome rearrangements, but T-FISH also detects balanced rearrangements. In our experiment the resolution power of CGH is too low for subtelomeric screening compared to T-FISH and MLPA.

UPORABA SUBTELOMERNE FISH, MLPA IN PGH ZA ISKANJE KROMOSOMSKIH SPREMEMB PRI IDIOPATSKI DUŠEVNI MANJRAZVITOSTI

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Subtelomerni kromosomski predeli so pogosto vključeni v kromosomske preureditve, ki vodijo do idiopatske duševne manjrazvitosti (IDM). Dosedanja najpogosteje uporabljena presejalna metoda za odkrivanje subtelomernih kromosomskih sprememb je metoda simultane fluorescenčne »in situ« hibridizacije (T-FISH), katere uporabnost smo preverjali z našo raziskavo. S T-FISH smo testirali 100 preiskovancev, otrok in mladostnikov iz severovzhodne Slovenije, ki so bili napoteni v genetski laboratorij z diagnozo IDM ali zaradi displastičnih znakov in so imeli po klasični citogenetski analizi normalen kariotip. Subtelomerne kromosomske spremembe smo našli pri 10 preiskovancih (10 %, 95 % CI 5, 0-17, 5 %). Štirje so bili nosilci subtelomerne delecije: del(2)(qtel), ki se je izkazala kot dedovani polimorfizem. Med klinično značilnimi subtelomernimi spremembami pri 6 % preiskovancev (95 % CI: 2, 5 – 12, 5 %) smo našli dve »de novo« nastali deleciji in štiri neuravnotežene translokacije (ena »de novo«). Metoda MLPA je potrdila vse s T-FISH najdene subtelomerne spremembe razen polimorfizma del(2)(qtel). S primerjalno genomsko hibridizacijo (PGH) smo preiskali celotni genom preiskovancev s subtelomernimi spremembami in potrdili spremembe, večje od 8 Mb. S preiskavo smo želeli določiti prevalenco subtelomernih sprememb pri preiskovancih z IDM ali z dizmorfnimi znaki ter določiti uporabnost treh metod, T-FISH, MLPA in PGH, pri kliničnem testiranju subtelomer. Ugotavljamo, da sta T-FISH in MLPA zelo uporabni in v določenih primerih zamenljivi metodi za rutinsko citogenetsko diagnostiko. Za pregledovanje subtelomer v primerih IDM ali kongenitalnih anomalij je najbolj zanesljiva metoda T-FISH, ki omogoča odkritje tudi uravnoteženih sprememb, ki jih lahko obenem lokaliziramo. PGH omogoča pregledovanje celotnega genoma sicer z večjo resolucijo v primerjavi s klasično kariotipizacijo, a je resolucija premajhna za preiskovanje subtelomer.

ULTRA-HIGH THROUGHPUT SNP ANALYSIS WITH BECKMAN COULTER'S GENOMELAB SNPSTREAM GENOTYPING SYSTEM

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As genome DNA sequence data become available, more studies are required to better understand the significance of the DNA variations among individuals. Single nucleotide polymorphisms (SNPs) may lead to individual differences between disease susceptibility and response to treatment. SNPs have been the focus of much attention in genetic studies because they are extremely abundant and well-suited for automated large-scale genotyping. In the human genome, for example, SNPs are estimated to occur every 500 -1000bp (~3,000,000 to 6,000,000 SNPs). Therefore, SNP detection provides a high probability of finding association with disease alleles over small distances. DNA sequencing is the gold standard technique to discover the SNP sites of a genome. SNP sites can be identified through comparison of sequences from different individuals in a population. The most common technique for SNP scoring/validation of pre-characterized DNA regions is primer extension which utilizes specific DNA primers that anneal to the template 5' adjacent to the target SNP site. The primer is then extended by a single base with the complementary dye-labeled, dideoxy-nucleotide terminator(s) using a DNA polymerase. Electrophoresis and is commonly used for the simultaneous analysis of multiple SNP sites using primers designed with different lengths. In this presentation , we will describe an effective approach to SNP discovery and scoring using the multi-functional CEQ 8000 Genetic Analysis System and Ultra high throughput SNP STREAM system.

GENETIC DISEASES I
GENETSKE BOLEZNI I

NEW STRATEGIES FOR MOLECULAR MEDICINE DEVELOPMENT

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There is an overall concern for the slow evolving from the basic knowledge in molecular biology to the applied molecular medicine. One of the major problem is the right collection of human tissues for biomarker research and validation. The fixed and paraffin-embedded tissues with any kind of disease stored in any hospital can be the solution. We are able today to use this material for immunohistichemistry, but also for molecular analysis at DNA and RNA level. There is already an European project that is starting to analyze this opportunity: "Archive's tissues: improving molecular medicine research and clinical practice - IMPACTS". In this project are already involved 20 of the major European university-hospitals. This type of research can be applied in many field of medicine starting from oncology. Also technical innovation can give us new opportunities: recently were developed new fixatives that maintain DNA, RNA and also proteins in fixed and paraffin-embedded tissues the same as in fresh frozen tissues (1). This fixative preserves also the morphology and the possibility of a good immunohistochemistry. The tissues can be analyzed at morphological level and, after a careful micro-dissection, it is possible to have DNA; RNA and proteins analysis the same as in fresh tissues. In this way tissues can be preserved at room temperature without very expensive freezing systems.

Reference:
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GENETIC SCREENING OF MICROSATELLITE INSTABILITY IN COLORECTAL CANCER

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Microsatellite instability (MSI) is a phenomenon characterized by small deletions or insertions within short tandem repeats in tumor DNA compared to matching normal DNA. Approximately 15% of all colorectal cancers (CRC) were found to be microsatellite instable, while MSI is a characteristic of more than 90% of tumors of patients with Lynch syndrome. Patients with MSI tumors have favorable prognosis and do not benefit from adjuvant chemotherapy with fluorouracil. Besides, MSI status of tumors is a prescreening step in detection of patients with Lynch syndrome. For determination of MSI we have developed a new multiplex PCR system with a set of five quasimonomorphyc mononucleotide markers and DHPLC analysis. Our subsequent strategy for screening of Lynch syndrome which has based on solely molecular genetic knowledge (methylation of *MLH1* promoter and mutation analysis of MMR genes) has increased mutation detection rate from approximately 65% to 87% and has enabled to identify new patients with Lynch syndrome. Lynch syndrome is the most common autosomal dominant inherited predisposition for colorectal cancer. Carriers of the mutation have 70-80% life-time risk to develop Lynch syndrome, so there is the need to determine who of the relatives in the Lynch syndrome family inherited the mutation. Genetic counseling and genetic testing of relatives at risk, together with surveillance and prevention enable that the disease is detected in earlier curable stages what is connected with decreased cost for medical treatment and most importantly with better survival of patients.

GENETSKO PRESEJANJE MIKROSATELITNO NESTABILNEGA KOLOREKTALNEGA RAKA

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Mikrosatelitna nestabilnost (MSI) je fenomen, za katerega so značilne majhne delecije in insercije znotraj kratkih tandemskih ponovitev v tumorski DNA v primerjav z normalno DNA istega posameznika. Približno 15% vseh kolorektalnih rakov je mikrosatelitno nestabilnih, medtem ko je MSI značilnost več kot 90% tumorjev bolnikov s sindromom Lynch. Bolniki z MSI tumorji imajo boljšo prognozo, medtem ko zdravljenje s fluoro uracilom pri njih ni uspešno. MSI status tumorjev pa predstavlja tudi prvo stopnjo pri odkrivanju bolnikov s sindromom Lynch. Za določanje MSI smo razvili nov multipleksni PCR sistem s petimi kvazimonomorfnimi mononukleotidnimi markerji in analizo DHPLC. Naša nadalnja strategija za presejanje sindroma Lynch, ki temelji izključno na molekularnogenetskih dognanjih (metilaciji promotorja gena *MLH1* in mutacijski analizi genov, vključenih v sindrom Lynch), je zvišala stopnjo določanja mutacij s približno 65% na 87% in nam omogočila odkriti nove bolnike s sindromom Lynch. Sindrom Lynch je najpogostejša dominantno dedovana predispozicija za kolorektalni rak. Nosilci mutacije imajo 70-80% doživljenjsko tveganje, da razvijejo sindrom Lynch, zato je pomembno, da ugotovimo, kdo od sorodnikov je podedoval mutacijo. Genetsko svetovanje in genetsko testiranje rizičnih sorodnikov ter spremljanje in preventivni ukrepi omogočajo, da se bolezen odkrije v zgodnejših, še ozdravljivih fazah, kar je povezano z manjšimi stroški zdravljenja in najpomembnejše z boljšim preživetjem bolnikov.

CLINICAL, GENETIC AND EPIDEMIOLOGIC CHARACTERISTICS OF MAJOR LIMB GIRDLE MUSCULAR DYSTROPHIES (LGMDS)

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LGMDs are a heterogeneous group of genetic disorders characterized by progressive muscle wasting and weakness with onset in the proximal muscles. These diseases present a large clinical variability regarding age of onset, rate of progression, pattern of skeletal muscle involvement, heart damage and mode of inheritance, with both autosomal recessive and dominant forms. The most common clinical forms are autosomal recessive classified as LGMD 2 (A-J). In general, they have more severe course compared to dominant forms so-called LGMD1 (A-F). All of them are incurable and often life-threatening disorders of children and young adults. Therefore they represent a considerable health and economic burden, not only on the patients and their families, but also on the whole community. Our inability to treat these affections effectively makes preventing any recurrence very important. For this reason it is necessary to know their exact genetic cause to provide adequate genetic counseling, to predict risks for the patient such as the development of cardiomyopathy, and to be able to take advantage of specific treatment when they become available. Difficulties in classification are often caused by the relatively common sporadic occurrence of autosomal recessive forms as well as by interfamilial clinical variability. Molecular genetic studies have demonstrated different causative mutations in the genes encoding a disparate collection of proteins involved in all aspects of muscle cell biology. These novel genes encode highly diverse proteins with different localization within or at the surface of the skeletal muscle fiber, such as the sarcolemma, the sarcomere, the muscle cytosol, the nucleus and the glycosilation pathway enzyme. Epidemiological data vary especially in LGMD2.

KLINIČNE, GENETSKE IN EPIDEMIOLOŠKE ZNAČILNOSTI NAJBOLJ POMEMBNIH OBROČASTIH MIŠIČNIH DISTROFIJ (LIMB-GIRDLE MUSCULAR DYSTROPHIES - LGMDS)

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LGMDs so heterogena skupina genetskih motenj, za katere je značilna progresivna mišična oslabitev in upad z začetkom v proksimalni muskulaturi.Te bolezni so zelo raznolike glede na starost ob začetku bolezni, hitrost napredovanja, vzorec vključevanja skeletnega mišičja, prizadetost srca in način dedovanja, s tako avtosomno recesivno kot dominantno obliko. Najpogostejše klinične oblike so avtosomno recesivne, klasificirane kot LGMD tip 2 (A-J). V splošnem imajo hujši potek v primerjavi z dominantnimi oblikami, imenovanimi LGMD tip 1 (A-F). Vse so neozdravljive in pogosto življenjsko ogrožajoče bolezni otrok in mladih odraslih. Zaradi tega predstavljajo občutno zdravstveno in ekonomsko obremenitev, ne samo pacientov in njihovih družin, ampak tudi celotne družbe. Ker nismo sposobni učinkovito zdraviti teh motenj, je izredno pomembno preprečevanje njihovega pojavljanja. V ta namen moramo poznati natančne genetske vzroke, da bi omogočili ustrezno genetsko svetovanje, predvideli tveganja za paciente, kot so razvoj kardiomiopatije, in da bi uporabili specifično zdravljenje, ko bo to enkrat na voljo. Težave s klasifikacijo povzroča razmeroma pogosto posamezno pojavljanje avtosomno recesivnih oblik kot tudi interfamiliarna klinična variabilnost. Molekularne genetske raziskave so pokazale raznovrstne vzročne mutacije v genih za različne proteine, ki so vključeni v vse vidike biologije mišične celice. Ti novo odkriti skeletno mišični geni nosijo zapis za vsakovrstne beljakovine z različno lokalizacijo znotraj ali na površini skeletno mišičnega vlakna, kot so sarkolema (sarkoglikani, disferlin, kaveolin 3), sarkomera (teletonin, miotilin, titin), mišični citosol (kalpain 3, TRIM32), jedro (emerin, lamin A/C) in encim glikolizacijske poti (FKRP). Epidemiološki podatki predvsem za LGMD2 so odvisni o populaciji.

EVALUATION OF HER2 GENE STATUS IN BREAST CANCER BY CHROMOGENIC IN SITU HYBRIDIZATION

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Her2 is a gene located on the long arm of chromosome 17, encoding a transmembrane 185 kDa protein with tyrosine kinase activity , involved in the signal transduction of cell growth. It has been amplified in approximately 10-34% of breast carcinomas and is considered to be an important biological marker of poor prognosis. The development of the new humanized monoclonal antibody against extracellular part of Her2 protein in the treatment of the breast carcinoma implied the necessity of laboratory assessment of Her2 gene status. The purpose of this study was to evaluate HER2 gene status with the chromogenic in situ hybridization (CISH) technique and to compare the results with those of immunohistochemical technique. Thirty three cases of breast carcinoma with an immunohistochemical HER2 protein score of 1+, 2+, 3+ (HercepTesttm) were investigated. Her2 gene status was evaluated on paraffin sections with the CISH technique using a digoxigenin-labeled DNA probe. Seventeen cases were negative and 16 were positive for Her2 gene amplification. Of these 6 cases showed low level amplification and 10 cases had high level amplification. The correlation of the results obtained by CISH and IHC showed that the concordance of the methods was highest in the 3+ group (100%) and lower in 1+ group, whereas a high degree of discordance was found in the 2+ group (65%). CISH is an accurate and practical technique for the evaluation of Her2 gene status and its application is considered necessary especially for the clarification of the 2+ results of immunohistochemical technique. In cases that are 3+ positive and negative by CISH, and relation to chromosome 17 polysomy should be done.

RARE MUTATIONS OF THE VMD2 GENE IN SLOVENIAN FAMILIES WITH BEST'S VITELLIFORM MACULAR DYSTROPHY.

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The aim of our study was to investigate the presence of the VMD2 mutation and to evaluate central retinal sensitivity in patients with Best's dystrophy, in which in advanced stages of the disease a shift of fixation to the preferred retinal locus (PRL) was observed. Eleven patients from five families with BVD were included in the study. Blood samples were taken for genetic analysis. Retinal sensitivity was tested by microperimetry (MP) and multifocal electroretinography (mfERG). The results were compared with retinal morphology seen by autofluorescence (AF). A new G15R mutation was found in exon 2 of the VMD2 gene in one of the families. Based on MP testing and AF, two groups of patients were formed. Patients in the first group (VA: 0,7 $\pm$ 0.2) were fixating centrally inside the non-uniform hypo- and hyperfluorescent area as seen with AF. With progression of the disease, there was an evident shift of fixation to the preferential retinal locus (PRL) in 8 eyes with visual acuity of 0.2 or less (Group 2). Electrophysiology testing in patients with central fixation (Group 1) showed reduced amplitudes of mfERG mostly in the inner two rings. Conclusions: Our results show a good correlation between morphology and visual function. In patients with advanced stages of BVD and visual acuity of 0.2 and lower, a fixation shift to the preferred retinal locus was observed. Cautious interpretation of mfERG is needed in patients with eccentric and non-stable fixation.

REDKE MUTACIJE GENA VMD2 PRI SLOVENSKIH DRUŽINAH Z BESTOVO VITELIFORMNO MAKULARNO DISTROFIJO

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Namen naše študije je bil ugotoviti prisotnost VMD2 mutacij in ocena delovanja centralne nevrosenzorične mrežnice pri bolnikih z Bestovo viteliformno distrofijo, kjer smo pri bolnikih z napredovalimi stadiji bolezni opazili ekscentričen premik fiksacije na nov preferenčni areal mrežnice. V študijo smo vključili 11 bolnikov iz 5 različnih družin. Vsem smo vzeli kri za genetske raziskave. Delovanje centralne mrežnice smo testirali z mikroperimetrijo (MP) in multifokalno elektroretinografijo (mfERG). Rezultate smo primerjali z morfološko sliko očesnega ozadja posneto z avtofluorescenco (AF). V družini M smo dokazali novo, zaenkrat v literaturi še neobjavljeno G15R mutacijo na drugem eksonu VMD2 gena. Bolnike smo na podlagi izvidov MP in AF razdelili v dve skupini. Bolniki v prvi skupini (VO: 0,7 $\pm$ 0,2) so fiksirali centralno znotraj nehomogenega hipo- oz. hiperfluorescentnega območja, vidnega z AF. Z napredovanjem bolezni je pri osmih očeh z vidno ostrino 0,2 in manj prišlo do ekscentričnega premika fiksacije na nov preferenčni areal mrežnice (Skupina 2). Elektrofiziološko testiranje je pokazalo dobro ujemanje izvidov mfERG in MP pri bolnikih s centralno fiksacijo (Skupina 1). Naši rezultati kažejo dobro ujemanje morfoloških testov s testi vidne funkcije. Pri bolnikih z napredovalimi stadiji BVD in vidno ostrino 0,2 in manj je prišlo do premika fiksacije na nov preferenčni areal mrežnice. Pri bolnikih z ekscentrično in nestabilno fiksacijo je potrebna pazljivost pri interpretaciji izvidov mfERG.

POPULATION GENETICS OF THE SMITH-LEMLI-OPITZ SYNDROME

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The Smith-Lemli-Opitz syndrome (SLOS, OMIM xx) is an autosomal recessive metabolic malformation disorder. The predominant clinical features are failure to thrive, mental retardation and typical malformations as anteverted nares, polydactyly, and 2,3 toe syndactyly. The severity of the disease varies from mild malformations and mild mental retardation to very severily affected patients and intrauterine death. The underlying biochemical defect concerns the cholesterol biosynthesis. Mutations in the gene for the delta-7 sterolreductase (DHCR7) have been detected in all SLOS patients. We analysed the genetic defect in about 250 SLOS patients from Europe and found specific mutational spectra for different European regions. Most frequent mutations as the IVS8-1G>C and the W151X have decreasing and increasing gradients respectively from Western to Eastern Europe. The T93M is a very frequent mutation in mediterranean regions as Greece, Italy and Spain with a decreasing gradient from East to West. For these three frequent mutations we estimated time of appearence and concluded for example for the T93M an appearence in Eastern regions of the mediterranean sea 8000 years ago and distribution to the West. Because of frequency and age of mutations we estimate that the distribution today of the SLOS is not only due to random drift but also to heterozygote advantage.

NCODE™ MIRNA ANALYSIS PLATFORM IDENTIFIES MIRNA BIOMARKERS IN COLON CANCER

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MicroRNAs (miRNAs) are 19-25 nt non-coding RNAs that regulate gene expression by inhibiting translation or triggering degradation of specific mRNA targets. miRNAs appear to play a critical role in directing cellular differentiation and have been implicated in several disease states. Here we describe the development of the NCode™ microRNA Analysis platform, an integrated solution that includes components for miRNA, labeling/detection, and a multi-species DNA array complete with exogenous controls. The NCode™ Multi-Species miRNA Microarray consists of 1344 probes printed in duplicate for interrogating validated miRNAs in human, mouse, rat, C. *elegans, drosophila,* zebrafish, plus additional predicted human miRNA. Array probes were designed using a proprietary algorithm to optimize hybridization at a uniform T_m, often allowing single mismatch discrimination during hybridization. The NCode™ miRNA Labeling System, in conjunction with the NCode™ Multi-Species miRNA Microarray, provides detection sensitivity of less than 0.3 fmol/rxn (180 copies/cell). Using the NCode™ miRNA Analysis products, we profiled human colon tumor. Comparing these profiles with adjacent normal tissue, we discovered multiple differentially expressed miRNAs that may be useful biomarkers of tumorigenesis. A subset of the differentially expressed miRNAs was validated using a novel quantitative, real-time SYBR® Green RT-PCR assay for miRNAs. These data demonstrate the power of the NCode™ miRNA Analysis platform for identifying disease related miRNAs which may serve as candidates for diagnostic or therapeutic targets.

GENETIC DISEASES II
GENETSKE BOLEZNI II

CENTRAL TOLERANCE AND AIRE: MUTATIONS IN APECED PATIENTS

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AIRE directs the ectopic expression of many tissue-specific genes in medullary thymic epithelial cells, which plays an important role in the negative selection of autoreactive T cells. Its structural and functional properties suggest that AIRE is a transcriptional activator. Indeed, AIRE regulates the elongation rather than initiation of RNA polymerase II. For these effects, AIRE binds and recruits the positive transcription elongation factor b to target promoters in mouse medullary thymic epithelial cells. In these cells, RNA polymerase II is already engaged on the insulin 2 promoter, but is unable to elongate in the absence of AIRE. Our findings reveal an important mechanism by which AIRE regulates the transcription of genes, which have been implicated in central tolerance in the thymus. Moreover, we have begun to analyze mutations in AIRE with regards to their effects on the multimerization, DNA-binding and transcriptional activation of the complicated protein.

A RARE VARIANT OF TIETZ SYNDROME

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A 4 year-old patient showing an intermediate Tietz-/ Waardenburg- type IIA syndrome phenotype with an additional general psychomotor retardation and mild dysmorphic anomalies is described. Conception of the patient was achieved following an intracytoplasmic sperm injection treatment. By routine cytogenetic analysis, a *de novo* deletion of the short arm of chromosome 3 was found. Using fluorescence *in situ* hybridisation and single-copy BAC probes as well as molecular techniques the extent of the deletion was determined in more detail. The present case has a del(3)(p12.2p14.1) with the distal breakpoint in BAC clone RP11-199N21 and the proximal breakpoint between BAC clones RP11-544A22 and RP11-217I10, indicating that the size of the deleted segment is about 14 Mb. DNA polymorphism analysis using twelve microsatellite markers revealed that the interstitial deletion 3p was of paternal origin. This is the first patient with an intermediate Tietz-/Waardenburg type IIA syndrome phenotype caused by a confirmed constitutional 3p microdeletion including the entire *MITF* gene.

THE MOLECULAR DIAGNOSTICS IN CHILDREN WITH HEREDITARY HEMATURIA: THE DIFFERENTIAL DIAGNOSTIC QUESTIONS AND ETHICAL DILEMMAS IN CLINICAL PRACTISE

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In patients, including small children, with microhematuria and positive history of microhematuria or renal disease in other family members, the diagnosis could be Alport syndrome (AS) or thin basement membrane nephropathy (TBMN). Both are caused by mutations in three type IV collagen genes and show specific changes of glomerular basement membrane. The renal biopsy is could show only thinning of the glomerular basement membrane in both diagnosis. Genetic screening for mutations in type IV collagen genes is non-invasive and could provide additional information useful for diagnosis and prognosis. In 112 patients from 43 unrelated families with suspected AS or TBMN we found six different mutations *COL4A5* gene in AS suspected patients (G198E, G310R, G624D, K664N, 1234+5 G>T, and 3615-3616delC); three mutations in *COL4A3* gene (G487C, G1015E, 3547-3548insGGA) and four in *COL4A4* (3353 G>C + 3354-3358delACCAG, 3068+2T>G, 3497+1G>A) all in heterozygous state, were identified only in patients with benign familial hematuria. That non-invasive method, the genetic analysis of collagen genes, particularly in the case of young patients, may be of great clinical importance and could diminish the need for invasive renal biopsy. However, an exact diagnosis determined before the clinical picture is expressed, might predispose the patient to years of unnecessary anxiety specially in cases when specific treatment is not indicated.

MOLEKULARNOGENETSKA ANALIZA PRI OTROCIH Z DEDNIMI HEMATURIJAMI: DIFERENCIALNO DIAGNOSTIČNA VPRAŠANJA IN ETIČNE DILEME V KLINIČNI PRAKSI

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Bolniki, tudi majhni otroci z mikrohematurijo in pozitivno anamnezo o mikrohematuriji ali ledvičnem obolenju v družini, imajo lahko Alportov sindrom(AS) ali bolezen tankih membran (TBMN). Obe bolezni sta posledici mutacij v enem izmed treh genov za kolagen tip IV in kažeta specifično spremenjeno glomerularno bazalno membrano. Odkrivanje mutacij v genih kolagena tipa IV je neinvazivna metoda, ki lahko olajša postavitev diagnoze in napoved prognoze bolezni. Pri 112 pacientih iz 43 družin s sumom na AS ali TBMN smo odkrili 6 različnih mutacij v genu *COL4A5* (G198E, G310R, G624D, K664N, 1234+5 G>T, and 3615-3616delC). 3 mutacije v genu *COL4A3* (G487C, G1015E, 3547-3548insGGA) in 4 v genu *COL4A4* (3353 G>C + 3354-3358delACCAG, 3068+2T>G, 3497+1G>A) vse v heterozigotnem stanju, pa smo našli le pri pacientih z benigno družinsko hematurijo. Neinvazivna genetska analiza kolagenskih genov v nekaterih primerih, posebno pri majhnih otrocih veliko pripomore k točni diagnozi in lahko odloži potrebo po invazivni ledvični biopsiji. Kljub temu pa je bolnik, pri katerem ugotovimo točno diagnozo, izpostavljen strahu še pred razvojem klinične slike lahko leta dolgo posebno v primerih bolezni, pri katerih ne poznamo specifičnega zdravljenja.

EVALUATION OF TELOMERASE ACTIVITY IN PATIENTS WITH CHRONIC B LYMPHOCYTIC LEUKEMIA VERSUS AGE MATCHED CONTROLS. CORRELATION BETWEEN THE TELOMERASE ACTIVITY AND BONE MARROW INFILTRATION

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Telomerase is a ribonucleoproteic enzyme associated with cellular immortality and malignancy. It is repressed in most normal somatic cells but reactivated in most of the malignant tumor cells and immortal cell lines. There is variable number of tandem repeats of the hexanucleotide "(TTAGGG)n" constituting chromosomal telomeres, present at the telomeric ends of mammalian chromosomes. Telomeres stabilize chromosomal ends and prevent their fusion, rearrangements and chromosomal loss. Telomerase, the enzyme that elongates telomeres, contains an RNA template complementary to TTAGGG repeats that permits de novo synthesis of telomeric DNA onto chromosomal telomeric ends. Recent findings have suggested that activation of telomerase is one of the most common and fundamental steps in carcinogenesis and attainment of cell immortality. Increased telomerase activity has been reported in CLL by many authors. In order to investigate the telomerase activity in patients with CLL and its correlation to commonly used morphologic prognostic markers, 47 frozen blood lymphocytes samples from patients with CLL, and 47 age matched controls were investigated for telomerase activity using the "Telomerase PCR ELISA-plus kit" from Roche. Trepanobiopsies from the same patients were analysed for the type of bone marrow infiltration as well. Analysis showed significant 3,5 fold increase in relative telomerase activity (RTA) in patients' peripheral lymphocytes, compared to the control group. The sex and age of the patients showed no influence on RTA in CLL patients, oppositely to the control group, where the age influenced telomerase activity. We found positive correlation between the RTA and disease stages (Binet), as well as between RTA and the type of BM infiltration.

RARE VSX1 GENE VARIATIONS IN SPORADIC AND HEREDITARY KERATOCONUS PATIENTS

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Keratoconus (KC) is a non-inflammatory progressive disorder of the cornea, which leads to progressive mixed myopic and irregular astigmatism. The estimated prevalence of KC is approximately 1:2000 in the general population. The *VSX1* gene is part of a gene subfamily that is involved in craniofacial and ocular development. Mutations in *VSX1*have been reported in keratoconus patients, although its role of *VSX1* in keratoconus has not been clarified. DNA extraction, PCR amplification of the coding region and borderlines and screening with SSCA (single stranded conformational polymorphism analysis) were performed in 113 patients, 70 male and 43 female, who were included in this study after the determination of diagnostic and other criteria. The changes were confirmed with direct sequencing. Thirty-seven patients had a positive family history of keratoconus and 76 were diagnosed with a sporadic form of the disease. We chose 157 blood samples of healthy blood donors as a control population. Through screening of exons and borderline intron regions of the *VSX1* gene we found only 3 changes already described: Asp144Glu, Ala128Ala and 627+23G>A. Asp144Glu was found in one patient and one healthy blood donor, Ala128Ala polymorphism was found more frequently in patients than in the control population, but the difference was not statistically significant. However, the frequency of 627+23G>A was statistically significant for the hereditary form of keratoconus. A new VSX1 variation 504-24C>T was found in the control group. The previously described, presumably pathogenic mutation, Asp144Glu, was in our case also identified in the control group of patients and is therefore probably a benign polymorphism. The absence of pathogenic mutations in the *VSX1* gene in a large number of unrelated KC patients indicates that there are likely to be other genetic factors involved in the development of this disorder.

REDKE VARIACIJE V GENU VSX1 PRI SPORADIČNI IN DEDNI OBLIKI KERATUKONUSA

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Keratokonus je najbolj pogosta ektatična distrofija roženice s pojavnostjo okoli 1:2000. Obolenje vodi do progresivnega mešanega miotopičnega in nepravilnega astigmatizma. Gen *VSX1,* ki je del poddružine genov vpletenih v kraniofacialni in očesni razvoj. V študijah analize gena in vpliva tega na razvoj bolezni, so bile najdene spremembe v genu pri bolnikih s keratokonusom, vendar pa vloga *VSX1* pri razvoju bolezni ni razjasnjena. Ob upoštevanju diagnostičnih in ostalih kriterijev smo v našo študijo vključili 113 pacientov, 70 moških in 43 ženskih, pri katerih smo potrdili obolenje. 37 pacientov je obolelo za dedno obliko keratokonusa, 76 pa za sporadično. Kot kontrolno populacijo smo uporabili 157 vzorcev krvi zdravih krvodajalcev. Po izolaciji DNA, pomnoževanju eksonov in mejnih intronskih regij gena *VSX1* smo analizirali gen z enoverižno konformacijsko analizo (SSCA), odkrite spremembe pa smo potrdili z direktnim sekveniranjem. S pregledom celotne kodirajoče regije smo odkrili 3 različne, že znane, spremembe; Asp144Glu, Ala128Ala in 627+23G>A. Asp144Glu smo odkrili v dveh primerih, pri vzorcu bolnika in pri kontrolnem vzorcu, polimorfizem Ala128Ala je v naši populaciji pogost, vendar pa pojav le-tega ni statistično značilen za to bolezen. Pojavnost 627+23G>A polimorfizma pa je statistično značilna za dedno obliko keratokonusa. Odkrili smo le eno novo spremembo, 504-24C>T, pri kontrolnem vzorcu. Pri pregledu gena VSX1 smo identificirali le eno potencialno patogeno spremembo v genu VSX1 (Asp144Glu), za katero se je izkazalo, da ni povezana z razvojem bolezni. Odsotnost sprememb v genu *VSX1* pri pacientih s keratokonusom, kaže na to, da na razvoj bolezni ne vplivajo spremembe v genu *VSX1,* temveč drugi genetski faktorji.

X CHROMOSOME MOSAICISM IN WOMEN WITH PREMATURE OVARIAN FAILURE AND RECURRENT PREGNANCY LOSS

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The frequency of X chromosome mosaicism in women with sporadic form of premature ovarian failure (POF) has been found between 3–13%. The same mosaicism was identified in 3–16% women with recurrent spontaneous abortion. In the present study a contribution of X chromosome abnormalities was evaluated in Slovene population of (a) women with sporadic idiopathic POF and of (b) women with a history of recurrent pregnancy loss. **Methods.** Karyotypes of peripheral lymphocytes in 122 patients with sporadic idiopathic POF and in 424 women with a history of recurrent pregnancy loss were analyzed at Department of Obstetrics and Gynaecology, Division of Medical Genetics, in the period between 1994 and 2005. **Results.** X chromosome mosaicism was found in 20.5% (25/122) women with POF. In 424 patients with regular menstrual cycle and a history of recurrent pregnancy loss, 9.2 % (39/424) X chromosome mosaicism was found; six of them (6/39) had aneuploid offspring. **Conclusion.** Cytogenetic analysis should be considered for women with unexplained sporadic POF. X aneuploidy has reproductive significance in phenotypically normal women with recurrent pregnancy loss and/or subfertility. The results have practical implications for genetic counseling and fertility treatment.

MOZAICIZEM KROMOSOMA X PRI ŽENSKAH S PREZGODNJO MENOPAVZO IN S PONAVLJAJOČIMI SPLAVI

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Mozaicizem kromosoma X je bil ugotovljen pri 3–13% bolnic s prezgodnjo menopavzo. Hkrati predstavlja pomembni delež med vzroki za ponavljajoče spontane splave (3–16%). V raziskavi smo želeli opredeliti delež navedenega mozaicizma v slovenski populaciji žensk s prezgodnjo menopavzo ter populaciji žensk s ponavljajočimi splavi. **Bolnice in metode dela.** Iz vzorcev periferne krvi 122 bolnic s prezgodnjo menopavzo in 424 bolnic s ponavljajočimi splavi , ki so bile zdravljene na Ginekološki kliniki Kliničnega centra v Ljubljani v obdobju 1994-2005, smo s klasičnimi citogenetskimi tehnikami analizirali kariotipe. **Rezultati.** Mozaicizem kromosoma X smo našli pri 20,5% (25/122) bolnic s prezgodnjo menopavzo. Prisoten je bil tudi pri 9,2% (39/424) bolnic s ponavljajočimi splavi, med njimi je šest bolnic (6/39) rodilo otroke s kromosomskimi nepravilnostmi. **Zaključek.** Mozaicizem kromosoma X je pomemben vzrok prezgodnje menopavze in ponavljajočih splavov v slovenski populaciji. Zato menimo, da so genetsko svetovanje in ustrezne genetske preiskave strokovno utemeljene kot sestavni del klinične obravnave parov z zmanjšano plodnostjo.

MOLECULAR-GENETICS AND MOLECULAR-CYTOGENETICS METHODS BY DIAGNOSIS OF HEREDITARY MOTOR AND SENSORY NEUROPATHY (HNSN) TYPE 1A

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The hereditary motor and sensory neuropathy (HMSN1), the most common neuropathy in human, is genetically rather heterogeneous. Hereditary neuropathy with liability to pressure palsies (HNPP) is genetically related to the HMSN1A, but phenotypically it is a rather different disorder. By a dosage mechanism, trisomic overexpression of gene PMP22 results in HMSN1A whereas its monosomic underexpression causes HNPP. Multiplex ligation-dependent probe amplification (MLPA) is new method to detect gene dosage differences. The aim of our study was to evaluate the applicability of MLPA for the detection of the specific 1.5 Mb duplication/deletion present in CMT1A/HNPP. The sample included 70 patients referred with diagnoses of HMSN1A or HNPP. A total of 9 duplications and 19 deletions were confirmed in 70 probands. There was 100% concordance between FISH and MLPA results. PCR method showed three false positive and one false negative result. In contrast to FISH, MLPA can be used as alternative method. PCR is not suitable for diagnosis of HMSN1A, while the change which seams to be specific for duplication occur in health population. The MLPA assay allows accurate detection of duplications of the gene PMP22. Therefore it should become an important method for molecular diagnosis of HMSN1A. MLPA is very multiplex, sensitive, reproducible and easy to perform. Large numbers of samples can be tested simultaneously. The equipment necessary for MLPA is present in most molecular genetic laboratories. MLPA can be employed as a tool in diagnostic procedures for any disease which arises from gene dosage changes in human genome.

MOLEKULARNO-GENETSKE IN MOLEKULARNO CITOGENETSKE TEHNIKE PRI DIAGNOSTIKI HEREDITARNE MIŠIČNO SENZORNE NEVROPATIJE (HMSN) TIPA 1A

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Hereditarna mišično senzorna nevropatija tipa 1A (HMSN1A) nastane zaradi podvojitve gena PMP22. Klinično različna, genetsko pa s HMSN1A povezana nevropatija, je dedna nagnjenost k utesnitvenim parezam (HNPP), ki je posledica delecije istega gena. Z uporabo hkratnega pomnoževanje od ligacije odvisnih prob (MLPA), je mogoče zanesljivo določiti število kopij posameznega odseka v človeškem genomu. Za odkrivanje specifične 1,5 Mb duplikacije/delecije prisotne pri HMSN1A/HNPP smo ovrednotili uporabnost metode MLPA in jo primerjali z obstoječima metodama FISH (fluorescentna ″in situ″ hibridizacija) in PCR (reakcija verižne polimerizacije), ter opravili natančno analizo rezultatov. V študijo smo vključili 70 preiskovancev s sumom na HMSN1A ali HNPP. Potrdili smo 9 duplikacij in 19 delecij gena PMP22. Primerjava rezultatov FISH in rezultatov MLPA je pokazala 100% ujemanje. Z metodo PCR smo odkrili 3 lažno pozitivne in 1 lažno negativen rezultat. Primerjava in ovrednotenje načina dela metode MLPA z metodo FISH, ter natančna analiza dobljenih rezultatov je pokazala, da sta metodi pri diagnostiki HMSN1A obolenja prosto zamenljivi. Metoda PCR ni primerna za diagnostiko HMSN1A, ker je sprememba, za katero smo mislili, da je specifična za duplikacijo, prisotna v vzorcu zdrave populacije. MLPA metoda je izredno robustna, fleksibilna in primerna za diagnostiko HMSN1A. Omogoča analize več pacientov v kratkem času, zajema osnovne postopke, običajne za delo v molekularno genetskem laboratoriju in je tako primerna za odkrivanje različnih bolezni in sindromov, katerih vzrok so spremembe v številu kopij točno določenih zaporedij nukleotidov v človeškem genomu.

GENETIC TESTING FOR CYSTIC FIBROSIS

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Background: Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the gene encoding cystic fibrosis transmembrane regulator (CFTR) protein. Over 1300 mutations found in the gene contribute to the complexity of the CF phenotypes ranging from a classic multiorgan disease commonly involving respiratory, gastrointestinal and reproductive tract to mild and monosymptomatic presentations. Pilocarpine iontophoresis is considered as standard diagnostic test for CF, but it often fails in atypical forms of CF. **Methods:** In order to provide an additional diagnostic test to assure the diagnosis and provide patients with a proper medical care we performed genetic testing on 16 adults suspected to have atypical form of CF. Following counselling, parents of patients with possible homozygote variant of mutations were tested. The allele specific polymerase chain reaction method was used to detect 29 most common mutations in the *cftr* gene. **Results:** The diagnosis was proved in 3 individuals, a homozygote for ΔF508, and two compound heterozygotes ΔF508/R1162X and ΔF508/3849+10kbC>T. In three cases only one mutation was found: I148T 2789+5G>A and ΔF508 in a heterozygote form. **Conclusions:** The genetic testing for CF is a valuable diagnostic tool in atypical forms of CF. Possible differential diagnosis need to be excluded because of a variable CF phenotype. In cases where only one or no mutation was detected a necessity of whole gene sequencing is indicated upon re-evaluation of clinical data to exclude rare mutations and polymorphisms that could be implicated in the pathogenesis of CF.

GENETSKO TESTIRANJE ZA CISTIČNO FIBROZO PRI ODRASLIH BOLNIKIH

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Izhodišča: Cistična fibroza je avtosomna recesivna bolezen povzročena z mutacijami v genu, ki kodira transmembranski regulator protein imenovan CFTR. Preko 1300 mutacij dokazanih v genu prispeva h kompleksnosti klinične slike od klasične multiorganske bolezni, ki pogosto prizadene respiratorni, gastrointestinalni ter reproduktivni trakt do monosimptomatskih fenotipov. Pilokarpinska iontoforeza, ki velja za standardni diagnostični test za CF, pogosto ni diagnostična pri atipičnih oblikah CF. **Metode:** Z namenom pridobitve dodatnega diagnostičnega testa za potrditev diagnoze CF ter s tem možnosti primerne medicinske oskrbe bolnikov smo izvedli genetsko testiranje pri 16-ih odraslih, pri katerih je bil postavljen klinični sum na atipično obliko CF. Po predhodnem posvetu smo testirali tudi starše bolnikov, pri katerih smo posumili na homozigotno obliko mutacije. Uporabili smo alelno-specifično polimerazno verižno reakcijo za detekcijo 29 najpogostejših mutacij v *cftr* genu. **Rezultati:** Diagnozo CF smo potrdili pri 3-ih preiskovancih, homozigotu za ΔF508, ter dveh sestavljenih heterozigotih z genotipom ΔF508/3849+10kbC>T in ΔF508/R1162X. V 3-ih primerih smo ugotovili le eno mutacijo: I148T, 2789+5G>A ter ΔF508 v heterozigotni obliki. **Zaključki:** Genetsko testiranje za CF je dragocen diagnostični test pri atipičnih oblikah CF. Zaradi raznolike klinične slike CF je potebno izključiti možne diferencialne diagnoze. V primeru velike predtestne verjetnosti CF bi pri bolnikih, pri katerih smo našli samo eno ali nismo našli nobene od 29 testiranih mutacij, morali sekvencionirati celoten gen za izključitev redkih mutacij ali polimorfizmov, ki so lahko vpleteni v patogenezo atipične CF.

GENETIC RESOURCES II
GENETSKI VIRI II

EVOLUTION OF *ESCHERICHIA COLI* VIRULENCE

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Escherichia coli is the most abundant representative of the non-anaerobic microbial flora of the human gut playing an important role in homeostasis. Beside this homeostatic function, however, well defined intestinal and extraintestinal pathotypes of *E. coli* may elicit severe infections. Functional genomic studies have facilitated the laboratory modelling of the sequence of events how a commensal bowel flora member could develop into a successful pathogen. Horizontal gene transfer between bacteria plays a pivotal role in this process. In uropathogenic *E. coli* (UPEC) strains virulence determinants are almost exclusively located on the chromosome. It means that after the transfer event the newly acquired DNA has to be integrated into the chromosome. Large sequences (10-100 kilobase pairs) may be involved in the transfer and recombination processes resulting in chromosomal regions called pathogenicicty islands (PAIs). Transfer RNA genes with an involvement in global regulatory machineries are privileged integration sites for PAIs. The above evolutionary events together with the sophisticated regulatory mechanisms of virulence gene expression are exemplified in this lecture.

EVOLUCIJA VIRULENCE BAKTERIJE *ESCHERICHIA COLI*

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Esherichia coli je eden najštevilčnejših predstavnikov fakultativno aerobne mikrobne flore v prebavnem traktu človeka in igra pomembno vlogo v homeostazi. Poleg vloge v homeostazi, pa specifični črevesni in izvenčrevesni patotipi *E. coli* lahko izzzovejo resne okužbe. Raziskave funkcijske genomike so olajšale modeliranje zaporedja dogodkov potrebnih, da se komenzal prebavnega trakta razvije v uspešnega patogena. Kjučno vlogo v tem procesu so igrali horizontalni prenosi DNA. V uropatogenih sevih *E. coli* (UPEC) so determinante virulence skoraj izključno locirane na kromosomu, kar pomeni, da se prenešena DNA mora vključiti v kromosom. Pri prenosih in rekombinacijah DNA so vpletena dolga zaporedja nukleotidov (10-100 kilobaznih parov) tako, da nastanejo odseki kromosomov imenovani otoki patogenosti (PAI – angl. pathogenicity islands). Nukleotidna zaporedja genov za tRNA so ob delovanju globalnih regulatornih mehanizmov pogosto mesta vključitve PAI v kromosome. V predavanju so predstavljene zgoraj opisane stopnje v evoluciji patogenih sevov *E. coli* kakor tudi dovršeni mehanizmi izražanja genov virulence.

HETEROGENEITY IN EXPRESSION OF THE *ESCHERICHIA COLI* COLICIN K ACTIVITY GENE *CKA* IS CONTROLLED BY THE SOS SYSTEM, LEXA BINDING AFFINITY, AND STOCHASTIC FACTORS

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Phenotypic heterogeneity is evident in all organisms and provides populations the flexibility required to adapt to environmental perturbations and survive adverse conditions. Colicins are plasmid-encoded bacteriocins, synthesized by cells of *Escherichia coli*. Among natural isolates colicin producing strains are found with high frequency and more than 20 colicin types have been characterized. Colicin K production is encoded by three genes: *cka* encoding the colicin activity protein, *cki* encoding the immunity protein which protects the producing strain, and *ckl* encoding the lysis protein. Synthesis of colicin K is primarily induced by an increase in the alarmone ppGpp due to nutrient depletion. Colicin synthesis is also characteristically regulated by the LexA protein, the key regulator of the SOS response. In the stationary phase transcription from the *cka* promoter is derepressed in only approximately 3 % of the colicinogenic population. Here we report on the molecular mechanism underlying heterogeneity in expression of the *cka* gene. Real time RT PCR showed that the SOS system, without exogenous DNA damage, induces moderate levels of *cka* expression. The use of *cka-gfp* fusions demonstrated that modifications in the conserved LexA boxes in the *cka* promoter region affected the affinity of LexA binding and the percentage of *cka-gfp* expressing cells. A *lexA-gfp* fusion showed that the *lexA* gene is highly expressed in a subset of bacteria. These results indicate that bistability in expression of the *cka* gene depends on (1) SOS induction without external DNA damaging agents, (2) the affinity of LexA binding to operator sequences as well as (3) on stochastic factors.

IZRAŽANJE GENA KOLICINA K PRI BAKTERIJI ESCHERICHIA COLI JE NADZOROVANO S SISTEMOM SOS, AFINITETO VEZAVE PROTEINA LEXA IN STOHASTIČNIMI FAKTORJI

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Fenotipska raznovrstnost je prisotna pri vseh organizmih in prinaša populaciji odzivnost, potrebno pri prilagajanju spremembam v okolju. Kolicini so bakteriocini, katerih zapis se nahaja na plazmidih pri bakteriji *Escherichia coli*. Med naravnimi izolati te bakterije se pojavljajo zelo pogosto. Znanih je več kot 20 tipov kolicinov. Produkcijo kolicina K zajemajo trije geni: *cka* nosi zapis za kolicinski protein, *cki* zapis za imunski protein, *ckl* pa zapis za litični protein. Sinteza kolicina K je inducirana predvsem zaradi povečanja koncentracije alarmona ppGpp ob pomanjkanju hrane. Značilna za sintezo kolicinov je reguliracija s proteinom LexA, ključnim regulatorjem SOS odziva. V stacionarni fazi je prepis preko promotorja cka dereprimiran le pri 3 % kolicinogene populacije. Poročamo o molekularnem mehanizmu, ki se skriva za heterogenostjo pri izražanju gena *cka*. Poskus s PCR v realnem času (»Real Time PCR«) je pokazal, da sistem SOS, če ne pride do poškodbe DNA, sproži nizek nivo izražanja gena *cka*. S pomočjo zlitja genov *cka-gfp* smo ugotovili, da spremembe v vezavnih mestih proteina LexA vplivajo na njegovo afiniteto do DNA in s tem na odstotek celic, ki izražajo zlita gena. Zlitje genov *lexA-gfp* je pokazalo, da je gen lexA močno izražen v določenem delu bakterijske populacije. Ti rezultati kažejo, da je nivo izražanja gena *cka* odvisen od (1) indukcije SOS sistema ne glede na poškodbo DNA, (2) afiniteto vezave proteina LexA na operatorsko zaporedje ter (3) stohastičnih faktorjev.

FUNCTIONAL AND STRUCTURAL POLYMORPHISM OF A QUORUM SENSING SYSTEM IN *BACILLUS SUBTILIS* STRAINS ISOLATED FROM A SOIL AGGREGATE

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Bacteria communicate by secreting and reacting to extra cellular chemical signals controlling gene expression in response to cell density. The quorum sensing system that induces expression of the *srfA* operon and the natural genetic competence in *Bacillus* subtilis involves the ComX pheromones, the ComP-ComA two-component regulators and the modification enzyme ComQ. A striking polymorphism has been implicated in the *comQXP'* loci of *B. subtilis* strains isolated from different desert soils, which may represent a sexual isolation mechanism. It was found that these loci cluster into four similarity groups corresponding to four different pherotypes (Ansaldi et al, 2002). We have examined functional and structural polymorphism of *comQXP'* loci in *B. subtilis* strains isolated from a small soil aggregate (0.5cm3). Sequencing of their *comQXP'* loci revealed only 56-64% similarity between loci at the DNA level, while *rpoB* genes were more than 99% similar indicating a high polymorphism of *comQXP' genes*. In addition, specificity of the quorum sensing response was tested using *B. subtilis* tester strains, which do not produce an active ComX pheromone but have the pherotype-specific ComP receptor and the *srfA-lacZ* reporter gene. The results confirm that the structural polymorphism correlates with the specificity of the quorum sensing response preventing the optimal communication between strains of different pherotypes even in a spatially limited environment such as the soil aggregate. The results also suggest that the level of variability in these loci is comparable to the one found in strains from spatially distant environments.

FUNKCIJSKA IN STRUKTURNA VARIABILNOST KOMUNIKACIJSKEGA SISTEMA TALNIH IZOLATOV *BACILLUS SUBTILIS*

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Bakterije med seboj komunicirajo z izločanjem in odzivanjem na kemijske signale, ki ob določeni celični gostoti sprožijo izražanje specifičnih genov. Sistem za zaznavanje celične gostote, ki sproži izražanje operona *srfA* in naravno kompetenco pri bakteriji *Bacillus subtilis* , vključuje feromon ComX, membranski receptor ComP, odzivni regulator ComA in modifikacijski encim ComQ. Nukleotidna zaporedja lokusa *comQXP* izolatov *B. subtilis* različnih puščav so zelo variabilna, kar bi lahko predstavljalo nov mehanizem seksualne izolacije med sevi iste vrste. Ansaldi s sod. (2002) so lokuse *comQXP* uvrstili v štiri skupine genskih grozdov, ki sovpadajo s štirimi različnimi ferotipi. V naših raziskavah smo proučevali funkcionalni in strukturni polimorfizem lokusov *comQXP* v sevih *B. subtilis,* izoliranih iz majhnega koščka tal (0,5 cm3). Sekvenciranje lokusa *comQXP* je pokazalo le 56-64% podobnost na nukleotidnem nivoju, medtem ko je bila podobnost genov *rpoB* več kot 99%, kar nakazuje na visoko stopnjo polimorfizma lokusa *comQXP* . Specifičnost sistema za zaznavanje celične gostote smo preverili tudi s testerskimi sevi *B. subtilis,* ki sami ne sintetizirajo feromona ComX, vsebujejo pa ferotipsko specifičen receptor ComP ter operon *srfA* z reporterskim genom *lacZ*. Rezultati potrjujejo, da se strukturni polimorfizem lokusa *comQXP* ujema s specifičnostjo odziva na gostoto celic, saj je le-ta manj učinkovita med bakterijami različnih ferotipov, tudi na majhnih medsebojnih razdaljah. Prav tako smo ugotovili, da je raven variabilnosti omenjenih lokusov bakterij, izoliranih iz majhnega koščka tal, primerljiva z variabilnostjo sevov, izoliranih iz prostorsko oddaljenih okolij.

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P 01

EMERGENCE OF THE LOW-LEVEL QUINOLONE RESISTANCE MEDIATING GENE *AAC(6')-IB-CR* IN SLOVENIA

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Flouroquinolones are broad spectrum antimicrobial agents are used in clinical medicine among others also for the treatment of urinary tract infections. The widespread use triggered an increase in bacterial resistance, which usually results from mutational changes in the chromosomally encoded type II topoisomerases and the expression of efflux pumps or porins. In 1998, *qnrA,* the first plasmid mediated low-level quinolone resistance gene was described, however. QnrA is a 218-amino acid protein that protects the target enzymes from the inhibitory activity of quinolones. Since then two distantly related Qnr determinants have been identified and the occurrence reported from few areas around the world. Additionally, a new mechanism of resistance for quinolones was described just recently. The new plasmid-associated gene, *aac(6')-Ib-cr,* encodes a new variant of a common aminoglycoside acetyltransferase that is capable of reducing the activity of certain fluoroquinolones. Slovenia is not an exception regarding the increasing quinolone resistance worldwide. The number of nonrepetitive ciprofloxacin resistant or intermediate uropathogenic ESBL producing *Klebsiella sp.* strains, collected at the IVZ (Institute of public health of the republic of Slovenia), increased between the years 2000 and 2005 from 8 to17. Several conjugation and PCR experiments were carried out to confirm that the increased resistance was partly due to the emergence of plasmid mediated determinants. None of the *qnr* genes was found in the strain collection. The *aac(6')-Ib-cr* gene was found just in one of 17 isolates collected until 2003, however, its prevalence increased to 14 out of 20 isolates, 11 out of 20 isolates and 10 out of 17 isolates collected in the years 2003, 2004 and 2005, respectively. This is in accordance with the higher number of isolated ciprofloxacin resistant strains and the dissapperance of the intermediate resistance phenotype.

POJAVLJANJE GENA *AAC(6')-IB-CR*, KI POSREDUJE NIZKO ODPORNOST PROTI KINOLONOM V SLOVENIJI

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Fluorokinoloni so protimikrobne snovi , ki se pogosto uporabljajo pri zdravljenju infekcijskih bolezni, med drugim tudi za zdravljenje infekcij mokril. Pogosta uporaba je najverjetnejši vzrok povečanja bakterijske odpornosti, ki običajno izhaja bodisi iz mutacijskih sprememb kromosomsko zapisanega gena za topoizomeraze tipa II, bodisi iz povečane ekspresije specializiranih membranskih črpalk ali porinov. 1998 leta so odkrili *qnr*A, prvi na plazmidu zapisani gen, ki posreduje odpornost proti nizkim koncentracijam kinolonov. QnrA je protein iz 218 aminokislin, ki ščiti tarčne encime pred inhibitorno aktivnostjo kinolonov. Odtlej so v nekaterih delih sveta odkrili še dve le daljno sorodni Qnr determinanti. Pred nedavnim pa so odkrili nov mehanizem odpornosti pred kinoloni. Novo odkriti gen, *aac(6')-Ib-cr*, je zapisan na plazmidu in kodira različico, sicer običajne, aminoglikozidne acetiltransferaze, ki zmanjša aktivnost nekaterih fluorokinolonov. Slovenija ni izjema, ko gre za povečevanje odpornosti bakterij proti kinolonom. Število proti ciprofloksacinu odpornih ali intermediarnih uropatogenih ESBL klebsiel, ki so jih na inštitutu za varovanje zdravja (IVZ) republike Slovenije izolirali med leti 2000 in 2005, se je povečalo iz 8 na 17. Da bi ugotovili, ali je vzrok za povečano odpornost vsaj deloma pojavljanje na plazmidih zapisanih determinant smo opravili konjugacijske in PCR poskuse. V zbirki sevov nismo odkrili niti enega od *qnr* genov. Gen *aac(6')-Ib-cr* smo odkrili le v enem od 17 sevov, ki so jih na IVZ izolirali pred letom 2003, ter v 14 sevih od 20, ki so bili izolirani v letu 2003, 11 od 20 sevov izoliranih v letu 2004 in 10 od 17 sevov, ki so bili izolirani v letu 2005. Večja prisotnost gena sovpada z večjim številom proti ciprofloksacinu odpornih izoliranih sevov in izginevanjem intermediarnega fenotipa v navedenih letih.

ISOLATION OF DINUCLEOTIDE MICROSATELLITE SEQUENCES IN COMMON FIG (*FICUS CARICA* L.)

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Revitalization of fig cultivation in Slovenia requires an inventory and characterization of fig germplasms in order to select fig varieties well suited for production of fresh and dried figs of commercial value. In the past, traditional cultivation and exchange of figs in the region contributed to the confusion of varietal denomination and several synonyms and homonyms have been noted. The limits of the morphological descriptors in identification process are well known and could be resolved by using of molecular markers. Currently, microsatellites are the most promising marker system for accurate and rapid identification of genotypes. This study reports on the development and characterization of enriched dinucleotide (GA/TC and GT/AC) microsatellite libraries. DNA extracted from leaves of local variety 'Miljska figa' was digested with eight different combinations of restriction enzymes to obtain good representation of the genome. Fragments containing microsatellites were fished out using probes complementary to the searched repeat attached to the nylon membranes and a small-insert library was constructed. Overall, 1328 GA and 1424 GT clones were screened and 49% of the GA clones and 71% of the GT clones in the enriched library contained microsatellite repeats. Sequencing of 71 clones selected for GA and 126 clones selected for GT repeats resulted in the identification of 51 uniqe GA and 66 unique GT sequences in the total length of 47 kb. Of the total 117 unique sequences, 112 (96%) contained microsatellite repeat. Isolated microsatellites will be further characterized and converted into PCR markers for fig identification.

IZOLACIJA DINUKLEOTIDNIH MIKROSATELITNIH ZAPOREDIJ FIGE (*FICUS CARICA* L.)

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Pri poskusu revitalizacije gojenja fig v Sloveniji se je pokazala potreba po inventarizaciji in karakterizaciji genskih virov fige. Namen odbire je izbrati tržno najprimernejše sorte tako za svežo porabo kot za sušenje. Tradicionalno gojenje fig in izmenjava rastlinskega materiala v preteklosti sta doprinesla k zmedi pri poimenovanju sort, zabeleženi pa so tudi številni sinonimi in homonimi. Znane so omejitve morfoloških znakov pri identifikaciji rastlin, kar lahko presežemo z uporabo molekulskih markerjev. Trenutno so mikrosateliti najbolj obetaven in uporaben markerski sistem za natančno in hitro razlikovanje genotipov. Pričujoča študija obravnava razvoj in karakterizacijo obogatenih dinukleotidnih (GA/TC in GT/AC) mikrosatelitskih knjižnic. Izolirano DNA iz listov lokalne sorte 'Miljska figa' smo razrezali z osmimi različnimi kombinacijami restrikcijskih encimov, z namenom pridobiti dobro pokritost genoma fige. DNA fragmente, ki so vsebovali mikrosatelitne ponovitve, smo ulovili s pomočjo komplementarnih sond pripetih na najlonske membrane in konstruirali genomsko knjižnico. Od skupaj pregledanih 1328 GA in 1424 GT klonov obogatene knjižnice jih je 49 % GA in 71 % GT vsebovalo mikrosatelitno ponovitev ali SSR bogato regijo. Nukleotidno zaporedje smo določili izbranim 71 GA in 126 GT klonom. Po ureditvi zaporedij smo dobili 51 edinstvenih GA in 66 edinstvenih GT zaporedij v skupni dolžini 47 kb. Od 117 edinstvenih zaporedij jih je 112 (96%) vsebovalo mikrosatelitno ponovitev. Izolirani mikrosateliti bodo v nadaljevanju ovrednoteni in uporabljeni kot PCR markerji za potrebe identifikacije fig.

HORIZONTAL GENE TRANSFER BETWEEN MYCOPLASMA SYNOVIAE AND MYCOPLASMA GALLISEPTICUM

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Major poultry pathogens *M. synoviae* and *M. gallisepticum* belong to different phylogenetic groups. A comparison of their genomes indicated horizontal gene transfer (HGT) of at least 14 regions, including *vlhA* genes encoding numerous haemagglutinin variants. *M. gallisepticum* (strain R) has five *vlhA* loci containing 43 *vlhA* genes, but only three are xenologs of the *vlhA* gene of *M.synoviae*. *M.synoviae* (strain53) has a single *vlhA* locus that includes the expressed *vlhA* gene and upstream, 55 *vlhA* pseudogenes lacking the 5'-*vlhA* region. It has been proposed that HGT of *vlhA* was from *M. gallisepticum* to *M. synoviae,* but our analyses do not support this proposal. In all *M. synoviae* isolates the gene order was: *nanA-nanH* (encoding sialidase) - *gapA1* (encoding glyceraldehide 3-phosphate dehydrogenase) - *vlhA*ps1. To certain *M. gallisepticum* strains at least one *vlhA* copy was transferred together with the 5'-*gapA* from *M. synoviae*. In S6 strain its *vlhA* sequence is almost identical to that of the first *vlhA* pseudogene of *M. synoviae* K2426D. *M. gallisepticum* ULB992 also revealed a *vlhA* gene linked to the 5'-*gapA*. This strongly suggests HGT and replacement of a previous *vlhA* with the *vlhA* gene of *M. synoviae*. Our analyses indicate HGT from *M. synoviae* also for the *nanH* gene. In *M. gallisepticum* strains R, PG31, A5969 the 3'- end *gapA* sequence is immediately upstream of their *nanH* genes. *M. synoviae* and *M. gallisepticum* also share genes for transposases (IS4 family) that might be involved in HGT and in rearrangments within their genomes.

HORIZONTALNI PRENOS GENOV MED MYCOPLASMO SYNOVIAE IN MYCOPLASMO GALLISEPTICUM

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Bakteriji *M. synoviae* in *M. gallisepticum* pogosto povzročata bolezni pri perutnini. Čeprav sodita v različni filogenetski skupini, je primerjava njunih genomov pokazala horizontalni prenos genov v najmanj 14 regijah, vključno z geni *vlhA,* ki kodirajo različice hemaglutinina. *M. gallisepticum* (sev R) ima 5 lokusov *vlhA,* ki vsebujejo 43 genov *vlhA,* vendar pa so glede na sekvenčno zaporedje samo trije podobni genu *vlhA M. synoviae*. *M. synoviae* (sev 53) ima en lokus *vlhA,* v katerem je izražen gen *vlhA* ter pred njim 55 psevdogenov *vlhA* z manjkajočim 5'-*vlhA* koncem. Po obstoječi teoriji naj bi horizontalni prenos genov *vlhA* potekal od *M. gallisepticum* k *M. synoviae,* vendar naši rezultati kažejo nasprotno. Pri vseh izolatih *M. synoviae* je bil vrstni red genov naslednji: *nanA - nanH* (gen za sialidazo) - *gapA1* (gen za gliceraldehid 3-fosfat dehidrogenazo) - *vlhA*ps1. K določenim sevom *M. gallisepticum* je bila iz *M. synoviae* prenesena vsaj ena kopija gena *vlhA* skupaj s 5'-*gapA*. Sekvenca takega *vlhA* pri sevu S6 je skoraj enaka prvemu psevdogenu *vlhA* pri *M. synoviae* K2426D. Tudi pri *M. gallisepticum* ULB992 obstaja gen *vlhA,* povezan s 5'-*gapA* delom. Te ugotovitve kažejo na zamenjavo predhodnega *vlhA* z ustreznim genom *vlhA* iz genoma *M. synoviae*. Pridobljeni podatki kažejo prav tako na horizontalni prenos gena *nanH* od *M. synoviae* k *M. gallisepticum,* pri kateri imajo sevi R, PG31, A5969 3'-konec sekvence *gapA* neposredno pred genom *nanH*. V preurejanje omenjenih genomov bi lahko bile vključene tudi transpozaze (IS4 družina), katerih enake gene najdemo pri *M. synoviae* in *M. gallisepticum*.

PRION PROTEIN POLYMORPHISMS GENOTYPING IN SLOVENIAN AUTOCHTONOUS SHEEP BREEDS

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Scrapie is a transmissible degenerative disease of the central nervous system occuring naturaly in sheep and goats. Breeding programs towards TSE resistance are conducted in many countries based on resistance rendering *Prnp* polymorphisms at codon 136 (A/V), 154 (R/H) and 171 (R,H,Q). Whole blood sample from 171 Slovenian breed of sheep Jezersko-solčavska, Bovška, Istrska pramenka and Belokranjska pramenka were collected. Unlabeled PCR primers and Taqman® MGB probe were designed for each of the 7 *PrnP* polymorphisms. Results from allelic discrimination *Prnp* gene assay for sheep prion protein were confirmed by direct sequencing of gene for sheep. The described method was performed alleles VRQ, ARQ, ARR, AHQ and ARH. Four allelic variants were determined in Belokranjska pramenka and Istrian pramenka and five and eight different genotypes have been determined. The examined Jezersko Solčavska sheep and Bovška sheep have five allelic variants and eleven and eight genotypes. The most frequent genotype in examined sheep population is ARQ/ARQ (28.65 %). Animals carrying this genotype are moderate susceptible to scrapie. Genotypes with VRQ variant known to carry very high risk of scrapie is only poorly represented in population (VRQ/VRQ 5.85 %). The allelic variant ARR typical for sheep resistant to scrapie have higher frequence than genotypes with VRQ alleles in the population of the examined sheep (ARR/ARR 6.43%).

DOLOČANJE POLIMORFIZMOV PRIONSKEGA PROTEINA *PRNP* PRI SLOVENSKIH AVTOHTONIH PASMAH OVAC

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Praskavec je prenosljiva degenerativa bolezen centralno živčnega sistema (TSE) pri ovcah in kozah. Rejski programi za povečanje odpornosti drobnice proti TSE temelijijo na določanju polimorfizmov gena *PrnP* na kodonu 136(A/V), 154 (R/H) in 171 (R, H, Q). Krvne vzorce smo odvzeli 171 živalim štirih slovenskih avtohtonih pasem. (Jezersko solčavska ovca, Bovška ovca, Istrska pramenka. Belokranjska pramenka). Neoznačeni začetni oligonukleotidi in sonde Taqman MGB so bili oblikovani za vse znane polimorfozme gena *PrnP*. Rezultati, ki smo jih dobili s testom ločevanja alelov gena *PrnP* smo potrdili z metodo sekevenciranja. Z opisano metodo smo določili alele VRQ, ARQ, ARR, AHQ in ARH. Štiri alele smo določili pri pasmah Belokranjske in Istrske pramenke ter pet različnih genetipov pri Belokranjskih in osem pri Istrskih pramenkah. Pri jezersko solčavskih ovcah in bovških ovcah smo določili pet alelov in enajst različnih geneotipov pri Jezersko solčavskih in osem pri Bovških ovcah. Najpogostejši genotip pri vseh pasmah je ARQ/ARQ (28.65 %). Živali s tem genotipom so občutljive na praskavec. Frekvenca genotipov z aleli VRQ, ki so odgovorni za visoko občutljivost ovac na praskavec je majhna (VRQ/VRQ 5.85 %). Genotipi z aleli ARR v preiskovani populaciji (ARR/ARR 6.43%), ki so odgovorni za odpornost ovac na praskavec imajo višjo frekvenco, kot genotipi z aleli VRQ.

ARE CROATIAN AND ALTAIC SIBIREA - THE SAME SPECIES?

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Our presentation dealing with molecular genetic investigation on a Sibireae croatica - terciar relict, very rare, disjunct & endemic species in Western Balkan region (Croatia, Bosnia) in relation to the more numerous species of Sibiraea altaiensis from Southern Sibiria. Both locations separate about 5000 km. Results are pointing to so close genetic relationship of Croatian and Altaic sibirea that taxa should be grouped within one species.

ALI STA HRVAŠKA IN ALTAJSKA SIBIREJA – ISTI VRSTI?

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Hrvaška sibireja (*Sibiraea croatica* Degen) je redka in endemična grmovna vrsta balkanske flore ter terciarni relikt. Ima ozek in disjunkten areal razširjenosti. Opisana je velika morfološka podobnost z Altajsko sibirejo (*Sibiraea altainensis* (Laxm.) C.K. Schneider.), ki uspeva v oddaljenosti ca. 5000 km proti vzhodu, v osrednji Aziji. Sistematika rodu Sibiraea ni dokončno raziskana. Namen naše raziskave je bil z uporabo sodobnih metod molekularne biologije ugotoviti ali so in kakšne so genetske razlike med disjunktnimi populacijami hrvaške in altajske sibireje. Z raziskavo smo preverjali hipotezo o nižjem taksonomskem statusu hrvaške sibireje z njenih naravnih rastišč na Hrvaškem ter v Bosni in Hercegovini v primerjavi z vrsto altajske sibireje z rastišč na območjih južnega predela Rusije (genski arhiv) in južne Sibirije. Primerjave genetskih struktur z analizo dolžine restrikcijskih fragmentov po pomnoževanju izbranih regij v jedrnem ali kloroplastnem genomu niso pokazale nikakršnih razlik niti znotraj posameznih populacij niti med geografsko oddaljenimi populacijami. Genetsko homogenost vzorčnega materiala potrjujemo tudi z analizami nukleotidnih zaporedij celotnih pomnoženih regij. Z rezultati raziskave zavračamo možnost, da se leta 1905 opisana vrsta *Sibiraea croatica* na molekularni ravni dovolj loči od vrste *Sibiraea altaiensis,* da bi jo lahko obravnavali kot ločeno vrsto in s tem potrjujemo hipotezo o njenem nižjem taksonomskem statusu.

IDENTIFICATION AND MOLECULAR DIVERSITY ASSESSMENT OF NATIVE TRUFFLES SPECIES (*TUBER* SPP.) FROM SLOVENIA COMPARED TO MATERIAL FROM HERBARIA

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The genus *Tuber* comprises about 230 species and subspecies. Some of them are of high economic value and all are able to form ectomycorrhiza with several tree and shrub species. The identification of species in mycorrhiza for scientific studies as well as separation of high quality from less quality species for sale is important. Slovenia is not yet well known as a country of trufficulture although some collections are known from the area in the last years, mainly collected for consumption by local collectors. Suitable tools, markers and reference material would be necessary for proper identification of the collections. Therefore the collected material was analysed for diversity of rDNA ITS spacers and ß-tubulin regions. The obtained sequences were compared to the material deposited in MA-Fungi herbarium at Real Jardín Botanico (RJB) in Madrid. We have analysed about 150 samples, mainly belonging to the genus *Tuber* but also some other hypogeous sporocarps, collected together with truffles, for identification. Using sequences of the ITS region in nuclear RNA genes we managed to separate and identify most of the commercially important species from the material collected from Slovenia and neighbouring areas, comparing them to material from herbarium RJB and available sequences in GenBank. Although the amplification of ß-tubulin region was not as successful, we still suggest further analysis of multiple amplified DNA fragments for possible variation and presence of pseudogenes on a larger scale.

IDENTIFIKACIJA IN UGOTAVLJANJE MOLEKULARNE PESTROSTI PRI RODU GOMOLJIK (*TUBER* SPP.) V SLOVENIJI, V PRIMERJAVI Z REFERENČNIM HERBARIJSKIM MATERIALOM

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V rodu gomoljik (*Tuber*) je znanih okoli 230 vrst in podvrst, od katerih imajo nekatere visoko komercialno vrednost. Domnevno vse vrste v rodu lahko tvorijo ektomikorizo z eno ali več drevesnimi in grmovnimi vrstami, njihova identifikacija v ektomikorizi v znanstvene in/ali komercialne namene (ločevanje komercialno zanimivih od ostalih vrst) pa je nujna oz. zaželjena. Podatkov o pojavljanju gomoljik v Sloveniji ni veliko, metode molekularne identifikacije in preverjanja materiala v raziskovalne ali tržne namene pa so še v razvoju. Za uspešno preverjanje identitete smo pridobili identificiran referenčni material lokalenga izvora, kot tudi herbarijske vzorce iz herbarija MA-Fungi, Botanični vrt v Madridu, Španija. Pri okoli 150 vzorcih hipogejih gliv pretežno iz rodu *Tuber,* ter hipogejih vrstah, najdenih na istih rastiščih, smo analizirali rDNK ITS regijo in del ß-tubulinskega gena z namenom pridobivanja vrstno specifičnih molekularnih markerjev. Z analizami nukleotidnih zaporedij smo uspešno ločiti in identificirali vse komercialno pomembne vrste, kot tudi ostale vrste. Predvsem pri vrsti *T. borchii* in *T. brumale* smo opazili precejšnjo znotrajvrstno variabilnost nukleotidnih zaporedij. Pomnoževanje ß-tubulinske regije je bilo pri rodu manj uspešno, saj smo uspeli pomnožiti le del regije, ki vsebuje manjše število informativnih mest, kljub temu bomo nadaljevali z analizami več regij v jedrni DNK, na večjem številu vzorcev.

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DEVELOPMENT OF EST DERIVED SSR MARKERS FOR MAPPING OF THE HOP GENOME (*HUMULUS LUPULUS* L.)

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Hop is an important, traditional crop in Slovenian agriculture, since most of the yield is recognized as a high quality product on the world market. Breeding a new variety usually takes thirteen years and more, so an efficient tool for marker assisted selecton (MAS) is needed. Microsatellite markers derived from ESTs may have advantages over those developed from traditional genomic libraries. They often have known or 'putative' functions and are gene targeted markers with the potential of representing functional markers in those cases where polymorphisms in the repeat motifs affect the function of the gene in which they reside. Unlike genomic SSRs, genic microsatellite markers are concentrated in gene rich regions. It is believed that the distribution of genic SSRs in the genetic maps mirrors the distribution of genes along the chromosme. In this work we mined with PERL script MISA the subset of hop EST sequences recently developed from subtractive libraries for presence of microsatellite repeat motifs. We screened 611 EST sequences (190 kb) and of those 91 sequences contained microsatellite motif. The most common repeat was trinucleotide, followed by mononucleotide and dinucleotide type of repeat. Selected sequences were further screened by TBLASTX algorithm against Arabidopsis or Rice chromosome databases to reveal the most similar hit in either genome and to discover the potential intron sites, which should be avoided during design of PCR primers. Totally, 25 primer pairs were developed and characterized on WTx2/1 family and 8 of them exhibit polymorphism, which can be applied for mapping.

RAZVOJ EST PRIDOBLJENIH SSR MARKERJEV ZA MAPIRANJE HMELJNEGA GENOMA (*HUMULUS LUPULUS* L.)

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Hmelj je pomembna, tradicionalna rastlina slovenskega kmetijstva, poleg tega pa se večina pridelka izvozi na svetovna tržišča, kjer je prepoznan kot visoko kvaliteten produkt. Žlahtnjenje novega kultivarja je izredno dolgotrajen proces, ki poteka 13 ali več let, zaradi tega so žlahtnitelji izredno zainteresirani za razvoj novih tehnologij za žlahtnjenje s pomočjo markerjev. Mikrosatelitni markerji, razviti na podlagi EST zaporedij, imajo prednosti, če jih primerjamo s tistimi, ki so razviti iz genomskih knjižnic. Za njih pogosto poznamo dejansko ali domnevno funkcijo in so markerji, ki so neposredno vezani z genom. V primeru, da sprememba ponovitve mikrosatelita dejansko vpliva na funkcijo gena, imajo potencial funkcionalnih markerjev. Genski mikrosateliti niso nanizani okrog centromer, kot je to v nekaterih primerih značilno za genomske mikrosatelite, ampak se, kot je pričakovati, nahajo v gensko bogatih regijah. Predvidevajo, da distribucija genskih mikrosatelitov odraža distribucijo genov na genetskih kartah. V pričujočem delu smo s PERL skripto MISA pregledali del hmeljevih EST zaporedij, razvitih iz subtrakcijske knjižnice, za prisotnost mikrosatelitnih ponovitev. Pregledali smo 611 EST zaporedij (190 kb), od teh jih je 91 zaporedij vsebovalo mikrosatelitno ponovitev. Najbolj pogoste so bile trinukleotidne ponovitve, sledile so mononukleotidne in dinukleotidne ponovitve. Z izbranimi zaporedji smo iskali podobnosti z Arabidopsisovimi in riževimi kromosomi s TBLASTX algoritmom, da smo odkrili najbolj podobna zaporedja in potencialna mesta intronov. Ta informacija je izrednega pomena pri izdelavi parov začetnih oligonukleotidov. Skupno smo razvili 25 parov začetnih oligonukleotidov, ki smo jih okarakterizirali pri WTx2/1 družini, osem jih je pokazalo polimorfizem in se bodo lahko uporabili pri kartiranju.

RESPONSE TO DROUGHT STRESS IN LEAVES OF COMMON BEAN (*PHASEOLUS VULGARIS* L.)

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Drought is an important factor in reducing yield of food legumes. Relative gene expression analysis using quantitative real-time PCR was performed for 34 distinct transcripts, which might be involved in the response to drought stress. The majority of transcripts (32) were identified by differential display RT-PCR; two were identified by blastn search of drought-inducible genes (identified in other plant species) against *P. vulgaris* sequences in the NCBI's EST database. Nine transcripts were confirmed as up-regulated in drought-stressed plants (in comparison to control plants); and eight transcripts were confirmed as down-regulated. Blast search revealed that up-regulated transcripts/genes belong to various previous reported functional categories characteristic for drought stress: LEA proteins, protein kinases, aldehyde dehydrogenases, AP2/EREBP transcription factors, osmoprotectant synthesis, and cellular- and carbohydrate metabolism; while five of eight down-regulated genes were photosynthesis-related genes. Eight genotypes of common bean were included in the expression analysis. $\Delta\Delta$Ct estimated for each genotype separately showed up- (or down-) regulation in all genotypes. This is suggesting common response of these 17 transcripts/genes to the drought stress in all *P. vulgaris* genotypes. Furthermore, similar transcripts were reported in other plant species. Therefore, these genes probably play the same role in common bean as in other plants.

ODZIV NA SUŠNI STRES V LISTIH NAVADNEGA FIŽOLA (*PHASEOLUS VULGARIS* L.)

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Suša je eden izmed pomembnejših dejavnikov, ki vplivajo na zmanjševanje pridelka pri stročnicah. Za 34 transkriptov, ki bi bili lahko povezani z odzivom na sušo, smo izvedli relativno analizo izražanja genov z metodo kvantitativni PCR v realnem času. Večino transkriptov (32) smo identificirali z diferencialnim prikazom RT-PCR, dva pa z iskanjem genov induciranih zaradi suše v podatkovni bazi nukleotidnih zaporedij EST fižola, v NCBI dbEST z algoritmom blastn. Povečano izražanje pri rastlinah, ki smo jih izpostavili sušnemu stresu (v primerjavi s kontrolnimi rastlinami), smo statistično potrdili za devet transkriptov; medtem ko smo znižano izražanje statistično potrdili za osem transkriptov. Transkripti, katerih raven se je zaradi suše povečala, so se uvrščali v iste funkcionalne skupine kot nekateri geni udeleženi v odziv na sušo pri drugih rastlinskih vrstah; to je v skupine: LEA proteini, protein kinaze, aldehid-dehidrogenaze, transkripcijski faktorji AP2/EREBP, sinteza snovi, ki varujejo pred osmozo, metabolizem celice in ogljikovih hidratov. Pet od osmih transkriptov, katerih raven se je zaradi suše znižala, je bilo povezanih s fotosintezo. V analizo izražanja je bilo vključenih osem genotipov navadnega fižola. Vrednosti $\Delta\Delta$Ct izračunane za vsak genotip posebej so pri vseh pokazale zvišano (ali znižano) raven izražanja; kar kaže, da je odziv teh 17 transkriptov/genov na stres zaradi suše skoraj enak pri vseh genotipih fižola. Zelo podobne transkripte so ugotovili tudi pri drugih rastlinskih vrstah; vloga teh genov je zato najbrž v fižolu podobna kot pri drugih rastlinah.

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MICROBIAL COMMUNITY STRUCTURE AND PHYLOGENETIC COMPOSITION IN THE SOILS OF THE LJUBLJANA MARSH

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Soil is a complex ecosystem with large microbial diversity. Peatlands are special soil ecosystems where the production of biomass exceeds its decomposition, resulting in the accumulation of soil organic carbon (SOC). Microbial community structures in three soils of the Ljubljana Marsh differing in SOC content were assessed using cultivation-independent molecular profiling methods. Samples of two fen soil types and one bog soil type were collected on three dates differing in watertable level and temperature. Bog soil is characterized by low pH and approximately 4-times higher SOC content as compared to the fen soil types. Denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP) of bacterial 16S rDNA gene fragments showed differences between microbial community structures of bog and fen soils but indicated very similar microbial community structure in the two fen soil types. In addition, clone libraries from the fen soil type with higher SOC content and from the bog soil type were constructed. Clones were affiliated with different phylogenetic groups according to BLAST searches and phylogenetic trees that were reconstructed. Phylogenetic compositions of the two soil types were different. Majority of the clones in the clone library of the fen soil type belonged to *Proteobacteria* (53%) while in the clone library of the bog we found many *Acidobacteria* (40%) and *Proteobacteria* (40%).

STRUKTURA IN FILOGENETSKA SESTAVA MIKROBNIH ZDRUŽB V TLEH LJUBLJANSKEGA BARJA

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Tla so kompleksen ekosistem z ogromno mikrobno diverziteto. Šotišča so posebni talni ekosistemi, v katerih produkcija biomase presega njeno razgradnjo. Posledično se v teh tleh kopiči organski ogljik (SOC). V treh tipih tal Ljubljanskega barja, ki se razlikujejo v vsebnosti SOC, smo z od kultivacije neodvisnimi molekularnimi metodami profiliranja določali strukture mikrobnih združb. Vzorce dveh tipov tal nizkega barja in enega tipa tal visokega barja smo odvzeli v treh različnih mesecih, ki se razlikujejo v nivoju podtalnice in v temperaturi. Tla visokega barja se značilno razlikujejo od obeh tipov tal nizkega barja v tem, da imajo nizek pH in okoli 4-krat večjo vsebnost SOC. Denaturacijska gradientna gelska elektroforeza (DGGE) in polimorfizem dolžine terminalnih restrikcijskih fragmentov (T-RFLP) bakterijskih genov za 16S rRNA sta pokazala razlike med strukturama mikrobnih združb v tleh visokega in nizkega barja, medtem ko sta bili strukturi obeh tipov tal nizkega barja zelo podobni. Pripravili smo tudi dve klonski knjižnici iz tal nizkega barja z višjo vsebnostjo SOC in iz tal visokega barja. Klone smo glede na iskanja z orodjem BLAST in skonstruirana filogenetska drevesa razvrstili v različne filogenetske skupine. Filogenetska sestava mikrobne združbe v tleh visokega barja se razlikuje od filogenetske sestave v tleh nizkega barja. Večino klonov iz knjižnice tal nizkega barja smo uvrstili v skupino *Proteobacteria* (53%), v knjižnici tal visokega barja pa smo našli največ predstavnikov skupine *Acidobacteria* (40%) in *Proteobaceria* (40%).

PEDIGREE ANALYSIS OF KRŠKOPOLJE PIG POPULATION

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The study investigates the genetic diversity in the Krškopolje pig population from pedigree information. The breed is only native pig breed in Slovenia and it was pursued in the past. Pedigree file included 488 animals, 127 males and 361 females. In the year 1992, the herd-book was established and the pedigree information before this year was unknown. The diversity measures: kinship and inbreeding coefficients, family size, effective number of founders (EF), and effective number of ancestors (EA) were calculated for reference population (RP) which consisted of alive animals. Males and females in the RP had on average 15.4 and 14.3 known ancestors, respectively. Equivalent number of known generations for both was 2.52 and it was consequence of incomplete pedigree. Family size for pairs was 1.60 progeny (variance 0.78), dams had 2.05 (1.44) offspring, while sires had 3.81 (15.69) offspring on average. Progeny which passed genes to the next generation were taken into account. Large variance for family size in sires was result of unbalanced usage of sires. Average kinship coefficient was low (between 0.042 and 0.049) and biased downward due to incomplete pedigree. EF was 22.0 in males and 16.9 in females of RP, while EA, derived from probabilities of gene origin, was 15.2 in males and 14.7 in females. Only five most important ancestors contributed 50% of genes to gene pool of the RP under study. The Krškopolje pig is genetically very small population. A lot of efforts will be needed to maintain the genetic variation left in the population to preserve the breed for longer period.

ANALIZA POREKLA V POPULACIJI KRŠKOPOLJSKIH PRAŠIČEV

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Študija proučuje genetsko pestrost v populaciji krškopoljskega prašiča na osnovi podatkov o poreklu. Pasma je edina avtohtona pasma prašičev v Sloveniji in je bila v preteklosti preganjana. Poreklo je vsebovalo 488 živali, 127 samcev in 361 samic. V letu 1992 je bila ustanovljena rodovniška knjiga pasme, pred tem letom je poreklo neznano. Mere pestrosti: koeficient sorodstva in inbridinga, velikost družin, efektivno število osnovalcev (EF) ter efektivno število prednikov (EA) smo izračunali za referenčno populacijo (RP), ki so jo predstavljale živeče živali. Samci in samice RP so imeli v povprečju 15,4 oz. 14,3 znanih prednikov. Ekvivalentno število znanih generacij je pri obojih 2,52 in je posledica nepopolnosti porekla. Velikost družin pri parih je bila 1,60 potomcev (varianca 0,78), svinje so imele 2,05 potomcev (1,44), medtem ko so imeli merjasci v povprečju 3,81 potomcev (15,69). Pri tem so bili upoštevani le potomci, ki so svoje gene prenesli v naslednjo generacijo. Velika varianca velikosti družin pri merjascih je posledica neenakomerne rabe samcev. Povprečni koeficient sorodstva je bil majhen, med 0,042 in 0,049, ter podcenjen, kar je posledica nepopolnosti porekla. EF znaša 22,0 pri samcih in 16,9 pri samicah RP, medtem ko je bil EA, ki temelji na verjetnosti izvora genov, 15,2 pri samcih in 14,7 pri samicah. Vsega pet najpomembnejših prednikov je prispevalo 50 % v sklad genov RP v raziskavi. Krškopoljski prašič je genetsko zelo majhna populacija. Veliko naporov bo potrebnih za zaščito preostale genetske variabilnosti v populaciji, da bomo pasmo ohranili na daljši rok.

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PLANT GENETIC RESOURCES PROGRAMME FOR FOOD AND AGRICULTURE IN SLOVENIA

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Early projects to collect Slovenian autochthonous populations, ecotypes and landraces of agricultural species were initiated about 40 years ago. Phytogeographic and historical background has supported the development of the national programme and through that conservation of plant genetic resources in Slovenia. In 1996 financing started for the Slovene Plant Gene Bank Programme with the goal to maintain, evaluate, regenerate and preserve Slovenian autochtonous species, ecotypes, populations and landraces of agricultural, medicinal and aromatic plants. They include Slovenian cultivars, old cultivars, landraces, various populations, clones and lines bred from autochtonous plants and ecotypes from the natural habitat important for food, agriculture. The signature and the ratification of the Convention on Biological Diversity obligated the Republic of Slovenia to conserve and use in a sustainable manner plant genetic resources. In the Slovene Plant Genetic Resources Programme for Food and Agriculture three institutions are involved: Agronomy Dept. at the Biotechnical faculty of the University of Ljubljana, Institute for Hop Research and Brewing of Slovenia, Žalec and Agricultural Institute of Slovenia, Ljubljana. All three institutions are housing more than 4000 accessions among other following species: *Fagopyrum, Humulus, Zea, Allium, Solanum, Triticum, Brassica, Lactuca, Rubus, Malus, Pyrus, Juglans, Prunus, Vitis* and several species of forage crops, grain legumes and medicinal and aromatic plants. All three Institutions are involved in the work of the ECP/GR FA (European Cooperative Programme for Genetic Resources used for Food and Agriculture) which is aiming at ensuring long term conservation and facilitating increased utilization of plant genetic resources in Europe.

PROGRAM OHRANJANJA GENSKIH VIROV KMETIJSKIH RASTLIN V SLOVENIJI

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Z zbiranjem avtohtonih populacij, ekotipov in krajevnih sort kmetijskih rastlin se je začelo v Sloveniji pred več kot 40 leti. Zaradi specifične fitogeografije in zgodovinske podlage se je razvil nacionalni program in z njim program ohranjanja rastlinskih genskih virov. V letu 1996 je pristojno ministrstvo začelo finančno podpirati program Slovenska rastlinska genska banka s ciljem da se vzdržujejo, evalvirajo, razmnožujejo in ohranjajo slovenske avtohtone vrste, ekotipi, populacije in krajevne sorte kmetijskih ter zdravilnih in aromatičnih rastlin. Le te vključujejo slovenske sorte, stare in krajevne sorte, različne populacije, klone in linije požlahtnjene z uporabo avtohtonega materiala pomembnega za kmetijstvo. Tudi podpis in ratifikacija Konvencije o biološki raznovrstnosti zavezuje Slovenijo, da skrbi za ohranjanje in trajnostno rabo genskih virov. Tri inštitucije, Oddelek za agronomijo biotehniške fakultete univerze v Ljubljani, Inštitut za hmeljarstvo in pivovarstvo Slovenije, Žalec in Kmetijski inštitut Slovenije, so vključene v program Slovenska genska banka kmetijskih rastlin. Vse tri skupaj hranijo več kot 4000 akcesij različnih vrst kmetijskih rastlin med katere spadajo naslednje vrste: *Fagopyrum, Humulus, Zea, Allium, Solanum, Triticum, Brassica, Lactuca, Rubus, Malus, Pyrus, Juglans, Prunus, Vitis* ter več različnih vrst krmnih rastlin, zrnatih leguminoz ter zdravilnih in aromatičnih rastlin. Vse tri inštitucije so povezane ter sodelujejo pri delu ECP/GR FA (European Cooperative Programme for Genetic Resources used for Food and Agriculture), ki si prizadeva na evropski ravni dolgoročno ohranjati genske vire kmetijskih rastlin ter spodbujati njihovo nadaljnjo uporabo.

"PLANTS FOR THE FUTURE" - EUROPEAN VISION FOR PLANT GENOMICS AND BIOTECHNOLOGY

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The main task of technology platforms (TP), a new initiative of the European Commission (EC) is to include all interested parties, with the industry as a key holder, in the preparation and realization of the further research and developmental politics of the EU, to increase private and to improve public investments. The European TP "Plants for the future" launched in 2004 is a stakeholder forum on plant genomics and biotechnology, which rose from the joint initiative of industry and EC in the course of Lisbon strategy and preparation for FP7. In the EC "Plants for the future vision paper" EU is to become the most competitive and sustainable knowledge based economy by 2010. Fallowing the lead, stakeholders of the new launched platform prepared a Strategic Research Agenda (http://www.epsoweb.org/Catalog/TP/index.htm) which addresses four main challenges **1st**: health, safe and sufficient food and feed, **2nd**: sustainable agriculture, forestry and landscape, **3rd**: green products and **4th**: competitiveness, consumer choice and governance. Fallowing the European initiative, three Slovene research institutions in the area of plant science, Agricultural institute of Slovenia, National institute of biology and Biotechnical Faculty have organised the Slovene initiative "Rastline za prihodnost" which brings new means of cooperation between interested parties and takes an active role in policy making and realisation of the goals set by the EU. Common advantages of the involved parties are acquiring guidelines for one's own developmental programmes, networking with other European companies and research institutions as well as potential partnerships in European projects (FP7).

"PLANTS FOR THE FUTURE" - EVROPSKA VIZIJA RASTLINSKE GENOMIKE IN BIOTEHNOLOGIJE

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Tehnološke platforme (TP) so nov instrument evropske razvojne politike, s katerim želi Evropska komisija z aktivnim sodelovanjem industrije in držav članic EU pospešiti vlaganja v nova znanja in inovativne tehnologije. Namen platforme je vključiti vse zainteresirane skupine, z industrijo kot ključnim nosilcem pobude, v pripravo in izvedbo raziskovalne in razvojno-tehnološke politike EU, povečati privatna vlaganja in izboljšati učinkovitost javnih vlaganj v raziskave in tehnološki razvoj. Evropska TP »Plants for the future« je nastala leta 2004 na področjih rastlinske genomike in biotehnologije, kot skupna iniciativa industrije in Evropske komisije procesu uresničevanja Lizbonske strategije in priprav na 7. okvirni program EU. Glede na vizijo predstavljeno v dokumentu "Plants for the future vision paper", bo EU postala visoko konkurenčna in tehnološko podprta ekonomija že do leta 2010. Zainteresirane skupine, ki sestavljajo forum nove TP so oblikovale Strateški raziskovalno razvojni program (Strategic Research Agenda: http://www.epsoweb.org/Catalog/TP/index.htm), v katerem so predstavljena štiri glavna področja delovanja: 1. zagotavljanje dovolj zdrave in varne hrane ter krme, 2. sonaravno kmetijstvo in gozdarstvo ter varovanje kulturne krajine, 3. zeleni produkti in 4. konkurenčnost, izbira potrošnika, ustrezna zakonodaja. Po zgledu evropske iniciative je na pobudo treh raziskovalnih inštitucij s področja rastlin, Kmetijskega inštituta Slovenije, Nacionalnega inštituta za biologijo in Biotehniške fakultete nastala slovenska TP "Rastline za prihodnost". Glavne prednosti sodelovanja v nacionalni platformi so sooblikovanje evropske razvojne politike in pridobivanje lastnih smernic razvoja, povezovanje v evropske raziskovlno razvojne konzorcije ter sodelovanje v evropskih projektih (7.OP).

ESTABLISHMENT OF A EUROPEAN INFORMATION SYSTEM ON FOREST GENETIC RESOURCES (EUFGIS)

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In the Biodiversity Action Plan for Agriculture the Commission proposed to launch a new Community programme on the conservation, characterisation, collection and utilisation of genetic resources in agriculture which was adopted in Council Regulation No (EC) 870/2004. Six proposals were successful in the first call; one of them being a project on forest genetic resources (FGR) titled Establishment of a European Information System on Forest Genetic Resources (EUFGIS). The project will be coordinated by International Plant Genetic Resources Institute (IPGRI) with six partner institutes in Austria, Denmark, France, Slovakia, Slovenia and UK. Data on FGR will also be provided by other EUFORGEN (European Forest Genetic Resources Programme) countries as the project will be implemented in close collaboration with EUFORGEN. The major goal of the EUFGIS project is to establish a Web-based, permanent and easily accessible information system to link national FGR inventories at pan-European level. The project will therefore create a network of national FGR inventories to provide data for the information system and develop minimum requirements for dynamic gene conservation units of forest trees, as well as common information standards for these units. In Slovenia, the information system will be linked to Slovenian agricultural gene bank through Slovenian Forest Gene Bank. Once operational, the new information system will support practical implementation of gene conservation of forest trees and sustainable forest management in Europe. It will help to identify gaps and overlaps in FGR conservation at pan-European level and ease various reporting and monitoring efforts at national level. The project also supports the work of the Ministerial Conferences on the Protection of Forests in Europe (MCPFE) and the SEBI2010 (Streamlining European Biodiversity Indicators) process.

VZPOSTAVITEV EVROPSKEGA INFORMACIJSKEGA SISTEMA O GOZDNIH GENSKIH VIRIH (EUFGIS)

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V okviru Akcijskega načrta za biodiverziteto v kmetijstvu je Evropska komisija predlagala vzpostavitev novega programa skupnosti za ohranjanje, karakterizacijo, zbiranje in uporabo genskih virov v kmetijstvu. Program je bil sprejet v uredbi sveta (ES) št 870/2004. Med šestimi sprejetimi predlogi prvega razpisa je tudi projekt o gozdnih genskih virih (GGV) z naslovom Vzpostavitev evropskega informacijskega sistema o gozdnih genskih virih (EUFGIS). Koordinator projekta je IPGRI (International Plant Genetic Resources Institute); v projektu sodeluje šest partnerskih institucij iz Avstrije, Danske, Francije, Slovaške, Slovenije in Velike Britanije, podatke o GGV pa bodo prispevale tudi ostale države EUFORGENa (European Forest Genetic Resources Programme), saj se bo projekt izvajal v tesnem sodelovanju z EUFORGEN. Poglavitni cilj projekta je vzpostavitev na medmrežju temelječega, trajnega in lahko dostopnega informacijskega sistema, ki bo povezal nacionalne inventure o gozdnih genskih virih (GGV) na panevropski ravni. V okviru projekta bo vzpostavljena mreža nacionalnih inventur GGV z namenom posredovanja podatkov v informacijski sistem, razvite bodo minimalne zahteve za enote dinamičnega varovanja gozdnih drevesnih vrst kot tudi skupni informacijski standardi za te enote. V Sloveniji se bo informacijski sistem preko slovenske gozdne genske banke povezal z gensko banko kmetijskih rastlin. Po vzpostavitvi bo novi informacijski sistem podpiral praktično izvedbo varstva genskih virov gozdnih dreves in sonaravno gojenje gozdov v Evropi. Omogočil bo identifikacijo vrzeli in prekrivanj v okviru varstva GGV na panevropski ravni ter olajšal monitoring in poročanje na nacionalni ravni. Projekt hkrati podpira delo Ministrske konference o varstvu gozdov v Evropi (MCPFE) in proces razvoja evropskih indikatorjev biodiverzitete (SEBI2010).

ASSESSMENT OF GENETIC VARIATION AMONG *VERTICILLIUM ALBO-ATRUM* ISOLATES BY USING MOLECULAR MARKERS AND VIRULENCE TESTING

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Verticillium albo-atrum Reinke & Berthold and *V. dahliae* are well-known soil borne plant pathogens causing vascular wilts in a wide range of mainly dicotyledonous hosts. In Europe, *V. albo-atrum* isolates infecting hop (*Humulus lupulus* L.) express different levels of virulence, inducing mild and lethal disease syndromes. The appearance of different strains or pathotypes requires more detailed knowledge of the population structure and virulence of *V. albo-atrum* isolates, which could contribute to resistance breeding, disease management and an understanding of the evolutionary behaviour of this organism. We reported here on the assessment of genetic variability among *V. albo-atrum* isolates obtained from different European hop growing regions, as well as among isolates from other hosts and *V. dahliae* isolates, by using AFLP molecular technique and virulence testing.

OCENA GENETSKE VARIABILNOSTI MED IZOLATI GLIVE *VERTICILLIUM ALBO-ATRUM* Z UPORABO MOLEKULARNIH MARKERJEV IN DOLOČANJEM VIRULENCE

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Verticillium albo-atrum Reinke & Berthold in *V. dahliae* sta talni fitopatogeni glivi, ki povzročata uvelost mnogih rastlin, med katerimi prevladujejo dvokaličnice. V Evropi, izolati glive *V. albo-atrum*, ki parazitirajo hmelj (*Humulus lupulus* L.), izražajo različno stopnjo virulence v obliki indukcije blagega in letalnega bolezenskega sindroma. Pojav različnih patotipov zahteva poglobljeno znanje o populacijski strukturi in virulenci izolatov glive *V. albo-atrum*, kar lahko pomembno prispeva pri žlahnjenju odpornih sort, kontroli nad boleznijo in razumevanju evolucijskih mehanizmov pri tem organizmu. V prispevku poročamo o oceni genetske variabilnosti med hmeljnimi izolati glive *V. albo-atrum* iz različnih hmeljarskih območij Evrope in med izolati iz nekaterih ostalih gostiteljskih rastlin, vključno z izolati glive *V. dahliae*, z uporabo AFLP molekularne tehnike in testiranjem virulence.

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MICROSATELLITE MARKERS IN PHYLOGENETIC AND FINGERPRINTING ANALYSES OF POTATO (*SOLANUM TUBEROSUM* L.)

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The ability to identify cultivars rapidly and reliably has appeal not only to those maintaining germplasm collections but also for quality inspections throughout the agro-chain. Therefore the requirement for a rapid method for the differentiation and identification of potato cultivars has become increasingly more important as an additional support to DUS testing (distinctness, uniformity and stability of morphological traits). Simple sequence repeat (SSR) markers have proved to be the most robust candidates for the rapid differentiation of potato cultivars. Therefore, we decided to use six microsatellite markers (STM1024, STM2022, STM2028, STM5148, STM5136, STM3012) for estimation of the genetic diversity of Slovene potato cultivars in comparison with foreign ones. In our study 34 potato cultivars from Slovene variety list were included. Fourteen of them were bred at Agricultural Institute of Slovenia, the rest were the most important foreign cultivars in Slovene potato production. Utilization of microsatellite markers in the analysis of 34 potato cultivars revealed a high level of polymorphism; on average five alleles per locus was found. The highest number of alleles (8) was observed at the locus STM5148. Polymorphism information content (PIC) value ranged from 0.59 (STM1024 and STM2022) to 0.82 (STM5148). With the use of six microsatellite markers 34 cultivars from Slovene national variety list were successfully and rapidly differentiated.

UPORABA MIKROSATELITNIH MARKERJEV ZA IDENTIFIKACIJO IN FILOGENETSKE ANALIZE KROMPIRJA (*SOLANUM TUBEROSUM* L.)

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Hitra in zanesljiva identifikacija sort je pomembna tako pri vzdrževanju genskih bank kot tudi pri kontroli kakovosti v celotni prehranski verigi. Različne molekularne tehnike, ki omogočajo hitrejšo identifikacijo in razlikovanje sort krompirja, postajajo vse pomembnejše tudi kot dopolnilo testiranju RIN (raznolikost, izenačenost in nespremenljivost morfoloških lastnosti). Kot najprimernejši so se v ta namen izkazali mikrosatelitni markerji (SSR). V naši raziskavi smo zato za oceno genetske raznolikosti 34 sort krompirja iz Slovenske sortne liste uporabili šest mikrosatelitnih markerjev: STM1024, STM2022, STM2028, STM5148, STM5136, STM3012. Od 34 sort vključenih v analizo jih je bilo 14 požlahtnjenih na Kmetijskem inštitutu Slovenije, preostale tuje sorte pa imajo pomemben delež v slovenski proizvodnji krompirja. Izbrani mikrosatelitni markerji so se izkazali za zelo polimorfne; v povprečju smo našli pet alelov na lokus. Največje število alelov (8) smo dobili pri lokusu STM 5148. Vrednost PIC (informacijska vrednost polimorfizma) je bila v razponu od 0,59 (STM1024 in STM2022) do 0,82 (STM5148). S pomočjo šestih mikrosatelitnih markerjev smo uspešno in hitro ločili vse sorte, ki smo jih vključili v raziskavo.

REPORTS ON SUCCESSFUL GENETIC RECOMBINATION WITHIN GENUS SAMBUCUS AND COMPLEX GENETIC EVALUATION OF INTERSPECIFIC HYBRIDS AND INDIVIDUAL SPECIES

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The aim of our studies was to produce interspecific hybrids among agriculturally important *Sambucus* species (i.e., *S. nigra, S. ebulus, S. caerulea, S. javanica*), and to estimate the genetic distances among these and other potential pollen parents. Obtained offspring plants were tested for their hybrid origin using several genetic approaches. The first attempts of hybridization among *S. nigra, S. caerulea* and *S. ebulus* were unsuccessful until the discovery of a genetic bridge: a genotype found on the Island of Espiritu Santo (Vanuatu, South Pacific), which was determined as *S. javanica* ″ES″. Finally, the corresponding author conducted successful crosses between *S. javanica* (used as a female parent) and other three species. The hybrids *S. javanica* ″ES″ × *S. nigra* were highly fertile, whereas the hybrids *S. javanica* ″ES″ × *S. caerulea* were partly sterile. The combination *S. javanica* ″ES″ × *S. ebulus* was fully sterile although the plants flowered vigorously. The genetic evaluation of putative hybrids was based on nrDNA and cpDNA phylogenetic relationships, genome size determination, sequencing of trnT-trnF region and restriction analysis of nuclear ITS region. The nuclear 2C content determined by flow cytometric analysis revealed a very low variation among nine species tested, the exception was *S. caerulea* (23.9 pg), genome size of other eight species ranged from 27.0 pg (*S. ebulus and S. tigranii*) to 28.6 pg (*S. nigra*). Phylogenetic analyses were conducted using two data sets: nucleotide sequence data of the internal transcribed spacer (ITS) of nuclear ribosomal DNA and sequence data of chloroplast DNA of trnT-trnF region. Two maximum parasimony trees of 7 Sambucus species showed similar clustering into distinct groups on both dendrograms. Seed parent and the maternal origin of cpDNA was confirmed for all hybrids, while pollen parent was confirmed in 21 out of 22 tested putative hybrid plants.

USPELA KRIŽANJA MED VRSTAMI RODU SAMBUCUS IN KOMPLEKSNO GENETSKO IZVREDNOTENJE KRIŽANCEV TER POSAMEZNIH SPECIESOV

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Medvrstna križanja znotraj rodu Sambucus, katerih cilj je bilo oblikovanje fertilnih potomstev, predstavljajo večletni projekt, ki ga vodi dopisni avtor. V tem delu poročamo o uspešnem oblikovanju medvrstnih križancev, ki obsegajo štiri vrste bezgov (*S. nigra, S. ebulus, S. caerulea* in *S. javanica*), o kompleksem genetskem izvrednotenju več izbranih vrst bezgov in ugotovitvi genetske pristnosti pridobljenih križancev. Za uspeh križanj je bila ključnega pomena introdukcija javanskega bezga, odkritega na tihomorskem otoku Espiritu Santo (država Vanuatu). Ta bezeg, determiniran kot *S. javanica* ″ES″, je bil uporabljen kot genetski most, ki je omogočil oblikovanje križancev s *S. nigra*, *S. ebulus* in *S. caerulea*. Potomstvo križanja *S. javanica* ″ES″ × *S. nigra* je bilo visoko fertilno, medtem ko je bila fertilnost križancev *S. javanica* ″ES″ × *S. caerulea* mnogo nižja. Kombinacija *S. javanica* ″ES″ × *S. ebulus* je bila povsem sterilna, čeprav so rastline bujno cvetele. Genetske raziskave so vključevale določitev velikosti genoma devetih vrst rodu *Sambucus*, določitev filogenetskih sorodstvenih razmerij med osmimi vrstami in potrjevanje materinske in očetovske komponente domnevnih križancev. Velikost 2C genoma je le malo variirala med vrstami, najmanjša je bila pri vrsti *S. caerulea* (23,9 pg), ostale pa so variirale med 27.0 pg (*S. ebulus in S. tigranii*) in 28,6 (*S. nigra*). Filogenetska analiza je bila izvršena s pomočjo analize sekvenc ITS jedrne regije in analize trn-T-trn-F kloroplastne regije. Kot rezultat predstavljamo dvoje dendrogramov narejenih na osnovi maksimalne parsimonije, ki kažeta veliko podobnost v razvrščanju vrst. S pomočjo analize velikosti genoma, sekvenciranja kloroplastne regije in restrikcijske analize ITS regije smo uspeli potrditi pravilnost vseh materinskih komponent ter očetovsko komponento pri 21 od 22 križancev.

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VIRULENCE FACTORS IN *ESCHERICHIA COLI* STRAINS ISOLATED FROM UTI IN SLOVENIA

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Urinary tract infections (UTI) are one of the most frequent infectious diseases. *Escherichia coli* (*E. coli*) is the major cause of UTI. Strains causing infection differ from commensal *E. coli* due to possession of virulence factors. To diminish the burden of UTI, using effective preventive measures, data on virulence factor prevalence in different geographic regions must be assessed. 110 *E. coli* isolates from humans with urinary tract infections collected at the Institute of Microbiology and Immunology, Medical Faculty, Ljubljana, Slovenia were analyzed for presence of virulence factor (related) gene sequences. *papC, sfa/focDE, afa, iucD, cnf1, papG III, cdt, fyuA, ibe* and PAI (pathogenicity island sequences from the uropathogenic strain CFT073) sequences were amplified with specific primers in PCR. Dot blot hybridization experiments were performed to confirm the PCR assays. The α-hemolysin production was tested by plating strains onto LB plates containing 2 % washed sheep blood erythrocytes. Capsules were detected on the basis of sensitivity to K- specific phages. Expression of the aerobactin iron uptake system, enterobactin and salmochelin was tested by siderophore cross-feeding tests. The significance of the results was established using the Fisher's exact test and the level of significance was set at a P value < 0.05. In summary, we have found, a prevalence of common virulence factors among the UTI *E. coli* strains isolated in Slovenia comparable to other studies from different geographic regions. A high prevalence of *fyuA* as well as of *iro* sequences was detected supporting their potential as targets for preventive intervention.

VIRULENTNI DEJAVNIKI SEVOV BAKTERIJE *ESCHERICHIA COLI*, IZOLIRANIH IZ BOLNIKOV Z OKUŽBO SEČIL V SLOVENIJI

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Escherichia coli (*E. coli*) je najpogostejša povzročiteljica okužb sečil, ki sodijo med najpogostejše okužbe. Sevi *E. coli,* ki povzročajo okužbe, se od sevov *E. coli,* ki so del normalne flore, razlikujejo po vsebnosti genetskih zapisov za virulentne dejavnike. Da bi lahko zasnovali učinkovite metode preventive, ki bi zmanjšale breme zaradi okužb sečil, potrebujemo podatke o razširjenosti virulentnih dejavnikov v različnih geografskih predelih. 110 sevov bakterije *E. coli,* ki so jih iz seča bolnikov z okužbo sečil izolirali na Inštitutu za mikrobiologijo in imunologijo Medicinske fakultete Univerze v Ljubljani, Ljubljana, Slovenija, smo pregledali za prisotnost genetskih zapisov dejavnikov virulence. Dele nukleotidnih zaporedij *papC, sfa/focDE, afa, iucD, cnf1, papG III, cdt, fyuA, ibe* in PAI (otok patogenosti uropatogenega seva CFT073) smo pomnoževali s specifičnimi začetnimi oligonukleotidi v verižni reakciji s polimerazo (PCR). Za potrditev rezultatov dobljenih s PCR smo izvedli hibridizacijo točkovnega odtisa. Sintezo α-hemolizina smo preverjali z nanosom bakterijskih sevov na plošče LB, ki so vsebovale 2 % spranih eritrocitov ovčje krvi. Obdanost bakterij s kapsulo smo odkrivali z občutljivostjo za K-specifične bakteriofage. Izražanje sistemov za privzem železa (aerobaktin, enterobaktin in salmohelin) smo zaznali v navzkrižnih testih sideroforjev. Pomembnost dobljenih rezultatov smo izkazali s Fisherjevim testom natančnosti, kjer je bila vrednost zaupanja $P < 0.05$. Če povzamemo rezultate, je razširjenost preiskovanih virulentnih dejavnikov v preučevanih sevih *E. coli* iz Slovenije podobna razširjenosti viruletnih dejavnikov v drugih študijah iz različnih geografskih področij. Odkrili smo visoko prevalenco *fyuA* in *iro,* kar nakazuje možno uporabo siderofornih receptorjev kot tarč v preventivi.

GENETIC AND MORPHOLOGICAL CHARACTERIZATION OF LAKE OHRID ENDEMIC SALMONIDS

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Lake Ohrid endemics (Salmo ohridanus and Ohrid trout, putative *S. letnica*) were characterized by uni- (mtDNA sequences) and bi-parentally (microsatellite loci) inherited genetic markers analysis, compared to 40 different morphological characters. Further exploring historical demography of both species, *Salmo ohridanus* was described as a highly divergent member of the genus *Salmo*. Based on comparative substitution rate differences in mtDNA and a rough age estimate of closely related *Salmo trutta* complex (i.e. at least 2 million years), the *S. ohridanus* probably split from a common ancestor of *S. trutta* $>$ 4 million years ago, which is consistent with minimum age estimate of the Lake Ohrid formation. Comparative analysis with Ohrid trout supports the notion that these fish have more recently colonized the lake and phylogenetically belong to the Adriatic lineage of *S. trutta*, which was estimated to expand 155,000 years ago. Nevertheless, based on microsatellite and mtDNA sequence variation the endemic Ohrid trout represents a monophyletic lineage, isolated from other Adriatic basin populations. In the interests of the unique biodiversity protection in this ancient ecosystem, we recommend retaining the taxonomic epithet *Salmo letnica* for the endemic Ohrid trout. Our results do not support the existence of population structuring within Lake Ohrid, even though samples include two putative intra-lacustrine forms. Evidence of rare hybridization between *S. ohridanus* and Lake Ohrid brown trout exist at both mtDNA and microsatellite markers, but does not support the extensive introgression.

GENETSKA IN MORFOLOŠKA KARAKTERIZACIJA ENDEMIČNIH VRST SALMONIDOV V OHRIDSKEM JEZERU

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Endemični salmonidni vrsti v Ohridskem jezeru (belvica, *Salmo ohridanus* , in ohridska postrv, *S. letnica*) smo opisali z analizo mtDNK, mikrosatelitov in 40 morfoloških znakov. Ti rezultati so nam dali tudi vpogled v zgodovinski demografski vzorec obeh vrst in potrdili vrsto *S. ohridanus* kot zelo divergentno linijo znotraj rodu *Salmo*. Na osnovi primerjalne analize substitucijske stopnje v mtDNK in starosti kompleksa vrste *S. trutta* (vsaj 2 milijona let), smo ocenili, da se je vrsta *S. ohridanus* od skupnega prednika *S. trutta* odcepila pred vsaj 4 milijoni let, kar sovpada z najnižjo oceno starosti Ohridskega jezera. Genetske analize ohridske postrvi pa so potrdile predvidevanja, da je leta naselila Ohridsko jezero mnogo kasneje. Na osnovi filogenetske analize se ohridska postrv uvršča znotraj Jadranske linije vrste *S. trutta*, za katero smo ocenili, da se je formirala in razširila šele pred 155,000 leti. Analiza variabilnosti mikrosatelitnih lokusov in mtDNK pa je vseeno pokazala, da endemična ohridska postrv predstavlja monofiletsko linijo, ki je ločena od preostalih populacij *S. trutta* v Jadranskem porečju. Čeprav obstoj samostojne vrste *S. letnica* ni podprt z genetskimi analizami, pa z namenom ohranitve edinstvene biodiverzitete starodavnega ekosistema v Ohridskem jezeru priporočamo, da se ohrani taksonomsko poimenovanje ohridske postrvi kot *S. letnica*. Naši rezultati pa ne podpirajo obstoja lokalno priznane populacijske strukture znotraj ohridske postrvi, čeprav smo v analizo vključili dve t.i. intra-lakustrični formi. Z analizo tako mtDNK kot mikrosatelitov smo ugotovili, da se vrsti *S. ohridanus* in ohridska postrv občasno križata; intenzivne introgresije, ki bi ogrozila obstoj obeh vrst, pa nismo zaznali.

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THE STANDARDIZATION AND COMPARISON OF RESULTS OBTAINED BY MICROSATELLITE MARKER ANALYSIS

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Molecular tools for grapes are available in many different resources or databases, such as: bioinformatics, markers, genetic mapping, BAC libraries, physical maps, genome sequences, ESTs, etc. There are a number of public databases of microsatellite molecular markers available: Italian - Istituto Agrario San Michele all'Adige, Greek - University of Crete, European and Bulgarian - AgroBioInstitute, Sofia. These databases are not, however, compatible or interchangeable, since we have found examples of the same cultivar analyzed with the same molecular markers but with different results being shown. The possibility of comparison of genetic resources, especially with neighboring countries, is of great importance for smaller regions such as Slovenia or ex-Yugoslavia countries. Results can be compared only if they are standardized, which is only possible if the same standard cultivars are analyzed as those in databases. In our analysis, 11 Macedonian grape varieties were analyzed using nine microsatellite markers. The resulting allelic profiles were included in the Bulgarian database, since Bulgaria is one of countries bordering on Macedonia. 'Chardonnay' was used as the standard variety, which means that the lengths of alleles were adjusted according to this variety. For two loci out of nine, the allelic lengths of 'Chardonnay' were identical, at the remaining 7 loci the lengths differed from 1 to 4 bp, while the allelic profiles were the same. After standardization, we combined varieties from the Bulgarian *Vitis vinifera* collection (74 cultivars and 2 clones) with Macedonian varieties into one dendrogram based on the proportion of shared allele genetic distances. Most of the Macedonian cultivars clustered together and were well separated from Bulgarian samples, with some exceptions, which require further study.

STANDARDIZACIJA IN PRIMERJAVA REZULTATOV ANALIZ MIKROSATELITSKE VARIABILNOSTI

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Molekularne analize grozdja so narejene v dokaj velikem obsegu in so dostopne v različnih podatkovnih bazah (bioinformatika, molekulski markerji, gensko kartiranje, BAC knjižnice, fizične karte, sekveniranje genoma, itd). V primeru molekulskih (mikrosatelitskih) markerjev so na medmrežju javno dostopne naslednje podatkovne baze: italijanska - Istituto Agrario San Michele all'Adige, grška – Univerza na Kreti, evropska in bolgarska - AgroBioInstitute, Sofia. Omenjene baze niso medsebojno usklajene oz. primerljive, kar je moč ugotoviti na osnovi nekaterih standardnih sort, ki se pojavljajo v vseh bazah. Možnost primerjave rezultatov molekularnih analiz med državami oz. pokrajinami ima še posebno velik pomen za manjše države, kjer je raznolikost rastlinskega materiala omejena in dobi vrednost šele po umeščanju v širše geografsko področje. V naši raziskavi smo na osnovi devetih mikrosatelitskih markerjev analizirali 11 sort vinske trte iz Makedonije. Rezultate analize oz. alelne profile smo primerjali z bolgarsko bazo podatkov in makedonske sorte umestili med bolgarske. Kot standard smo uporabili sorto 'Chardonnay', kar pomeni da smo na osnovi primerjave dolžin alelov sorte 'Chardonnay' prilagodili dolžine alelov makedonskih sort. Dolžine alelov sorte 'Chardonnay' so bile pri primerjavi z bolgarskimi analizami enake na dveh mikrosatelitskih lokusih izmed devetih, na preostalih sedmih lokusih pa so se dolžine razlikovale za 1 do 4 bp. Alelni profili so bili na vseh lokusih enaki. Po standardizaciji smo združili sorte bolgarske kolekcije (74 kultivarjev, 2 klona) z makedonskimi sortami in na osnovi koeficienta oddaljenosti (Dps') naredili dendrogram. Večina makedonskih sort se je združila v eno skupino, ločeno od bolgarskih vzorcev. Par makedonskih sort pa se je tesneje povezal z bolgarskimi, kar je potrebno podrobneje proučiti.

HIGH PREVALENCE OF MULTIDRUG RESISTANCE AND RANDOM DISTRIBUTION OF MOBILE GENETIC ELEMENTS AMONG UROPATHOGENIC *ESCHERICHIA COLI* (UPEC) OF THE FOUR MAJOR PHYLOGENETIC GROUPS

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Of serious concern and an increasing health problem, on a global basis, is the appearance and spread of antimicrobial resistance. *Escherichia coli* strains are part of the normal flora of the gastrointestinal tract of humans and various animals. However, they are also the major cause of extraintestinal infections such as urinary tract infections, meningitis, infections associated with intravascular devices as well as osteomyelitis. As extraintestinal pathogenic *E. coli,* (ExPEC), including uropathogenic *E. coli* (UPEC), yearly affect a large proportion of the population they are a major target of antimicrobial therapy. Resistance genes are disseminated by plasmids or by transposons and also can be integrated into DNA elements designated integrons. Integrons are composed of a site specific recombination system capable of integrating and expressing genes in cassettes. In an effort to study the prevalence and distribution of antibiotic resistances in ExPEC strains, 110 UPEC strains from Ljubljana, Slovenia, were examined for antimicrobial susceptibility, mobile genetic elements involved in dissemination of antibiotic resistances, serotype as well as phylogenetic origin. A high prevalence of drug resistance and multidrug resistance was found. Twenty six percent of the isolates harbored a class 1 integron, while a majority of the strains (56%), harbored *rep* sequences characteristic of F-like plasmids. *int* as well as *rep* sequences were found to be distributed in a random manner among strains of the four major phylogenetic groups (A, B1, B2 and D) indicating that all groups have a similar tendency to acquire and maintain mobile genetic elements frequently associated with resistance determinants.

POGOSTNOST POJAVLJANJA ODPORNOSTI PROTI VEČJEMU ŠTEVILU ANTIBIOTIKOV IN NAKLJUČNA RAZPOREDITEV MOBILNIH GENETSKIH ELEMENTOV MED UROPATOGENIMI SEVI *ESCHERICHIA COLI* (UPEC) ZNOTRAJ ŠTIRIH FILOGENETSKIH SKUPIN

Matija Rijavec[1], Marjanca Starčič Erjavec[1], Rolf Reissbrodt[3], Angelika Fruth[3], Jerneja Ambrožič Avguštin[1], Veronika Križan-Hergouth[2] in Darja Žgur-Bertok[1]
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Pojav in širitev odpornosti proti antibiotikom je zaskrbljujoč in vedno večji zdravstveni problem na globalni ravni. Bakterija *E. coli* je del normalne flore prebavnega trakta ljudi in različnih živali. Hkrati je tudi glavna povzročiteljica zunajčrevesnih okužb, kot so okužbe urinarnega trakta, meningitis, okužbe povezane z intravaskularnimi napravami ter osteomielitis. Zunajčrevesni patogeni sevi *E. coli* (ExPEC), vključno z uropatogenimi *sevi E.* coli (UPEC), letno okužijo velik del populacije in so zato glavna tarča protimikrobnih terapij. Geni, odgovorni za odpornost proti antibiotikom, se razširjajo s plazmidi ali transpozoni in se lahko vklučujejo v dele DNA, imenovane integroni. Integroni so sestavljeni iz sistemov za mestnospecifično rekombinacijo, ki so sposobni vključevanja in izražanja kasetnih genov. V študijo o prisotnosti in širjenju odpornosti proti antibiotikom pri sevih ExPEC je bilo vključenih 110 sevov UPEC iz Ljubljane, Slovenija. Pri sevih smo proučevali pojavljanje odpornosti proti antibiotikom, prisotnost mobilnih genetskih elementov vključenih v prenašanje le-te, serotipe in filogenetski izvor. Veliko število preiskovanih sevov je bilo odpornih proti enemu ali celo večjemu številu antibiotikov. Šestindvajset odstotkov izolatov je imelo integron razreda 1, medtem ko je večina sevov (56%) imela zaporedje rep značilno za plazmide tipa F. Zaporedja *int* in *rep* se med sevi znotraj štirih poglavitnih filogenetskih skupin (A, B1, B2 in D) pojavljajo naključno. To kaže na to, da vse štiri skupine lahko sprejemajo in vključujejo mobilne genetske elemente, ki imajo pogosto determinante odpornosti.

MUTAGENESIS
MUTAGENEZA

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CIPROFLOXACIN INDUCES BACTERIOCIN SYNTHESIS IN *ESCHERICHIA COLI*

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The effect of antibiotics, which remain in the host after antibiotic treatment, could contribute to the physiological state known as the post-antibiotic effect. Antibiotics that interfere with DNA replication, as well as cell wall synthesis, induce the SOS response. We investigated the influence of subinhibitory concentrations of ciprofloxacin, a fluoroquinolone, on bacteriocin production in *Escherichia coli*. The bacteriocins of *E. coli* are designated colicins and are active against cells of the same and closely related species. They are found with high frequency among natural isolates. Colicin synthesis is characteristically regulated by the LexA protein, the key regulator of the SOS response. Recently, colicins have been shown to have an in vivo antagonistic role promoting microbial diversity within *E. coli* populations in the mammalian colon and the potential to promote microbial genetic diversity. The results of our study show that sublethal concentrations of ciprofloxacin induce colicin expression in an SOS-dependent manner and imply that SOS-inducing antibiotics could thus affect microbial strain diversification, as well as promote the acquisition and dissemination of antibiotic resistance. Thus, our results, even though anticipated, reinforce the need for great caution in the use of SOS-inducing antibiotics.

CIPROFLOKSACIN INDUCIRA SINTEZO BAKTERIOCINOV BAKTERIJE *ESCHERICHIA COLI*

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Antibiotiki, ki ostanejo v gostitelju po zdravljenju, lahko prispevajo k fiziološkemu stanju opisanem kot postantibiotični učinek. Antibiotiki, ki ovirajo podvojevanje DNA in sintezo celične stene bakterij, izzovejo SOS odziv. Proučevali smo vpliv subinhibitornih koncentracij ciprofloksacina, antibiotika iz skupine florokinolonov, na sintezo bakteriocinov bakterije *Escherichia coli*. Bakteriocini bakterije *E. coli* so imenovani kolicini in delujejo proti istim in ozko sorodnim vrstam. Velika večina naravnih izolatov bakterijskih vrst jih proizvaja in izloča. Izražanje kolicinov je značilno uravnano z LexA proteinom, ključnim regulatorjem SOS odziva. Pred kratkim je bilo pokazano "in vivo", da kolicini antagonistično vplivajo na diverziteto mikrobne združbe bakterij *E. coli* v črevesju sesalcev in imajo potencial, da vplivajo na genetsko diverziteto mikroorganizmov. Rezultati naše študije so pokazali, da subinhibitorne koncentracije ciprofloksacina, ki inducirajo SOS sistem, lahko preko indukcije sinteze kolicinov vplivajo na diverziteto mikrobne združbe v določenem okolju ter lahko pospešijo pridobivanje in širjenje odpornosti proti antibiotikom. Zato naši rezultati, kot že pričakovano, krepijo potrebo po veliki previdnosti pri uporabi SOS-inducirajočih antibiotikov.

Reference / Viri:

Jerman B., Butala M., Zgur-Bertok D. 2005. Sublethal concentrations of ciprofloxacin induce bacteriocin synthesis in Escherichia coli. Antimicrobial Agents and Chemotherapy, 49(7):3087-90.

PROTECTIVE EFFECT OF PLANT ANTIOXIDANTS AGAINST OXIDATIVE DNA DAMAGE AND MUTAGENESIS IN PROKARYOTIC AND EUKARYOTIC *IN VITRO* TESTS

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The protective effects of antioxidative substances from plants: eucalyptol, linalool and myrcene against oxidative DNA damage and mutagenesis was investigated in bacterial and mammalian cells *in vitro*. The bacterial assay was performed with repair proficient strain of E. coli WP2 (IC185) and its *oxyR* derivative (IC202), deficient in induction of antioxidative enzymes. The cells were co-treated t-BOOH and antioxidant substances. The comet assay with human hepatoma cell line (HepG2 cells) was used to measure the potential of antioxidants to reduce t-BOOH induced DNA damage. The cells were A) pre-treated with different concentrations of antioxidants (0, 0.01, 0.1 and 1 μg/ml) for 21 hours and than co-treated with antioxidants and t-BOOH (1μM) for 20 minutes and B) the cells were co-treated with antioxidants (0.01, 0.1 and 1 μg/ml) and t-BOOH (1μM) for 20 minutes. The reduction of t-BOOH-induced mutagenesis was obtained in both bacterial strains with all tested substances. The highest inhibition of mutagenesis was obtained in IC202 strain: 40% eucalyptol (0.05 mg/p), 60% linalool (1mg/p), 69% myrcene (1 mg/p), indicating that antimutagenic potential is based primarily on their antioxidative properties. Linalool and eucalyptol significantly reduced t-BOOH-induced DNA damage in HepG2 cells in both approaches used. The higher effect against t-BOOH induced DNA damage was observed when the cells were pretreated with the oxidants for 21 hours prior to the exposure to t-BOOH (protocol A), indicating that tested supstances can act both by scavenging oxidant and increasing antioxidant cell pool.

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MODIFIED COMET ASSAY FOR DETECTING GENOTOXIC AND ANTIGENOTOXIC EFFECTS IN HUMAN AND RAT PRECISION-CUT LIVER SLICES

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The comet assay is a rapid and sensitive method for measuring deoxyribonucleic acid (DNA) strand breaks at the level of individual cells. Cultured liver cells are usually deficient in metabolic activity. Therefore, we have adapted the comet assay for use in precision-cut human and rat liver slices, where metabolic activity is preserved and all the liver cell types are present, maintaining the tissue architecture. With the modified comet assay we detected concentration-dependent DNA damage in human and rat liver slices, induced by three procarcinogens, that cause genotoxicity by two distinct mechanisms: *tert*-butyl hydroperoxide (*t*-BOOH) causes oxidative damage to DNA, metabolites of 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) form adducts with DNA and benzo(a)pyrene (BaP) metabolism is responsible for DNA adduct formation and oxidative damage. In rat liver slices we then studied the protective effect of the prenylated flavonoid xanthohumol (XN) against genotoxicity of the three procarcinogens. XN has been suggested to have cancer chemopreventive activities, acting as an antioxidant and inhibitor of metabolic activation of procarcinogens. Exposure of rat liver slices to concentrations lower than 10 μM XN did not affect their viability and did not induce DNA damage. At concentration as low as 0.001 μM XN nearly completely prevented the DNA damage, induced by BaP and IQ, while no protection against *t*-BOOH induced DNA damage was observed. We have shown that the modified comet assay with precision-cut liver slices is a useful tool to study genotoxic and antigenotoxic effects, which also enables detection of species differences in susceptibility to procarcinogens.

MODIFICIRANI TEST KOMET ZA MERJENJE GENOTOKSIČNIH IN ANTIGENOTOKSIČNIH UČINKOV V ČLOVEŠKIH IN PODGANJIH REZINAH JETER

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Test komet je hitra in občutljiva metoda za kvantifikacijo eno- in dvoverižnih prelomov deoksiribonukleinske kisline (DNA) na nivoju posamezne celice. Jetrne celice v kulturi imajo večinoma okrnjeno metabolno aktivnost. Zato smo test komet prilagodili za uporabo na človeskih in podganjih rezinah jeter, kjer je metabolna aktivnost ohranjena, kjer so prisotni vsi tipi jetrnih celic in je ohranjena prvotna struktura jeter. Z modificiranim testom komet smo ugotovili koncentracijsko odvisne poškodbe DNA v človeskih in podganjih rezinah jeter, ki smo povzročili s tremi karcinogeni, ki povzročajo poškodbe DNA na dva različna načina. *Tert*-butil hidroperoksid (*t*-BOOH) povzroča oksidativne poškodbe, metaboliti 2-amino-3-methylimidazo[4,5-f]quinolina (IQ) tvorjo adukte z DNA, medtem ko je metabolizem benzo(a)pyrena (BaP) odgovoren tako za tvorbo aduktov na DNA kot za oksidativne poškodbe. V podganjih jetrnih rezinah smo nato ugotavljali zaščitno delovanje preniliranega flavonoida ksantohumola (XN) pred genotoksičnostjo zgoraj uporabljenih mutagenov. Dosedanje raziskave nakazujejo, da je ksantohumol učinkovina, ki deluje preventivno pri nastanku in razvoju raka. Deloval naj bi kot antioksidant in kot inhibitor metabolne aktivacije prokarcinogenov. Izpostavitev podganjih jetrnih rezin koncentracijam XN, nižjim od 10 μM, ni vplivala na preživelost celic in ni povzročila poškodb DNA. XN je že pri koncentraciji 0.001 μM skoraj povsem preprečil nastanek poškodb DNA, povzročenih z mutagenoma BaP in IQ, je, medtem ko proti *t*-BOOH ni deloval zaščitno. Pokazali smo, da je modificirani test komet na rezinah jeter uporabno orodje za preučevanje genotoksičnih in antigenotoksičnih učinkov, pri čimer lahko ugotavljamo tudi razlike v vrstni občutljivosti za različne prokarcinogene.

INDUCED ADAPTIVE SURVIVAL RESPONSE BY CYCLOHEXIMIDE IN CELLS EXPOSED TO MYTOMICIN C AND TAXOL LEAD TO GENOMIC INSTABILITY

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Cycloheximide as a inhibitor of protein synthesis can reduce cytotoxicity of various antitumor drugs. We argue that adaptation (pro-life processes) can overwhelm its positive aspects (antimutagenic and anticarcinogenic) by increasing a population of cells with chromosome aberrations (chromosome instability) by apoptotic inhibition. To evaluate an adaptive response induced by CHX in peripheral blood lymphocytes exposed to Taxol (TX) and Mytomicin C (MMC) we used the CB-micronucleus test and the CA test. We have found that CHX in a dose of 10 μg/ml induces a adaptive response in human peripheral blood lymphocytes exposed to increasing doses of Taxol (0.01 μM, 0,05μM and 0,2μM) and Mytomicin C (0.05 μM, 0,15μM and 0,6μM). Even though, genotoxicity of TX and MMC measured by the percentage of micronuclei in binuclear (BN) cells was not elevated in the presence of CHX, the CA analysis showed an increased level of cells with chromosomes expressing premature centromere separation (chromosome instability). CHX induced a highly statistical difference ($p < 0,001$) in the proliferation index (NDI) compared to cells that were exposed to TX and MMC alone. Also, the increase in the NDI index was correlated to a decrease in nuclear fragmentation. The observed differences in nuclear fragmentation and the NDI index between two groups shows that adaptation to TX and MMC induced by CHX can be a consequence of inhibition of apoptosis. At present, these investigations can help us to better assess the genotoxic risk of combined therapies and therefore exploit new paths for a better control of cancer.

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DETECTION OF ANTIGENOTOXIC EFFECT OF BASIL (*OCIMUM BASILICUM* L.) WITH MICROBIAL SHORT-TERM TESTS

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Sweet basil (Ocimum basilicum L.) is employed as a folklore remedy for a wide spectrum of ailments in many traditional medicines. The mutagenic and antimutagenic properties of essential oil of basil (EO) and its major constituent, terpenoid alcohol Linalool, were examined in bacterial tests. In preliminary experiments both basil derivatives showed no mutagenic effect, with or without metabolic activation, in the Salmonella/microsome assay in TA100. Antimutagenic effect against spontaneous and t-BOOH-induced mutagenesis was examined in mismatch repair (MMR) deficient and repair proficient Escherichia coli K12 strains. In wild type strain SY252 the reduction of t-BOOH-induced mutagenesis was 49% for EO and 36% for Linalool. The spontaneous mutagenesis was reduced in its MMR deficient counterpart (mutH) and inhibition ranged from 27% for EO to 44 % for Linalool. Reduction of spontaneous and t-BOOH-induced microsatellite instability was obtained in mutH and repair proficient strains (30-35% of inhibition). Inhibitory potential of EO and Linalool was also tested in E. coli WP2 strain deficient in induction of antioxidative enzymes (oxyR). The reduction of t-BOOH-induced mutagenesis was 30% for EO and 60% for Linalool. Antigenotoxic potential of Linalool was tested in Comet assay on Saccharomyces cerevisiae. Linalool exhibited protective capacity against H_2O_2-induced comets, more in pre-treatment than in co-treatment experiments. Results obtained with EO, Linalool and Vitamin E, used as a model antioxidant, indicate that antimutagenic and antigenotoxic potential of basil derivatives could be attributed to their antioxidative properties.

SELECTIVE CYTOTOXICITY OF XANTHOHUMOL FOR NORMAL AND CANCER CELLS

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Xanthohumol (XN), one of the principal prenylflavonoids of the hop plant and flavouring agent in beer, has recently been suggested as cancer chemopreventive agent. We have confirmed its protective effects on cancer initiation at low (0.01-10 μM) concentrations (1). The aims of this study were to establish the cytotoxicity of higher concentrations of XN on normal and neoplastic human cell lines and its effects on apoptosis, *in vitro* cell adhesion and invasion, that play crucial role in cancer progression. We chose two non-cancerous (HUVEC and MCF10A) and three neoplastic cell lines (MCF10AneoT, U87 and SNB-19) and exposed them to 1-50 μM XN. XN was cytotoxic for cancer cells at 15 μM and for the normal cells at 50 μM concentration. XN initiated apoptosis at 30 μM, however, after 48 h the proportion of apoptotic cells was significantly higher in neoplastic (50%), compared with normal cells (20%). At subtoxic concentrations, XN had a significant impact on adhesion of U87 cells to Matrigel and fibronectin, but it did not affect the adhesion of MCF10AneoT. At subtoxic concentrations, XN did not significantly affect invasiveness of U87 and MCF10AneoT monolayers into Matrigel and SNB-19 spheroids into collagen, but impaired the invasion at higher concentration, due to increased apoptosis. Our data disprove the suggested effects of XN on invasion (2) and confirmed the hypothesis on selective cytotoxicity of XN for cancer cells (3). If this is confirmed for all cancer cells, XN may be good candidate for prevention and treatment of cancer.

SELEKTIVNA CITOTOSKIČNOST KSANTOHUMOLA ZA NORMALNE IN RAKAVE CELICE

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Ksantohumol (XN), eden pomembnejših prenilflavonoidov hmelja in ojačevalec okusa v pivu, je bil predlagan tudi kot snov za kemopreventivo raka. Pokazali smo že na njegove učinke proti iniciaciji raka pri nizkih koncentracijah (0,01-10 μM) (1). Namen te raziskave pa je bil določanje citotoksičnosti višjih koncentracij XN na normalne in rakave celice in njegov vpliv na apoptozo, *in vitro* celično adhezijo in invazivnost, ki so ključne za napredovanje raka. Izbrali smo dve nerakavi (HUVEC in MCF10A) in tri rakave celične linije (MCF10AneoT, U87 in SNB-19) in jih izpostavili delovanju 1-50 μM XN. XN je bil citotoksičen za rakave celice pri 15 μM in za normalne celice pri 50 μM koncentraciji. XN je sprožil apoptozo pri 30 μM koncentraciji, vendar je bilo po 48 h prizadetih več rakavih (50%) celic kot normalnih (20%). Pri subtoksičnih koncentracijah, je imel XN značilen vpliv na adhezijo U87 celic na Matrigel in fibronektin, ni pa vplival na adhezijo MCF10AneoT. V subtoksičnih koncentracijah XN ni značilno spremenil invazivnosti monoslojev U87 in MCF10AneoT v Matrigel in SNB-19 sferoidov v kolagen, pač pa je znižal invazivnost pri višjih koncentracijah zaradi naraščajoče apoptoze. Naši podatki so ovrgli predlagane učinke XN na invazijo (2) in potrdili hipotezo o selektivni citotoksičnosti XN za rakave celice (3). Če to drži za vse rakave celice, je XN dober kandidat za preprečevanje in zdravljenje raka.

Reference / Viri: (1) Plazar et al., Mut.Research (submitted); (2) Vanhoecke et al., Int J Cancer, 2005,117, 889; (3) Gerhauser, Eur J Cancer, 2005, 4, 1941.

THE CYTOTOXIC AND GENOTOXIC EFFECTS OF MICROCYSTIN-LR IN DIFFERENT CELL LINES

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Microcystins (MCs) are the most common hepatotoxic cyclic heptapeptides produced by different cyanobacterial species. They are inhibitiors of protein phosphatases 1 and 2A and are tumour promoters. Increasing evidence suggests that oxidative stress plays an important role in MCLR induced hepatotoxicity. In the present study we investigated the toxic and genotoxic effects of MCLR in four different cell lines. Using the MTT assay we observed that MCLR decreases human hepatoma (HepG2) and human colon adenocarcinoma (CaCo-2) cell proliferation, while it has no effect on human B lymphoblastoid (NC-NC) and human astrocytoma (IPDDC) cell proliferation. With the single cell gel electrophoresis (Comet assay) we found that MCLR induced dose dependent transient increase of DNA damage in HepG2 and Caco-2 cells. No DNA damage was observed in NC-NC and IPDDC cells. We further explored whether the toxin increased the level of intracellular reactive oxygen species (ROS) in exposed cells. Using the fluorescent probe 2',7'-dichlorofluorescin diacetate (DCFH-DA) we detected dose and time dependent increase of ROS in HepG2 and CaCo-2 cells, but not in NC-NC and IPDDC cells. The results show, that the differences in susceptibility of different cell types towards MCLR induced toxic and genotoxic effects correlated with the differences of ROS formation between the cell types. Subsequent studies will be conducted to explore if differences in ROS formation are due to different absorbtion of MCLR by different cells and/or it is due to differences in enzymatic activities and redox status of different cell types.

CITOTOKSIČNO IN GENOTOKSIČNO DELOVANJE MIKROCISTINA-LR PRI RAZLIČNIH CELIČNIH LINIJAH

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Mikrocistini (MC) so najpogostejši hepatotoksični ciklični heptapeptidi, ki jih tvorjo različne vrste cianobakterij. So inhibitorji fosfoprotein fosfataz 1 in 2A in povzročajo napredovanje tumorjev. Vse več je dokazov, da igra oksidativen stres pomembno vlogo pri hepatotoksičnosti MCLR. Ugotavljali smo citotoksično in genotoksično delovanje MCLR pri štirih različnih celičnih linijah. S testom MTT smo ugotovili, da MCLR zmanjša proliferacijo rakavih celic človeških jeter (HepG2) in debelega črevesa (CaCo-2), medtem ko na proliferacijo človeških B limfoblastoidnih celic (NC-NC) in celic človeškega astrocitoma (IPDDC) ni vplival. S testom elektroforeze posameznih celic (test komet) smo ugotovili, da je MCLR je povzročil od koncentracije odvisno prehodno povečanje poškodb DNA pri HepG2 in CaCo-2 celicah, medtem, ko pri NC-NC in IPDDC celicah poškodb DNA nismo opazili. Nadalje smo ugotavljali, ali toksin poviša nivo znotrajceličnih reaktivnih kisikovih zvrsti (ROS) pri izpostavljenih celicah. S fluorescenčno probo 2',7'-dichlorofluorescin diacetate (DCFH-DA) smo potrdili od časa in koncentracije odvisno povišanje ROS pri HepG2 in CaCo-2 celicah, ne pa tudi pri NC-NC in IPDDC celicah. Rezultati kažejo, da je različna občutljivost celic na toksične in genotoksične učinke MCLR je povezana z razlikami v tvorbi ROS v različnih vrstah celic. Z nadalnjimi raziskavami bomo ugotovili ali so razlike v tvorbi ROS povezane z razlikami v sposobnosti absorbcije MCLR in/ali z razlikami v encimskih aktivnostih in redoks statusu različnih tipov celic.

GENOMIC TECHNOLOGIES
GENOMSKE TEHNOLOGIJE

A NEW HIGH-THROUGHPUT SNPS GENOTYPING SERVICE IS AVAILABLE IN TRIESTE

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A new high-throughput SNPs genotyping service is available in Trieste. Thanks to an agreement between CBM and IRCCS Burlo-Garofolo, we have set up a comprehensive genotyping core with the latest technologies available. The core is equipped with an ABI Taqman 7900 HT, ILLUMINA and Affymetrix platform and a liquid handler for samples processing. Thanks to this comprehensive set of technologies, the core can accomplish studies of any size. In particular, Taqman technology is extremely useful in studies with a large number of samples and few polymorphisms (usually association studies), whereas ILLUMINA and Affymetrix are indicated in studies with less samples and a huge number of SNPs (up to 550.000) like linkage studies. In conclusion, we think that the availability of our genotyping service in a neighborhood country can be an important resource to the Slovenian scientific community.

NOV CENTER ZA SNPS GENOTIPIZACIJO V TRSTU

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Zahvaljujoč sporazumu med CBM in IRCCS Burlo-Garofolo, je v Trstu ustanovljen izpopolnjen center za SNPs genotipizacijo. Center razpolaga z ABI Taqman 7900 HT, ILLUMINA in Affymetrix platformo ter z opremo za podrobno obdelavo tekočih vzorcev. Z navedeno tehnološko opremo, ki v tem trenutku velja za najsodobnejšo na tržišču, se v centru lahko opravljajo najobširnejše raziskave. Podrobneje, se tehnologijo Taqman uporablja za raziskave s številnimi vzorci z majhnim številom polimorfizmov (ponavadi, so to asociacijski študiji) medtem, ko se tehnologiji ILLUMINA in Affimetrix uporabljata za raziskave z manjšim številom vzorcev in z velikim številom SNPs (do 550.000), tako kot pri linkage analizah.Menimo, da razpoložljivost nasega centra za genotipizacijo v sosednji državi Italiji, predstavlja pomembno oporo za slovensko znanost.

EFFECT OF ELECTROGENE THERAPY WITH *P53* ALONE AND IN COMBINATION WITH ELECTROCHEMOTHERAPY USING CISPLATIN ON SURVIVAL OF HUMAN PROSTATIC CARCINOMA CELLS

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Electroporation is an established method for the delivery either of chemotherapeutic drugs cisplatin and bleomycin (electrochemotherapy) or DNA (electrogene therapy) into the cells *in vitro* and *in vivo*. p53 plays crucial role in diverse cellular pathways in response to DNA damage, one of them being apoptotic cell death. The aim of our study was to evaluate electrogene therapy with *p53* alone or combined with electrochemotherapy using cisplatin in two human prostatic carcinoma cell lines, PC-3 and DU145 with different *p53* status. To evaluate cytotoxic effect of combined treatment, cells were treated either with plasmid DNA, cisplatin or electroporation and combinations of these treatments. Status of *p53* in the cell lines was determined immunocytochemically and *p53* gene sequencing, electroporation efficiency of drug uptake by measurement of propidium iodide fluorescence, cytotoxicity of the treatments by colony forming assay and bystander effect of transfected cells by MTT assay. Morphological changes in cellular structures were evaluated on cytospins stained using Giemsa's, immunostaining and acridine orange/ethidium bromide mixture. Results of our study show that electrogene therapy with *p53* alone had synergistic cytotoxic effect regardless of the status of *p53* in prostatic carcinoma cells whereas electrogene therapy in combination with electrochemotherapy exerted antagonistic cytotoxic effect in PC-3 (*p53 null*) cell line and additive effect in DU145 (*p53 mt*). Transfected cells exerted bystander effect and in cells exposed to treatments combined with cisplatin cytological analysis showed greater proportion of apoptotic death. To conclude, our study showed that electrogene therapy with *p53* had synergistic cytotoxic effect in both prostatic carcinoma cell lines, whereas in combination with electrochemotherapy the best effect observed was additive.

VPLIV ELEKTROGENSKE TERAPIJE S *P53* V KOMBINACIJI Z ELEKTROKEMOTERAPIJO S CISPLATINOM NA PREŽIVETJE HUMANEGA RAKA PROSTATE

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Z elektroporacijo v celice in tkiva vnašamo kemoterapevtike, kot sta cisplatin in bleomicin (elektrokemoterapija) ali plazmidno DNK (elektrogenska terapija). Celice se s procesom apoptoze odzovejo tudi na poškodbe DNK pri tem pa protein p53 igra ključno vlogo. Namen raziskave je bil preučiti vpliv elektrogenske terapije s *p53* v kombinaciji z elektrokemoterapijo s cisplatinom na preživetje celic humanega raka prostate PC-3 in DU145, ki se med seboj razlikujeta po endogenem statusu p53. Da bi preučili vpliv kombiniranega zdravljenja smo celice izpostavili delovanju plazmidne DNK, cisplatinu ali elektroporaciji ter kombinacijam posameznih terapij. Status endogenega p53 smo določili z imunskim barvanjem in sekveniranjem gena *p53*, učinkovitost vnosa kemoterapevtikov z elektroporacijo z merjenjem fluorescence propidijevega jodida, citotoksičnost terapij s testom klonogenosti in učinek na sosednje celice s testom MTT. Morfološke spremembe v celicah smo opazovali na citospinih pobarvanih po Giemsi, imunocitološko in po barvanju z mešanico akridin oranža in etidijevega bromida. Dokazali smo, da je kombinacija elektroporacije in genske terapije učinkovala sinergistično na zmanjšanje preživetja celic neodvisno od statusa *p53*. Kombinacija elektrogenske terapije s *p53* in elektrokemoterapije s cisplatinom je učinkovala antagonistično na zmanjšanje preživetja celic PC-3 (p53 odsoten) in aditivno na celice DU145 (p53 mutiran). Transfecirane celice so s sproščanjem snovi vplivale na zmanjšanje preživetja sosednjih celic, pri celicah izpostavljenih terapijam s cisplatinom pa smo opazili večji delež celic, ki so umrle z apoptozo. Zaključimo lahko, da je elektrogenska terapija s *p53* sinergistično zmanjšala delež preživelih celic pri obeh vrstah celic raka prostate, največji učinek kombinacije elektrogenske terapije in elektrokemoterapije pa je bil le aditiven.

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ENCHANCED ELECTRICALLY ASSISTED PLASMID DNA DELIVERY TO LPB TUMOURS USING COLLAGENASE AND HYALURONIDASE PRE-TREATMENT OF TUMOURS

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Electrogene therapy represents a promising way for treatment of cancer but the usefulness of such therapy depends on effective delivery of genetic material to tumours. Tumour's extracellular matrix could impede effective gene delivery to tumours. Therefore, our aim was to evaluate whether pretreatment of tumours with collagenase or hyaluronidase can lead to increase in transfection efficiency of electrically-assisted gene delivery. Subcutaneously implanted LPB fibrosarcoma tumours syngenic to C57Bl/6 mice were treated with intratumoural injection of collagenase 24 h and/or hyaluronidase 2 h before electrically-assisted gene delivery. Plasmid DNA (50μg/50μl), encoding green fluorescence protein-GFP, was injected intratumorally and after 1 minute tumours were exposed to 8 square wave electric pulses (600 V/cm, 5 ms, 1 Hz). GFP expression in the tumours was measured 2, 9 and 15 days post-transfection using a stereo fluorescence microscope. Transfection efficiency was defined as the percentage of tumour area expressing GFP with regard to the total tumour area. Mean fluorescence intensity of transfected area was also determined. Treatment of tumours with both enzymes prior to electrically-assisted gene delivery lead to $\sim$ 5-fold increase in GFP expression compared to pre-treatment of tumours with either of the enzymes alone. Futhermore, pretreatment of tumours with only one enzyme did not yielded better transfection efficiency compared to electrically-assisted GFP delivery alone. Regardless of the transfection method used, the transfection in tumours lasted up to 15 days. In conclusion, modification of tumour extracellular matrix by treatment of tumours with collagenase and hyaluronidase contributes toward successful electrically-assisted GFP delivery into the tumours.

ELEKTROPORACIJA POVEČA VNOS PLAZMIDNE DNA V TUMORJE LPB PO PREDHODNEM TRETIRANJU TUMORJEV S KOLAGENAZO IN HIALURONIDAZO

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Elektrogenska terapija predstavlja obetajoč način zdravljenja raka, vendar je njena uporabnost odvisna od učinkovitosti vnosa genskega materiala v tumorje. Zunajcelični matriks tumorjev lahko vpliva na učinkovitost vnosa genov v tumorje. Zato je bil naš namen ugotoviti ali predhodno tretiranje tumorjev s kolagenazo in hialuronidazo vpliva na povečanje transfekcije v tumorjih po vnosu genskega materiala z elektroporacijo. V podkožne tumorje LPB, ki so sokrvni z mišmi C57Bl/6, smo vbrizgali kolagenazo 24 ur in hialuronidazo 2 uri pred vnosom genskega materiala z elektroporacijo. Potem smo v tumor vbrizgali plazmidno DNK (50μg/50μl), ki nosi zapis za zeleno fluorescirajoči protein-GFP (pEGFP-N1) in po 1 minuti smo tumor izpostavili 8 pravokotnim električnim sunkom (600 V/cm, 5 ms, 1 Hz). Izražanje proteina GFP v tumorjih smo določali 2., 9. in 15. dan po transfekciji s stereo fluorescentnim mikroskopom. Učinkovitost transfekcije smo izrazili kot odstotek področja tumorja, kjer se je GFP izražal, glede na celotno področje tumorja. Določili smo tudi srednjo vrednost intenzitete fluorescence v transfeciranem področju tumorja. Izražanje proteina GFP je bilo v tumorjih, ki smo jih tretirali z obema encimoma pred vnosom gena za GFP z elektroporacijo, približno 5-krat višje kot pri tumorjih tretiranih samo s posameznim encimom. Predhodno tretiranje tumorjev samo z enim encimom ni izboljšalo učinkovitosti transfekcije v primerjavi z vnosom gena za GFP z elektroporacijo. Ne glede na vrsto tretiranja tumorjev pred vnosom gena za GFP z elektroporacijo, je bila transfekcija vidna 15 dni. Zaključimo lahko, da spreminjanje zunajceličnega matriksa tumorjev s kolagenazo in hialuronidazo pripomore k uspešnejšemu vnosu gena za GFP v tumor z elektroporacijo.

MECHANISMS OF ERYTHROMYCIN AND CIPROFLOXACIN RESISTANCE IN *CAMPYLOBACTER JEJUNI* AND *C. COLI* FROM DIFFERENT SOURCES

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The incidence of human *Campylobacter* infections is increasing in many developed countries, as well as the proportion of isolates resistant to fluoroquinolones and/or macrolides, the drugs of choice to treat campylobacteriosis. The main source of human infection is food, particularly poultry meat. In Slovenia, the data about the prevalence and antibiotic resistance of *Campylobacter* from food are not collected routinely, but we confirmed previously frequent contamination of Slovenian poultry meat and antibiotic (multi)resistance of *Campylobacter* isolates (1, 2). Quinolones and macrolides resistance of campylobacters are mostly due to target mutations in the GyrA subunit of the gyrase and in the 23S rRNA gene, respectively. In addition, campylobacters possess CmeABC and CmeDEF efflux systems that may act in synergy with target mutations or *per se* to confer antimicrobial resistance. Additional problem is overexpression of efflux system(s), possibly confering a multidrug resistence pattern of *Campylobacter strains*. We studied 35 *Campylobacter jejuni* and *C. coli* food, animal and human isolates with determined MICs to erythromycin and ciprofloxacin with standardized microdilution method, in combination with efflux pump inhibitor, Phe-Arg-ß-naphthylamide (PaßN) to get a broader view in the mechanisms of strain resistance. Mutations in target genes were confirmed with PCR-RFLP, MAMA-PCR and partly by sequencing procedures. High heterogeneity was found among strains and will be presented for illustration of possible mechanisms involved in antibiotic resistance of *C. jejuni* and *C. coli*.

MEHANIZMI ODPORNOSTI BAKTERIJ *CAMPYLOBACTER JEJUNI* IN *C. COLI* IZ RAZLIČNIH VIROV PROTI ERITROMICINU IN CIPROFLOKSACINU

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Incidenca humanih kampilobakterioz narašča v mnogih razvitih deželah, prav tako pa tudi odpornost kampilobakterjev proti fluorokinolonom in/ali makrolidom, ki se uporabljajo za zdravljenje kampilobakterioz. Glavni vir okužb je hrana, predvsem perutninsko meso. V Sloveniji nimamo dolgoletnih podatkov o prevalenci in odpornosti bakterij *Campylobacter* iz živil, a smo odkrili velik delež kontaminiranih vzorcev piščančjega mesa na trgu in odpornost izolatov proti antibiotikom (1,2). Vzrok odpornosti proti kinolonom in makrolidom so točkaste mutacije v genih GyrA podenote giraze in 23S rRNA, poleg tega pa k odpornosti prispevajo membranske izlivne črpalke – te so možen razlog večkratne odpornosti sevov na različna protimikrobna sredstva. Preiskovali smo 35 živilskih, živalskih in humanih kliničnih izolatov bakterij *C. jejuni* in *C. coli* z mikrodilucijsko metodo in v kombinaciji z inhibitorjem efluksne črpalke, fenil-alanin-ß-napftilamidom (PaßN). Mutacije smo potrdili z metodami PCR-RFLP, MAMA- PCR in deloma sekvenciranjem pomnožkov. Odkrili smo veliko heterogenost sevov, na osnovi katere bomo ilustrirali možne mehanizme odpornosti bakterij *Campylobacter* na izbrana antibiotika.

Preferences / Viri:
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DNA MICROARRAY TECHNOLOGY: APPLICATION TO ECOTOXICOLOGY

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Molecular diagnostic technologies play a significant role in the practice of medicine, public health, pharmaceutical industry and more recently in the environmental field. DNA Microarray is one of these significant technologies which has progressed rapidly in the hands of biological researchers for assessing gene expression analysis. Microarrays are providing insights into areas such as toxicology, pharmacology and tumorigenesis. The application to Ecotoxicology is still at an early stage but already many applications have been published. Molecular biomarkers offer the possibilities, of early detection of environmental stressor, inferred mechanisms of action and to improve the monitoring of environmental stressors. We are currently performing DNA Microarray analysis to identify new biomarkers for the detection of chemical stressors in environment. The budding yeast *Saccharomyces cerevisiae* is one of the organism used as model since the genome has been sequenced and mutants are characterized as well as metabolic pathways and more recently the protein interactions. The presentation will be an overview of the technology and will show the gene expression profile of S. cerevisiae exposed to two class of chemicals which can affect human and environment health.

Reference: Teresa Lettieri. "*Recent applications of DNA Microarray technology to Toxicology and Ecotoxicology*" Environ Health Perspect 114:4-9 (2006).

FINE GENETIC MAPPING OF QTLS ON MOUSE CHROMOSOME 15 IN THE POLYGENIC MODEL OF OBESITY USING BIOINFORMATICS TOOLS

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Mouse models are a powerful tool for understanding common complex diseases, including obesity. Polygenic forms of obesity present a growing health problem in the developed world and also an unwanted component of growth in farm animals. Quantitative trait locus (QTL) analysis has proven to be an efficient method for mapping obesity QTL in mice, which can, in turn, predict the locations of human obesity genes. Our previous studies identified major QTL effects on several mouse chromosomes (Chr) in an F_2 cross between the high fat (F) and low fat (L) selected strains and the QTL region on mouse Chr 15 was further mapped into two separate QTLs. The aim of the present study was to find candidate genes and narrow down the genetic intervals containing the two mouse obesity QTL regions on Chr 15. We combined a number of recently developed bioinformatics tools including haplotype analysis, comparative genomics and *in silico* mapping. We have mouse obesity QTL positions from other studies and publicly available SNPs, including SNPs for F and L-lines, to perform the haplotype analysis. Comparative genomics of human obesity QTL syntenic to mouse Chr 15 QTL further narrowed the target regions. In addition, using *in silico* fat-pad mapping including a number of different inbred mouse strains identified associations between small SNP haplotype blocks and fat pad weights. Application of these new bioinformatics strategies should facilitate and accelerate identification of obesity causing genes in the F and L lines in the future studies.

NATANČNEJŠE GENETSKO KARTIRANJE KVANTITATIVNIH LOKUSOV NA KROMOSOMU 15 PRI POLIGENEM MIŠJEM MODELU DEBELOSTI Z UPORABO BIOINFORMACIJSKIH METOD

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Mišji modeli predstavljajo učinkovito orodje za raziskave pogostih kompleksnih bolezni kot je na primer debelost. Poligene oblike debelosti predstavljajo vedno večji zdravstveni problem v razvitem svetu, poleg tega pa je nalaganje maščevja nezaželjena komponenta rasti pri domačih živalih. Z analizo kvantitativnih lokusov (*quantitative trait locus,* QTL) lahko uspešno kartiramo kvantitativne lokuse, ki vplivajo na nalaganje maščevja pri miših in na osnovi rezultatov predvidimo mesta homolognih genov pri človeku. Predhodne raziskave na F_2 populaciji linij F (*fat,* debel) in L (*lean,* suh) so v mišjem genomu potrdile številne kvantitativne lokuse. Področje na kromosomu 15 so kasneje natančneje kartirali in odkrili dva ločena kvantitativna lokusa. Cilj naše raziskave je bil poiskati kandidatne gene in zožiti genetska intervala omenjenih kvantitativnih lokusov na kromosomu 15 pri miših. V raziskavi smo uporabili kombinacijo novejših bioinformacijskih metod, med drugim analizo haplotipov, primerjalno genomiko ter *in silico* kartiranje. Za analizo haplotipov smo uporabili lokacije kvantitativnih lokusov za debelost iz predhodnih študij in javno dostopne SNP (*single nucleotide polymorphism*) podatkovne baze, ki vsebujejo tudi SNP linij F in L. S primerjalno genomiko smo področja kromosomov, ki vsebujejo kvantitativne lokuse, primerjali s homolognimi področji v človeškem genomu in s tem še dodatno zmanjšali tarčne intervale. Z *in silico* kartiranjem smo raziskali možne povezave manjših SNP blokov z masami različnih maščobnih depojev pri številnih inbridiranih linijah miši. Pričakujemo, da bo uporaba omenjenih novih bioinformacijskih metod olajšala in pospešila identifikacijo genov, ki vplivajo na nalaganje maščevja pri linijah F in L.

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SUCCESSFUL DNA ELECTROTRANSFER INTO MURINE SKELETAL MUSCLE

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Electrically assisted gene delivery to skeletal muscles is an attractive approach in two therapeutic applications: gene therapy and DNA vaccination. Prolonged expression and secretion from skeletal muscle is crucial for systemic distribution of therapeutic proteins. The aim of this study was to determine the optimal treatment protocol for electrically-assisted delivery of plasmid DNA into murine skeletal muscle for long term expression of proteins. To determine optimal treatment parameters for successful transfection of murine skeletal muscle, evaluation of different sets of electrical parameters, time interval between plasmid DNA injection and application of electric pulses as well as different plasmid DNA concentration were tested in tibialis cranials muscle of C57Bl/6 mice using DNA plasmid encoding green fluorescent protein (GFP). Different sets of combinations of high voltage (HV: 600 V/cm, 100 μs) and low voltage (LV: 20-80 V/cm, 20-40 ms) pulses were used for electroporation. Transfection efficiency was followed by *in vivo* imaging system using fluorescence stereo microscope. In addition, transfection efficiency was assessed on 24 frozen tissue sections/muscle 1 week after the electrically-assisted gene delivery using fluorescence microscope. The pictures were analyzed using the Image J software tool. Fraction of transfected area and mean fluorescence intensity for this area were determined. The study showed that electrically assisted gene delivery is a successful method for plasmid DNA transfer into skeletal muscle. The optimal treatment parameters for electrically assisted gene delivery into skeletal muscle of C57Bl/6 mice in our experiments were 1HV (600 V/cm, 100 μs) and 4 LV (80 V/cm, 100 ms), time interval 5 s and plasmid DNA concentration of 30 μg/muscle. We achieved long term expression of GFP, since fluorescence was still present 31 weeks after transfection. These results are the basis for evaluation of therapeutic protein efficiency in gene therapy and DNA vaccination.

USPEŠNA *IN VIVO* TRANSFEKCIJA GENOV Z ELEKTROPORACIJO V MIŠJO SKELETNO MIŠICO

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Vnos genov v skeletne mišice z elektroporacijo je učinkovita metoda za gensko zdravljenje in DNK cepiva. Skeletne mišice so najprimernejše tkivo za dolgotrajno izražanje in sistemsko distribucijo terapevtskih proteinov. Namen raziskave je bil določiti najbolj optimalne pogoje za uspešno in dolgotrajno transfekcijo v mišjo skeletno mišico z elektroporacijo. Določali smo 3 parametre, ki vplivajo na uspešnost transfekcije z elektroporacijo: vrsto električnih pulzov (visokonapetostni pulz = HV (600 V/cm, 100 μs) in nizkonapetostni pulzi LV (20-80 V/cm, 20-40 ms) in njune kombinacije), časovni interval med injiciranjem plazmidne DNK in elektroporacijo, ter količino injicirane plazmidne DNK. Za določitev uspešnosti transfekcije smo opazovali izražanje reporterskega gena za zeleni fluorescirajoči protein (GFP), kodiranega na plazmidu pEGFP-N1, ki smo ga injicirali v mišico *tibialis cranialis* pri miškah C57Bl/6 pred elektroporacijo. Izražanje proteina smo opazovali z neinvazivno metodo s fluorescentno stereolupo, hkrati pa tudi z določanjem fluorescence na zmrzlih rezih iz odvzetega tkiva mišice. Zajete slike smo analizirali s programom Image J in določili delež območja s transfekcijo ter povprečno intenziteto fluorescence v tem območju. Poskusi so potrdil, da je elektroporacija učinkovita metoda za vnos genov v mišjo skeletno mišico. Transfekcija je bila najuspešnejša pri uporabi električnih pulzov : 1 HV(600 V/cm, 100 μs) in 4 LV (80 V/cm, 100 ms), časovnem intervalu med injiciranjem plazmidne DNK in elektroporacijo 5 s, ter koncentraciji plazmidne DNK 30 μg/mišico. Izražanje proteina GFP v mišji skeletni mišici je bilo dolgotrajno, saj je bila po 31 tednih fluorescenca še vedno prisotna. Ti rezultati so osnova za nadaljnje določanje učinkovitosti terapevtskih proteinov pri genskem zdravljenju in DNK cepivih.

BIOINFORMATICS
BIOINFORMATIKA

ESTIMATION OF GENETIC PARAMETERS FOR TEST-DAY MILK RECORDS OF THE FIRST LACTATION CHURRA GALEGA BRAGANÇANA EWES USING RANDOM REGRESSION ANIMAL MODEL

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The objective of the study was to compare different models in the estimation of genetic parameters for test-day milk records in Churra Galega Bragançana ewes. Data comprising 10700 test-day measurements from the first lactation of 3096 ewes were used in analyses of morning milk yield (MMY), afternoon milk yield (AMY), and daily milk yield (DMY). Records before 30 and after 150 days in milk were discharged. Average milk yield was 217.1 g, 198.3 g, and 415.4 g for MMY, AMY, and DMY, respectively. Pedigree file contained 5494 animals. Simple fixed regression animal model (FRM) was compared to random regression animal models (RRM), where orthogonal Legendre polynomials of order 1 were used. The REML method as implemented in the VCE-5 programme was applied for estimation of (co)variance components. Statistical models contained linear regression on days in milk and number of lambs born as fixed effects, while flock-test-day, permanent environment of a ewe, and direct additive genetic effect were treated as random. Estimates of heritability from FRM were low, from 2.9% for AMY to 8.2% for MMY. Heritability estimates from RRM for MMY, AMY, and DMY decreased from 42.4%, 22.3%, and 32.5% at 30 days to close to zero at the end of lactation (150 days). There is a potential for using random regression to model additive genetic and permanent environmental effects for genetic evaluation in Churra Galega Bragançana ewes, especially from the first two thirds of lactation when decision on mating has to be taken.

OCENA GENETSKIH PARAMETROV ZA MERITVE KOLIČINE MLEKA NA KONTROLNI DAN IZ PRVE LAKTACIJE OVC PASME CHURRA GALEGA BRAGANÇANA Z MODELOM ŽIVALI Z NAKLJUČNO REGRESIJO

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Cilj raziskave je bila primerjava različnih modelov pri oceni genetskih parametrov za količino mleka na kontrolni dan ovc pasme Churra Galega Bragançana. Podatke, ki so obsegali 10700 meritev na kontrolni dan od 3096 ovc iz prve laktacije, smo uporabili v analizi jutranje (MMY), popoldanske (AMY) in dnevne količine namolženega mleka (DMY). Zapise pred 30. in po 150. dnevu laktacije smo izločili. Povprečna količina mleka je bila za MMY 217,1 g, za AMY 198,3 g in za DMY 415,4 g. Podatki o poreklu so zajemali 5494 živali. Enostavni model živali s sistematsko regresijo (FRM) smo primerjali z modelom živali z vključeno naključno regresijo (RRM), pri čemer smo uporabili ortogonalne Legendrove polinome reda 1. Pri ocenjevanju komponent (ko)variance smo se poslužili metode REML, kot je vgrajena v programu VCE-5. Statistični modeli so vsebovali linearno regresijo za stadij laktacije in število rojenih jagnjet kot sistematska vpliva, medtem ko so bili trop-kontrolni-dan, permanentno okolje ovce in direktni aditivni genetski vpliv obravnavani kot naključni vplivi. Ocene heritabilitete po FRM so bile nizke, od 2,9 % za AMY do 8,2 % za MMY. Heritabilitete ocenjene z RRM so se od 30. dneva laktacije proti koncu laktacije (150. dan) zmanjševale od 42,4 % (MMY), 22,3 % (AMY) oz. 32,5 % (DMY) proti nič. Uporaba naključne regresije za modeliranje permanentnega okolja in aditivnega genetskega vpliva nakazuje prednost pri genetskem vrednotenju ovc Churra Galega Bragançana, posebno v prvih dveh tretjinah laktacije, ko se sprejema odločitev o nadaljnjih pripustih.

HERD MANAGEMENT AND INFORMATION SYSTEM

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The main objective was to develop software for herd management within the information system. Database structure was derived on information retrieved from various pig, horse, and rabbit production systems. A system analysis was performed in four steps: information analysis, normalization of database structure, and software development for data collection, browsing, and data analyses. To avoid mistakes and to speed up the information flow, direct data collection on input forms was introduced on family farms. Three types of browsers were developed in order to retrieve information used for monitoring herd production, evaluating efficiency of certain groups of animals or to summarize individual performance. Herd activities include monitoring of herd structure, planning of various activities, supporting decision making, etc. Group and individual control can be used to detect problems or to support decisions on group or animal level, respectively. Group activities were adjusted to treatment of animals classified by production phase, origin, age, time period, etc. They were meant to support regular activities or to detect certain problems like delayed events, prolonged production phase, small litter size, etc. By defining time period and some other variables like genotype, origin, location, parity, etc., the user can narrow down the group. Individual performance can be evaluated to check the data consistency and to regulate replacement of breeding stock. Suggestions are done on the basis of herd statistics or standards based on population parameters at any critical point. Data exchange was built in to submit data to or receive them from partners or from the computing center.

INFORMACIJSKI SISTEM PRI URAVNAVANJU ČREDE

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Namen raziskave je, znotraj informacijskega sistema, razviti programsko opremo za uravnavanje reje. Strukturo podatkovne zbirke smo vzpostavili na podlagi informacij, ki jih zbirajo rejci prašičev, konjev in kuncev v različnih proizvodnih sistemih. Uporabili smo metodologijo sistemske analize, ki je sestavljena iz štirih večjih sklopov: informacijske analize, normalizacije podatkovne zbirke, zajema podatkov (vnos in prenos) in izdelave orodij za pregled podatkov. Vnos podatkov preko vnosnih oken poteka pri rejcu, s čimer se zmanjša pogostost napak in pospeši tok podatkov. Razvili smo pregledovalnike in analize, s katerimi rejec pridobi informacije potrebne za uravnavanje reje. Lahko jih razdelimo na tri tipe: spremljanje proizvodnosti črede, ocenjevanje učinkovitosti posameznih skupin živali in spremljanje individualne proizvodnosti. Spremljanje proizvodnosti črede vključuje uravnavanje starostne ter pasemske strukture črede, načrtovanje prireje (obnova, pripusti, kotitve …), kontrolo in reševanje problemov v reji ipd. S spremljanjem določenih skupin ali posameznih živali lahko rejec zazna probleme v reji, kot so zapozneli izidi dogodkov, podaljšana obdobja, majhna velikost gnezda, velik delež pregonitev ipd., ali pa dobi dodatno podporo pri sprejemu svojih odločitev. Na pregledovalnikih in analizah je omogočen izbor časovnega obdobja in ostalih faktorjev kot so genotip, rejec, lokacija, zaporedna kotitev, ipd., s čimer lahko uporabnik omeji izbor opazovanih živali. Spremljanje individualne proizvodnosti je namenjeno preverjanju točnosti podatkov in uravnavanju remonta. Predlogi temelijijo na podlagi statistike črede ali standardov, ki so posledica parametrov populacije ob posameznih kritičnih točkah. Izdelali smo aplikacije, ki omogočajo izmenjavo podatkov med rejcem in računalniškim centrom.

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GENETIC PARAMETERS FOR BODY WEIGHT IN DIVERGENTLY SELECTED LINES OF CHICKENS

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The objective of this study was presenting the response of selection over 31 generations for high (D+ line) and low (D- line) body weight at 56th day of age. Selection experiment started in 1979 from a commercial heavy sire line of the Slovenian provenance Prelux-bro. Genetic parameters were compared between sexes and lines. Statistical model included fixed effects with classes: generation, sex, interaction between generation and sex, and the hatching group nested within generation as random effect. Body weight of each generation was shown as least square mean. Selection response was calculated as difference of least square means between D- and D+ lines. Realized heritability was defined as ratio of the single-generation progress of selection to the selection differential of the parents. Body weight was changing along generations similar in males and females. In females, selection differential (SD) of D+ line was linearly decreasing along generations -2.28 ± 0.78 g per generation, whereas in males SD was not changing. In D- line, the quadratic regression was used. Males had larger SD in both lines, but in line D- the difference between sexes was decreasing along generations. In 31st generation the selection response amounted 2011 g. Realized heritability for body weight was 0.090 ± 0.011 in line D+, 0.161 ± 0.012 in line D- for males, and 0.194 ± 0.022 in line D+, and 0.370 ± 0.026 in line D- for females.

GENETSKI PARAMETRI ZA TELESNO MASO PRI DVOSMERNO SELEKCIONIRANIH LINIJAH KOKOŠI

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Namen prispevka je prikaz spreminjanja učinka selekcije skozi 31 generacij piščancev, selekcioniranih na večjo (linija D+) in manjšo (linija D-) telesno maso pri 56. dnevu starosti. Selekcijski poizkus smo pričeli leta 1979 na očetovski liniji (D) prelux-bro. Genetske parametre smo primerjali med spoloma in linijama. V statistični model smo vključili sistematske vplive z nivoji: generacijo, spol živali, interakcijo med generacijo in spolom ter vpliv skupine valjenja, ugnezdene znotraj pripadajoče generacije kot naključni vpliv. Telesno maso piščancev v določeni generaciji smo prikazali kot ocenjene srednje vrednosti po metodi najmanjših kvadratov. Učinek dvosmerne selekcije smo prikazali kot razliko med ocenjenimi srednjimi vrednostmi telesnih mas D+ linije ter D- linije. Realizirano heritabiliteto smo izračunali kot regresijo srednjih vrednosti, ocenjenih po metodi najmanjših kvadratov, na selekcijski diferencial. Trend spreminjanja telesne mase z generacijami je bil pri petelinčkih in jarkicah podoben. Selekcijski diferencial (SD) linije D+ je z generacijami linearno padal, pri jarkicah -2.28 ± 0.78 g/generacijo, pri petelinčkih pa se SD ni značilno spreminjal. Pri liniji D- je bil uspešnejši model s kvadratno regresijo. Pri obeh linijah je bil SD večji pri petelinčkih, vendar se je pri D- liniji razlika med spoloma z generacijami zmanjševala. Selekcijski učinek je v 31. generacijah znašal 2011 g. Pri petelinčkih linije D+ je realizirana heritabiliteta za telesno maso 0.090 ± 0.011, za D- linijo 0.161 ± 0.012, za jarkice v D+ liniji 0.194 ± 0.022 in 0.370 ± 0.026 za D-.

THE R GENETICS PROJECT: BIOCONDUCTOR FOR GENETICS

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The R Genetics Project is an open-source collaborative effort to develop a complete set of tools for storing, accessing, manipulating, and analysing genetic data, from small scale candidate gene studies consisting of a few genetic markers to large scale whole genome studies containing hundreds of thousands of markers. The project is being developed upon R, a language and environment for statistical computing and graphics. The initial goal is to provide a foundation of efficient data structures and manipulation functions that are easy to use. We intend this foundation to allow developers of methods to quickly and easily develop packages implementing their own techniques, while maintaining interoperability. This will reduce the burden on both applied data analysis and developers, as one must currently move data between numerous program packages and data formats outside as well as inside R. The core R Genetics packages have reached sufficient maturity for introduction to the community. This talk will describe the R Genetics project, and provide an outline of the data structures, features and methods for pedigree data within package GeneticsPed.

PROJEKT R GENETICS: BIOCONDUCTOR ZA GENETIKO

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R Genetics je odprtokodni projekt z namenom razvoja celotnega seta orodij za shranjevanje, dostop, ravnanje in analizo genetskih podatkov. Podpora zajema tako majhne sete podatkov študij kandidatnih genov z le nekaj markerji kakor tudi velike sete podatkov genomskih študij z več sto tisoč markerji. Projekt temelji na R-ju, programskem jeziku in okolju za analizo podatkov in grafiko. Namen projekta je najprej razviti osnovno strukturo za učinkovito shranjevanje in enostavno ravnanje s podatki. Ta osnova bo omogočila razvijalcem programov hitrejši in enostavnejši razvoj paketov z njihovimi metodami hkrati pa ohranila zmožnost enostavnega prenosa podatkov. Pričakujemo, da bo to olajšalo ravnanje in analizo genetskih podatkov. Sedaj je namreč tako izven kot tudi v R-ju potrebno prenašati podatke med številnimi programi in jih pretvarjati v različne formate. Osnovni paketi v okviru projekta R Genetics so dosegli zadostno stopnjo razvoja za predstavitev skupnosti. V okviru predstavitve bomo predstavili projekt R Genetics, splošen pregled podatkovnih struktur, značilnosti in metod za porekla v paketu GeneticsPed.

GENOTYPE BY ENVIRONMENT INTERACTION FOR YIELD TRAITS IN SLOVENIAN HOLSTEIN CATTLE USING REACTION NORMS

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The objective of the study was to evaluate genotype by environment interaction (GxEI) for yield traits in Slovenian Holstein cattle using reaction norm. In this model the phenotypic expression of genotype in different environments is described as a function of environment. The regression of genotype performance in each environment on environmental gradient is usually used. In the study, a linear random regression model with heterogeneous environmental variances and regression of phenotypic observations of daughters within sire on herd environment was used. A total of 85515 first to third lactation records of 49947 cows were in the data. Only records of cows offspring of sires with at least 50 observation of daughters were included. The phenotypic measures were 305 days milk, fat and protein yield. The environmental value was the herd-year average of each trait. Estimated correlation between level and slope of reaction norms were 0.65, 0.72 and 0.67 for milk, protein and fat yield, respectively. Residual variances increased approximately linearly with increasing value of the herd environment. The heritabilities as a function of the production environment ranged from 0.20 to 0.25 with similar shape for all yield traits. The lowest heritability was found in the environments one standard deviation below the average of environmental variables analyzed. Distinctive crossing of sire's reaction norms for protein and fat production occurred, indicating GxEI in population analyzed. That is agreement with estimated rank correlations between predicted offspring performances in average and deviating environments (higher than 0.89). For milk yield, crossing of reaction norms was less distinctive.

VREDNOTENJE INTERAKCIJE GENOTIP OKOLJE ZA LASTNOSTI MLEČNOSTI PRI ČRNO-BELI PASMI Z UPORABO REAKCIJSKE NORME

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Namen raziskave je bil izvrednotiti interakcijo genotip okolje (IGxE) za lastnosti mlečnosti pri slovenski črno-beli pasmi z uporabo reakcijske norme. Reakcijska norma prikazuje fenotipsko ekspresijo genotipa v različnih okoljih kot funkcijo okolja. Pogosto se uporablja kot regresija lastnosti genotipa v različnih okoljih na okoljski gradient. V raziskavi je bil uporabljen linearni model z naključno regresijo in heterogenimi variancami za ostanek ter regresijo opazovanj hčera znotraj očetov na okolje v čredi. Vključene so bile prva do tretja laktacija 49947 krav (85515 laktacij), potomk 179 očetov. Po očetu je bilo vključenih najmanj 50 opazovanj. Preučevane lastnosti so prireja mleka, beljakovin in maščobe v 305 dneh laktacije. Kot spremenljivka okolja je bilo vključeno povprečje čredaleto za posamezno lastnost. Dobljene ocene genetskih korelacij med nivojem in nagibom reakcijske norme so 0,65 za prirejo mleka, 0,72 za beljakovine in 0,67 za mleko. Okoliška varianca narašča skoraj linearno z boljšim okoljem. Heritabiliteta, kot funkcija okolja, se giblje od 0,20 do 0,25 s podobno obliko pri vseh treh lastnostih prireje. Heritabiliteta je najnižja v okoljih, ki so za eno standardno deviacijo nižje od povprečja analizirane spremenljivke okolja. Prihaja do izrazitega križanja reakcijskih norm za beljakovine in maščobo, kar nakazuje prisotnost IGxE v preučevani populaciji. To je tudi v skladu z dobljenimi rangi korelacije med napovedmi lastnosti potomcev v povprečnem in odstopajočih okoljih (0,89 in več). Križanje reakcijskih norm za mleko je manj izrazito.

STRUCTURAL MOTIFS OF DISEASE RESISTANCE GENE ANALOGS FROM HOP *HUMULUS LUPULUS* L.

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The majority of isolated plant resistance genes code for proteins of NBS-LRR class that provide resistance to viruses, funghi, bacteria and nematodes. The sequence analysis of cloned *R* genes from a broad range of plant species revealed common structural motifs to which *R* genes are divided to two different subclasses, TIR-NBS-LRR and CC-NBS-LRR. In order to discover common aminoacid motifs of 86 resistance gene analog sequences (RGAs) from hop cv. Wye Target and wild hop accession 2/1 with *R* genes and RGA sequences from other plant species, MEME analysis was carried out (Bailey and Gribskov, 1998). According to patterns of 15 different motifs, RGA hop sequences can be assigned to 9 distinct groups. Five motifs are characteristic for CC-NBS-LRR and four for TIR-NBS-LRR gene class in different plant species, while three motifs were detected only in hop. Some sequences are differing in single gene segments where a simple duplication and subsequent divergence may be involved, while at sequences with more diverged motifs other types of rearrangement may also have occured such as recombination or unequal crossing-over. Sequence analysis of hop RGAs suggested insertions/deletions and point mutations. The motif search analysis confirmed high degree of motif conservation among hop RGAs, *R* genes and RGAs from different plant species, with the highest sequence and structural similarity of RGA hop sequences with *R* genes of *Solanaceae* family members.

STRUKTURNI MOTIVI ANALOGOV GENOV ZA ODPORNOST PROTI ŠKODLJIVIM ORGANIZMOM PRI HMELJU *HUMULUS LUPULUS L.*

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Večina kloniranih genov za odpornost proti škodljivim organizmom (*R* genov) kodira proteine, ki spadajo v NBS-LRR razred. Analize zaporedij kloniranih *R* genov odkrivajo številne skupne strukturne motive, na podlagi česar jih delimo na dva manjša razreda, v CC-NBS-LRR in TIR-NBS-LRR razred. Z namenom odkrivanja motivov pri 86 analognih sekvencah R genov hmelja (RGA) cv. Wye Target in divje akcesije 2/1 z R geni in RGA sekvencami pri drugih rastlinskih vrstah, smo izvedli analizo MEME (Bailey and Gribskov, 1998). Na podlagi vzorcev 15 različnih motivov lahko RGA zaporedja hmelja razdelimo v 9 skupin. Pet motivov je značilnih za CC-NBS-LRR in štirje za TIR-NBS-LRR razred genov pri različnih rastlinskih vrstah, tri strukturne motive pa smo detektirali le pri hmelju. Nekatera zaporedja se razlikujejo le v enem genskem segmentu kot posledica enostavnih duplikacij in posledične diverzifikacije, zaporedja z večjim številom različnih motivov pa so verjetno nastala z drugimi načini preureditve, z rekombinacijo in/ali neenakim prekrižanjem homolognih kromosomov. Analize RGA zaporedij hmelja kažejo na insercije/delecije in točkovne mutacije. Z analizo iskanja motivov smo potrdili visoko stopnjo ohranjenosti motivov med RGA hmelja z *R* geni ali RGA zaporedji pri drugih rastlinskih vrstah, z največjo podobnostjo zaporedij in strukturnih motivov z *R* geni predstavnikov družine *Solanaceae*.

GENETIC PARAMETERS FOR GROWTH OF CHAROLAIS CALVES

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Genetic parameters for birth weight (BW), weight at the beginning (WB), in the middle (WM), at the end of grazing season (WE), and weight at the age of one year (WY) were analysed. Data were collected on 319 Charolais calves (171 bulls and 148 heifers) reared on Educational and Research Animal Husbandry Centre Logatec (Slovenia). Calves were born in years 1995 – 2005. Pedigree data included parents and grandparents, together 377 animals. Variance and covariance components were estimated by REML method in the VCE-5 package. The effects of sex, parity and year of birth were included in models for all traits. Age of calves at the beginning of grazing season was included as linear regression in models for all traits except for the birth weight. Age of calves in the middle, at the end of grazing season, and age at one year were included as linear regression in the models for corresponding weights. Direct additive genetic effect was included in models for all traits as random effect. Estimated heritabilities for BW, WB, WM, WE and WY were 0.62, 0.23, 0.35, 0.29 and 0.23, respectively. Genetic correlations between BW and WB and WM were 0.43, between BW and WE it was 0.65 and between BW and WY 0.64.

GENETSKI PARAMETRI ZA TELESNO MASO PRI TELETIH ŠAROLE PASME

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Analizirani so bili genetski parametri za rojstno maso (RM), telesno maso na začetku (MZ), sredini (MS) in na koncu paše (MK) ter ob starosti enega leta (ML). Podatki so bili zbrani na 319 teletih šarole pasme (171 bikov in 148 telic), rejenih na Pedagoško raziskovalnem centru za živinorejo v Logatcu. Teleta so bila rojena v letih od 1995 do 2005. Podatki o poreklu so vključevali starše in stare starše, skupaj 377 živali. Komponente varianc in kovarianc so bile ocenjene z metodo REML v VCE-5 paketu. Vpliv spola, zaporedne telitve in leta rojstva so bili vključeni v modele za vse proučevane lastnosti. Starost telet na začetku paše je bila kot linearna regresija vključena v modele za vse lastnosti razen v model za rojstno maso. Starost telet na sredini in na koncu paše ter ob enem letu pa je bila kot linearna regresija vključena v modele za pripadajoče telesne mase. Direktni aditivni genetski vpliv je bil vključen v modele za vse lastnosti kot naključni vpliv. Ocenjene heritabilitete za RM, MZ, MS, MK in ML so bile 0,62, 0,23, 0,35, 0,29 in 0,23. Genetske korelacije med RM in MZ ter MS so bile 0,43, med RM in MK 0,65 in med RM in ML 0,64.

ESTIMATION OF GENETIC PARAMETERS FOR MILK TRAITS IN CATTLE USING TEST DAY RECORDS IN CROATIA

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The objective of this paper was estimation of genetic parameters for daily milk yield, fat and protein content from test day records for Simmental and Holstein-Friesian cattle in Croatia. Data consisted of 333189 daily records of 26095 cows born between 1992 and 2002 taken from the central database of the Croatian Livestock Centre. There were a total of 58655 animals included into pedigree. The average daily milk yield was 16.2kg with standard deviation of 5.9kg and the average contents were 4.11% (SD 0.73%) for fat and 3.49% (SD 0.42%) for protein. Editing of data was performed with a program written in SAS environment. Variance components were estimated by REML as implemented in the VCE-5 program package. Statistical model was determined with the following fixed effects with classes: breed, parity, region and calving season. Days in milk (DIM) was fitted in the model using Ali-Schaeffer function for lactation curve. Direct additive genetic effect and permanent environment were included in the model as random effects. The estimated heritabilities for daily milk yield, fat and protein component were 0.27 $\pm$ 0.003, 0.20 $\pm$ 0.002 and 0.18 $\pm$ 0.003, respectively.

VREDNOTENJE GENETSKIH PARAMETROV ZA LASTNOSTI MLEČNOSTI NA KONTROLNI DAN PRI KRAVAH NA HRVAŠKEM

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Namen tega prispevka je vrednotenje genetskih parametrov za dnevno količino mleka, vsebnost maščobe in beljakovin na kontrolni dan pri lisasti in črno-beli pasmi goveda na Hrvaškem. V analizo smo vključili podatke 333189 dnevnih meritev pri 26095 kravah, rojenih med letoma 1992 in 2002 iz centralne baze Hrvaškega stočarskega centra. V poreklo je bilo vključeni 58655 živali. Povprečna količina mleka na kontrolni dan je bila 16,2kg standardnim odklonom 5,9kg, povprečna vsebnost je znašala 4,11% (SD 0,73 %) za maščobo in 3,49% (SD 0,42 %) za beljakovine. Preverili smo kakovost podatkov v statističnem programu SAS. Komponente varianc so bile ocenjene z metodo REML v VCE-5 programskem paketu. V statistični model smo vključili sistematske vplive z nivoji: pasmo, zaporedno laktacijo, regijo in sezono telitve. Dan laktacije je opisan z Ali-Schaefferjevo funkcijo za laktacijsko krivuljo. Direktni aditivni genetski vpliv in permanentno okolje smo vključili v model kot naključna vpliva. Ocenjene heritabilitete za dnevno količino mleka, vsebnost maščobe in beljakovin so bile 0,27 $\pm$ 0,003, 0,20 $\pm$ 0,002 and 0,18 $\pm$ 0,003.

BIOTECHNOLOGY
BIOTEHNOLOGIJA

REGULATION OF BOVINE κ-CASEIN GENE EXPRESSION

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Molecular basis of the regulation of casein gene expression is of great interest for the advancement of milk production. Identification of crucial regulatory regions governing casein gene expression would provide valuable information for marker assisted selection in dairy cattle. The interest of our work was predominantly regulation of bovine κ-casein gene expression. At first, we performed series of cell transfection studies on different cell lines (BME, HC11, CaCO2, COS7) by employing pGL3 luciferase reporter vector constructs, containing different lengths of κ-casein gene promoter. Expression of κ-casein promoter constructs was compared to expression of bovine β-casein gene promoter-containing pGL3 vector construct. It was observed that the most common κ-casein variants, κ-casein A and κ-casein B, are synthesized differentially in the lactating mammary gland of heterozygous animals (κ-casein AB). Differences were observed on mRNA and also protein levels. Numerous allele-specific polymorphisms found in 3' untranslated region of κ-casein gene lead us to assumption that they may influence allele specific expression. We constructed eight pGL3 reporter vector constructs, containing variety of combinations with different lengths of κ-casein gene promoter fragments and A- or B-genotype variant of κ-casein gene 3'UTR. Two to three times higher expression was observed when using constructs containing B-genotype specific variant of 3'UTR. We hope that mutagenesis experiments will finally help us define, which polymorphism in 3'UTR is of greater importance for allele specific expression of bovine κ-casein gene.

URAVNAVANJE IZRAŽANJA GENA ZA κ-KAZEIN PRI GOVEDU

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Molekularna osnova uravnavanja izražanja kazeinskih genov je nadvse pomembna za napredek v proizvodnji mleka. Z določitvijo odločilnih mest, ki uravnavajo izražanje kazeinskih genov, bi prišli do pomembnih informacij, ki bi jih lahko uporabili za selekcijo mlečnih pasem goveda, zasnovano na osnovi markerjev. Naše delo je bilo usmerjeno zlasti v določanje najpomembnejših regulatornih regij, ki vplivajo na izražanje κ-kazeina pri govedu. Naredili smo niz transfekcijskih poizkusov na različnih celičnih linijah (BME, HC11, CaCO2, COS7) z vektorskimi konstrukti v pGL3 plazmidu, ki so vsebovali različne dolžine promotorja gena za κ-kazein. Izražanje luciferaze pri konstruktih s κ-kazeinskim promotorjem smo primerjali z izražanjem luciferaze pri konstruktu, ki je vseboval del bovinega β-kazeinskega promotorja. Ugotovljeno je bilo, da se v mlečni žlezi med laktacijo najpogostejši alelni obliki κ-kazeina pri govedu, oblika A in oblika B, pri heterozigotnih živalih (AB) sintetizirata v različnih količinah. Razlike so opazili tako na ravni mRNA, kot tudi na beljakovinski ravni. V 3+ neprevedeni regiji gena za κ-kazein se nahajajo številni alelno-specifični polimorfizmi, ki bi lahko pomembno vplivali na izražanje gena. Da bi potrdili to hipotezo, smo pripravili osem različnih konstruktov, ki so vsebovali različne dele promotorjev ter A oziroma B alelno obliko 3' neprevedene regije. Opazili smo, da je bilo izražanje dva- do trikrat močnejše pri konstruktih, ki so vsebovali B alelno obliko. Upamo, da bomo z mutagenezo uspeli določiti polimorfizem, ki je v največji meri odgovoren za alelno-specifično izražanje gena za κ-kazein pri govedu.

THE ROLE OF RAIDD DURING MAMMARY GLAND DEVELOPMENT

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A successful lactation in mammalian species depends on a controlled process of cell proliferation during the period of mammary gland growth and development at pregnancy and apoptosis during involution. Majority of apoptotic proteins exhibit high variations in mammary gland synthetic capacity suggesting their functional role, but their effect on the mammary gland development and milk production is still unresolved. Hence characterising the mechanisms of apoptotic protein action during mammary gland development using transgenic models could provide novel insights on mammary gland biology. RAIDD – a dual domain adapter protein is known to interact with the cell apoptotic machinery via PIDD and TNFR-1 death complexes. It has been shown to be present during the processes of embryonic organ remodelling, herein opening the possibility of being involved in mammary gland development and the processes of involution after the period of lactation as well. To evaluate Raidd's role during mammary gland development whole mount staining experiments were performed utilizing transgenic *RaiddGeo,* Rosa26 and knockout *Raidd-/-* mouse lines. Our study demonstrated that *Raidd* may participate in the remodelling of the post lactating gland, via apoptotic restructuring of the formed alveoli. In the later, *Raidd* expression decreases once regressed ductal architecture is achieved implying that the rate of the localised degradation of lobulo-alveolar branching after lactation may be *Raidd*-dependent. In conclusion, our results suggest that *Raidd* may play a role during mammary gland involution after the successive periods of lactation, but not during its prepubertal development.

VLOGA GENA *RAIDD* MED RAZVOJEM MLEČNE ŽLEZE

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Obseg laktacije pri sesalcih je v veliki meri odvisen od kontrole procesov proliferacije med obdobjem intenzivnega razraščanja mlečne žleze v obdobju brejosti in apopotoze ali programirane celične smrti med involucijo v post-laktacijskem obdobju. V zaporednih obdobjih rasti in regresije mlečne žleze sinteza apoptoznih proteinov v mlečni žlezi zelo niha, kar podpira idejo o ključni vlogi posameznih apoptotskih proteinov v različnih obdobjih oblikovanja mlečne žleze. Vendar vpliv večine apoptotskih proteinov na razvoj mlečne žleze in produkcijo mleka še ni potrjen. Upraba transgenih živalskih modelov zato omogoča natančnejše overednotenje mehanizmov delovanja apoptotskih proteinov med razvojem mlečne žleze in ustvarja z dodatnim pristopom nove poglede na biologijo mlečne žleze. RAIDD - apoptotski adapterski protein sodeluje v signalni poti programirane celične smrti preko vezave na proteinske komplekse s faktorjem PIDD ali TNFR1. Prisotnost proteina RAIDD v procesih zgodnjega preoblikovanja organov med embriogenezo podpira idejo, o potencialni vlogi proteina RAIDD tudi v zgodnjem razvoju mlečne žleze in v kasnejših pre- in post-laktacijskih obdobjih. Z namenom overednotenja vloge gena *Raidd* med razvojem mlečne žleze je bil opravljen poskus reporterskega barvanja mlečne žleze pri transgenih *Raidd/Geo,* Rosa26 in knockout *Raidd-/-* miših. Rezultati kažejo na potencialno vlogo gena *Raidd* pri preoblikovanju mlečne žleze v procesu apoptozne regresije mlečnih alveolov. Stopnja izražanja gena *Raidd* se zniža ob koncu regresije, kar nakazuje možnost, da je stopnja razgradnje lobulo-alveolarnih razvejitev po obdobju laktacije odvisna od proteina RAIDD. Slednje podpira domnevo o vlogi gena *Raidd* v procesu involucije mlečne žleze po laktaciji, kar pa ne velja za razvoj mlečne žleze med puberteto.

MOLECULAR DETECTION AND IDENTIFICATION OF *EUTYPELLA PARASITICA*, THE CAUSAL AGENT OF EUTYPELLA CANKER OF MAPLES

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Eutypella canker of maple was for the first time reported in Slovenia and consequently in Europe in the year 2005 (Jurc et al., 2006). Canker wounds on different maple species are caused by fungal species *Eutypella parasitica,* which is a member of family Diatrypaceae (Ascomycota). This family includes different saprophytic fungal species, typically occurring on a broad range of dead or declining woody angiosperms; and two known parasitic species *E. parasitica* and *Eutypa lata* (usually pathogenic to grapevine). Species have quite alike morphological characteristics and signs on diseased hosts. *E. lata* was also detected on maples, but there are no reports of this fungus being pathogenic to maples. DNA regions (rDNA ITS), containing ITS1 and ITS2 regions and 5.8S rDNA, amplified with universal fungal primers were digested with different restriction endonucleases. Restriction patterns of all collected strains of E. parasitica at Slovenian Forestry Institute were identical and did also not show any deviations from the CBS strain. Furthermore, restriction patterns of related species, also *E. lata,* revealed different patterns as did *E. parasitica.* PCR-RFLP-ITS method is as shown useful as confirmation method of proper identification of *E. parasitica* or / and can be used as detection method. Faster method of *E. parasitica* detection was performed with species-specific primers that have been constructed on the basis of sequenced rDNA ITS regions of different *E. parasitica* isolates. Specificity of constructed primers was tested with different fungal species, usually associated with wood; some fungal species found on Eutypella cankers and with some *E. parasitica* related species. We have successfully amplificated the expected length of polymerase chain reaction (PCR) product with *E. parasitica*-specific primers from total DNA, extracted directly from infected maple wood.

MOLEKULARNA DETEKCIJA IN IDENTIFIKACIJA GLIVE *EUTYPELLA PARASITICA*, POVZROČITELJICE JAVOROVEGA RAKA

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V letu 2005 je bila prvič zabeležena najdba javorovega raka v Sloveniji in s tem hkrati v Evropi (Jurc in sod., 2006). Rakave rane na različnih vrstah javorjev povzroča gliva *Eutypella parasitica,* ki jo uvrščamo v družino Diatrypaceae (Ascomycota), kjer najdemo različne saprofitne vrste gliv, ki se pojavljajo običajno na odmrlih ali odmirajočih listavcih, patogeni vrsti sta *E. parasitica* ter *Eutypa lata* (običajno patogen trte). Vrsti imata podobne morfološke lastnosti ter povzročata podobne simptome na gostiteljih. *E. lata* je bila izolirana tudi iz javorjev, vendar glede na literaturo na omenjeni drevesni vrsti ne povzroča bolezenskih znakov. Z univerzalnimi glivnimi začetnimi oligonukleotidi smo pomnožili DNA fragment (rDNA ITS), ki vključuje ITS1 in ITS2 regiji ter vmesni 5.8S rDNA odsek. Restrikcijski vzorci ITS-fragmentov vseh izolatov *E. parasitica,* shranjenih na Gozdarskem inštitutu Slovenije, so bili enaki ter so se skladali z restrikcijskim vzorcem tipskega seva iz zbirke CBS, medtem ko so se razlikovali od restrikcijskega vzorca sorodnih vrst, tudi vrste *Eutypa lata.* Metoda PCR-ITS-RFLP je torej učinkovita za potrditev pravilnosti identifikacije povzročitelja javorovega raka ter jo lahko uporabimo kot detekcijsko metodo. Hitrejša metoda detekcije je bila izvedena z vrstno specifičnimi začetnimi oligonukleotidi, ki so bili skonstruirani na osnovi določenega zaporedja rDNA ITS regij različnih izolatov *E. parasitica.* Specifičnost oligonukleotidov smo preverili z različnimi glivnimi vrstami, ki se običajno pojavljajo na odmrlih ali živih drevesih, z nekaterimi glivami, ki se lahko pojavljajo na rakavih ranah javorjev ter sorodnimi vrstami vrsti *E. parasitica.* Z verižno reakcijo s polimerazo (PCR) z uporabo *E. parasitica*-specifičnih začetnih oligonukleotidov smo uspešno pomnožili produkt pričakovane dolžine iz ekstrakta okuženega javorovega lesa.

Vir / Reference: Jurc D., Ogris N., Slippers B., Stenlid J. 2006. First report of Eutypella canker of in Europe. Plant Pathology, 55, 4: 577

INSERTION OF MUTATED TRUNCATED *PFK*A GENE FROM ASPERGILLUS NIGER INTO ESCHERICHIA COLI

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There are two forms of 6-phosphofructo-1-kinase (PFK1) in *Aspergillus niger* cells, the 85-kD native enzyme and the 49-kDa shorter fragment that is formed after posttranslational modification of the native enzyme. Modification is a two stage process. Firstly, the native protein is cleaved by proteases which results in a formation of an inactive shorter fragment. Secondly, the fragment must be phosphorylated by cAMP-dependent protein kinase in order to retain activity. The shorter fragment has been reported to have more favourable kinetic characteristics enabling undisturbed metabolic flux through glycolysis. Detailed kinetic analyses have revealed that the modified enzyme is resistant to citrate inhibition. So far, truncated *pfk*A genes have only been tested in eukaryotic microorganisms, testing the activity of inserted genes in prokaryotes has just recently been established. In order to obtain an active shorter fragment avoiding complicated posttranslational modifications a truncated *pfk*A gene without introns was prepared and two sets of mutations were introduced. By site directed mutagenesis codons for amino acid residues that function as targets for phosphorylation were specifically changed. Native *pfk*A gene without introns (n-*pfk*A), two truncated *pfk*A genes without introns, one mutated in codons for two amino acids (mt-*pfk*A) and the other with wider domain of nucleotides changed (Mt-*pfk*A) were inserted into an expression vector pALTER-Ex1 and cloned in *pfk*A disrupted *Escherichia coli* strain (DF1010). The results of physiological tests performed on the transformants will be presented on the poster.

VNOS MUTIRANEGA KRAJŠEGA GENA *PFK*A IZ GLIVE *ASPERGILLUS NIGER* V BAKTERIJO *ESCHERICHIA COLI*

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V celicah glive *Aspergillus niger* obstajata dve obliki 6-fosfofrukto-1-kinaze (PFK1), 85 kDa velik nativni encim in 49 kDa velik krajši fragment, ki nastane s posttranslacijsko modifikacijo nativnega encima. Modifikacija je dvostopenjski proces. Najprej proteaze cepijo nativni protein, s čimer nastane neaktivni krajši fragment. Fragment mora biti nato fosforiliran s cAMP-odvisno proteinsko kinazo, da ponovno pridobi aktivnost. Objavljeno je bilo, da ima krajši fragment boljše kinetične lastnosti, ki omogočajo neoviran metabolni pretok preko glikolize. Podrobne analize kinetike so pokazale, da je modificiran encim odporen proti inhibiciji s citratom. Do sedaj smo krajše gene *pfk*A testirali le v evkariontskih mikroorganizmih, nedavno pa smo začeli tudi s proučevanjem delovanja vnešenih genov v prokariontih. Da bi pridobili aktivni krajši fragment brez zapletenih posttranskacijski modifikacij, smo pripravili krajši gen *pfk*A brez intronov in vanj uvedli dva niza mutacij. Z mestnospecifično mutagenezo smo specifično spremenili aminokislinske ostanke, ki so tarča fosforilacije. Nativni gen *pfk*A brez intronov (n-*pfk*A), dva krajša gena *pfk*A brez intronov, enega s spremenjenimi kodoni za dve amino kislini (mt-*pfk*A) in drugega s spremenjeno širšo domeno nukleotidov (Mt- *pfk*A), smo vnesli v ekspresijski vektor pALTER-Ex1 in jih klonirali v sevu bakterije Escherichia coli z okvarjenim genom *pfk*A (DF1010). Rezultati fizioloških testov transformant bodo predstavljeni na plakatu.

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GENOME-WIDE EXPRESSION PROFILING OF B LYMPHOCYTE RECEPTOR ACTIVATION

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B cell antigen receptor (BCR) is a signaling complex that mediates differentiation stage-specific cell fate decisions in B lymphocytes. While recognition of autoantigens by BCR on self-reactive immature B cells leads to their deletion, foreign antigen-specific mature B lymphocytes respond to BCR engagement by clonal expansion. Here we define transcriptional changes induced by BCR in immature ($CD93^+CD23^-$) and mature($CD93^-CD23^+$) B splenic murine cells. We analyzed transcriptional changes in response to BCR ligation using cDNA microarrays containing 15,247 unique oligo(dT)-primed cDNA clones, primarily derived from early embryonic cDNA libraries. By comparing gene expression profiles of isolated mature versus immature B lymphocytes, we identified 44 genes which differed significantly between both differentiation stages, notably *CD24a, Fcgr2b, Id2, Myc* and *Prkcd*. We have also identified 24 genes discriminating both cell types in their responses to BCR, stimulation among those *Myc, Marcksl1, Hrb* in *Ptger4*.. Collectively, our results provide evidence that developmental changes in the BCR-operated transcriptional changes play a crucial role in the control of B-cell function, leading either to apoptosis induced cell tolerance or immune proliferative responses.

ANALIZA DIFERENCIALNE EKSPRESIJE GENOV PO AKTIVACIJI ANTIGENSKEGA RECEPTORJA LIMFOCITOV B

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Antigenski receptor limfocitov B (BCR) je del signalne kaskade, ki v odvisnosti od stopnje zrelosti odloča o usodi limfocitov B. Interakcija med avtoantigenom in BCR vodi pri nezrelih limfocitih v celično smrt, medtem ko se zrele celice po aktivaciji receptorja odzovejo s klonalno ekspanzijo. Cilj naše raziskave je bil definirati fenotipsko različne odzive primarnih vraničnih nezrelih ($CD93^+CD23^-$) in zrelih ($CD93^-CD23^+$) limfocitov B po aktivaciji BCR. Pri tem smo uporabili cDNA mikromreže s 15,247 različnimi cDNA kloni, ki povečini izvirajo iz embrionalnih cDNA knjižnic. Analiza transkriptoma je pokazala, da obstajajo razlike v transkripciji, ki so posledica različne razvojne stopnje celic, tako v mirujočem stanju in še veliko bolj po zamreženju BCR. Identificirali smo 44 genov, katerih izražanje se je značilno razlikovalo med zrelimi in nezrelimi celicami, med drugim geni za *CD24a, Fcgr2b, Id2, Myc* and *Prkcd*. Identificirali smo tudi 24 genov specifičnih za BCR- induciran celični odgovor v nezrelih oziroma zrelih primernih limfocitih B, med drugim geni *Myc, Marcksl1, Hrb* in *Ptger4*. Analiza transkriptoma bo prispevala tako k razumevanju procesa razvoja tolerance do telesu lastnih antigenov, kjer igra ključno vlogo apoptoza, kakor tudi k razumevanju učinkovitega imunskega odziva organizma na vdor tujih antigenov, za kar je bistvena antigensko inducirana proliferacija limfocitov B.

GENETIC DISEASES
GENETSKE BOLEZNI

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THE ROLE OF THE *ATP2A3* GENE IN THE DEVELOPMENT OF DIFFERENT MALIGNANT TUMOURS

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The *ATP2A3* gene encodes sarco/endoplasmic reticulum ATPases type 3 (SERCA3) which maintain a low cytosolic Ca^{2+} concentration by actively transporting Ca^{2+} from the cytosol into the sarco/endoplasmic reticulum lumen. SERCA3 has been identified as a non-muscular SERCA type (SERCA3a according to the new nomenclature) and is expressed in various cell types, such as lymphoid cells, platelets, endothelial cells, intestinal epithelial cells and cerebellar Purkynje neurons. Six different isoforms, differing in carboxy terminal sequences have been identified. Down-regulation of SERCA3 expression has been reported on colon cancer cell lines and tissue in connection with dedifferentiation of transformed cells. On the basis of this study, we decided to analyze the *ATP2A3* gene for alterations. We investigated patients with head and neck, colon, lung and prostate cancers, patients with brain tumours as well as a control group of healthy individuals. Among 8 different alterations, we found three missense mutations, two silent mutations, and three intronic single nucleotide alterations. All alterations were germline. We also found a correlation between a low expression level in the *ATP2A3* gene and two changes in the intronic region of the *ATP2A3* gene. The results of our research show that changes in the *ATP2A3* gene are statistically significantly more common in patients with head and neck carcinoma ($p = 0.0021$), lung cancer ($p = 0.0026$), brain tumour ($p = 0.0045$) and colon cancer ($p = 0.0052$), while we did not find any mutations in prostate cancer. Our results therefore suggest that changes in the *ATP2A3* gene may be involved as an early event in cancer development in humans.

VLOGA GENA *ATP2A3* PRI RAZVOJU RAZLIČNIH MALIGNIH TUMORJEV

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Gen *ATP2A3* kodira Ca^{2+}-ATPaze sarko/endoplazemskega retikuluma tipa 3 (SERCA3), ki z aktivnim transportom vzdržujejo nizko koncentracijo Ca^{2+} v citosolu. Z alternativnim cepljenjem v karboksi terminalnem delu nastane šest izooblik, ki so prisotne je v številnih nemišičnih tkivih. Raziskave izražanja proteina SERCA3 so pokazale negativno uravnano izražanje pri različnih celičnih linijah in tkivu raka širokega črevesa v povezavi z dediferenciacijo transformiranih celic. Na podlagi teh raziskav smo se odločili preveriti ali so spremembe v genu *ATP2A3* povezane z nastankom raka. Z metodama konformacijske analize na poliakrilamidnem gelu in DHPLC smo pri bolnikih z rakom glave in vratu, širokega črevesa, pljuč, in prostate, bolnikih z možganskim tumorjem ter kontrolno skupino zdravih posameznikov pregledali gen *ATP2A3*. Z verižno reakcijo s polimerazo v realnem času smo preverili izražanje gena *ATP2A3*. Mutacijska analiza je razkrila 8 različnih sprememb v genu *ATP2A3* od katerih so bile tri mutacije, dve tihi spremembi in tri zamenjave nukleotida v intronskem delu gena *ATP2A3*. Vse spremembe so bile prisotne že v zarodni liniji. Našli smo tudi povezavo med dvema spremembama v intronskem delu in znižanim izražanjem gena *ATP2A3*. Rezultati naših raziskav kažejo, da so spremembe v genu *ATP2A3* statistično značilno pogoste pri bolnikih z rakom glave in vratu ($p = 0{,}0012$), s pljučnim rakom ($p = 0{,}0026$), z rakom na možganih ($p = 0{,}0045$) in z rakom širokega črevesa ($p = 0{,}0052$), medtem ko pri raku prostate nismo našli sprememb. Iz naših rezultatov lahko zaključimo, da spremembe v genu *ATP2A3* predstavljajo enega izmed zgodnjih dogodkov v razvoju raka pri človeku.

GENETIC DIAGNOSTICS OF HEMOPHILIA A

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Hemophilia A is a chromosome X-linked bleeding disorder due to mutations in the FVIII gene. There are 170 hemophilia A patients in Slovene registry for Hemophilia, of which 74 have severe hemophilia A. 50 % of patients with severe hemophilia A have inversion of intron 22, which is detected with LD-PCR method. Point mutations are detected with amplification of exons of factor VIII gene followed with direct sequencing technique. In the Slovene population we managed to determine genetic mutation in 81/170 patients, 35/81 have inversion of intron 22 and another 46/81 have 21 different mutations in FVIII gene which cause hemophilia A - six of them are so far found only among Slovene patients. The determination of hemophilia mutations is of great importance for the timely discovery of the hemophilia carriers and the pre-natal diagnostics of hemophilia A.

GENETSKA DIAGNOSTIKA HEMOFILIJE A

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Hemofilija A je recesivna dedna bolezen, vezana na kromosom X, z motnjo v strjevanju krvi. Hemofilija A je posledica različnih mutacij v genu za faktor VIII. V Slovenskem registru je zabeleženih 170 bolnikov s hemofilijo A, 74 jih ima težko obliko bolezni. Približno 50 odstotkov bolnikov s težko obliko bolezni ima inverzijsko mutacijo introna 22, ki jo ugotavljamo s pomnoževanjem dolgih odsekov DNK. Točkovne mutacije iščemo s pomnoževanjem različnih eksonskih zaporedij v genu za faktor VIII in nato z direktnim sekvenciranjem. V slovenski populaciji smo do sedaj odkrili gensko mutacijo pri 81/170 bolnikih s hemofilijo A, od tega pri 35/81 inverzijsko mutacijo in še 21 različnih mutacij pri 46/81 bolnikih. Šest od dokazanih mutacij je bilo opisanih samo pri slovenskih bolnikih. Določitev mutacij pri hemofilikih je zelo pomembna zaradi pravočasnega odkrivanja prenašalk hemofilije in diagnosticiranja hemofilije A pred rojstvom.

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GENETICS OF POLYCYSTIC OVARY SYNDROME- A ROLE OF VNTR IN THE *INS* GENE

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Polycystic ovary syndrome (PCOS) is the most common endocrinopathy in women of reproductive age. Regarding its extremely heterogeneous clinical picture and its segregation in families, a multifactorial and oligogenic pathogenesis has been proposed. Insulin resistance and compensatory hyperinsulinemia, the features being frequently observed in PCOS women, could be genetically determined by VNTR in the *INS* gene. The aim of the present study was to investigate a possible association of the polymorphism with the presence of PCOS and with serum fasting insulin concentrations ([insulin]) in PCOS. The study group consisted of 118 women with PCOS, while the control group consisted of 110 healthy volunteers. For all participants, [insulin] was measured (immunoradiometric assay), BMI calculated and *INS* VNTR genotyping performed (indirectly through the -23 *HphI* polymorphism in the *INS* gene, with real-time PCR). The *INS* VNTR class III allele frequencies were 79.2 % in PCOS patients and 70.4 % in controls, while class I allele frequencies were 20.8 % in the study and 29.6 % in the control group; between the two groups, the difference in the allele distribution differed significantly ($p = 0.030$). Appropriate linear regression model (*adjusted* $R^2 = 0.40$) showed that BMI and interaction of BMI with *INS* VNTR genotype are significant predictors for [insulin] in PCOS patients ($p < 0.001$ for both predictors), while the influence of the genotype as an independent predictor was not significant. To conclude, *INS* VNTR class III alleles might be a significant risk factor for the development of PCOS. In PCOS patients, higher ITM and a combination of III/III genotype with obesity significantly predict higher [insulin].

GENETIKA SINDROMA POLICISTIČNIH JAJČNIKOV- VLOGA POLIMORFIZMA VNTR V GENU *INS*

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Sindrom policističnih jajčnikov (PCOS) je najpogostejša endokrina motnja pri ženskah v rodnem obdobju. Glede na izrazito heterogenost klinične slike PCOS ter glede na njegovo pojavljanje v družinah je patogeneza PCOS najverjetneje multifaktorska in oligogenska. Bolnice s PCOS pogosto razvijejo inzulinsko rezistenco in kompenzacijsko hiperinzulinemijo, za kateri bi genetsko nagnjenost lahko predstavljal minisatelitski polimorfizem (VNTR) v genu za inzulin (gen *INS*). Namen raziskave je bil preveriti morebitno povezavo navedenega polimorfizma s prisotnostjo PCOS ter pri bolnicah ovrednotiti njegov vpliv na serumske koncentracije inzulina na tešče ([inzulin]). V študijsko skupino smo vključili 118 bolnic s PCOS, v kontrolno skupino pa 110 zdravih prostovoljk. Za vse preiskovanke smo z imunoradiometrično metodo določili [inzulin], izračunali ITM ter izvedli molekularno genetsko analizo polimorfizma VNTR *INS* (preko polimorfizma -23 *HphI* v genu *INS*, s PCR v realnem času). Frekvenca alelov VNTR *INS* razreda III je bila 79,2 % v študijski in 70,4 % v kontrolni skupini, frekvenca alelov razreda I pa 20,8 % v študijski in 29,6 % v kontrolni skupini; razlika v porazdelitvi alelov med skupinama je bila značilna ($p = 0,030$). Ustrezni linearni regresijski model (*prilagojen* $R^2 = 0,40$) je pokazal, da sta ITM in interakcija ITM-genotip VNTR *INS* pri bolnicah s PCOS značilna napovedna parametra za [inzulin] ($p < 0,001$ za oba parametra), medtem ko vpliv genotipa kot samostojnega dejavnika ni značilen. Ugotavljamo, da so aleli VNTR *INS* razreda III verjetno povezani z večjim tveganjem za razvoj PCOS. Pri bolnicah večji ITM ter kombinacija genotipa III/III in prekomerne telesne teže napovedujeta višji [inzulin].

DETERMINATION OF DEL(13) IN MULTIPLE MYELOMA BY CIG-FISH

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Multiple myeloma is a hematological malignancy that affects B-linage lymphocytes. Some recurrent chromosomal aberrations have an important diagnostic and prognostic value in clinical course of multiple myeloma. They are usually determined by interfase FISH. Especially important are abnormalities that result in poor survival of afflicted patients, among them also deletion of chromosome 13. However, bone marrow infiltration by plasmocytoma cells can vary considerably depending on type and progress of multiple myeloma. Due to inability of FISH to distinguish between plasmocytoma and the rest of bone marrow cells, the amount of cells possessing chromosomal changes can be underestimated. Therefore, we decided to introduce cIg-FISH method in patients with low proportion of cells with del(13) ($<$15%). cIg-FISH is a combination of immunolabeling of cell cytoplasm Ig light chain (λ or κ) and FISH. We modiffied different procedure steps and applied the optimized protocol on cells, previously prepared for standard cytogenetic analysis. Using a modified method a good labeling of plasmacytoma cells was obtained as well as intensive signals for chromosome 13 on cell nuclei. The method was introduced into routine diagnostics and is used whenever a low proportion of plasmacytoma cells in bone marrow is determined already by cytological examination or when the amount of cells containing del(13) is at the detection limit.

CIG-FISH POSTOPEK DOLOČANJA DEL(13) PRI PLAZMACITOMU

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Plazmacitom je novotvorba, pri kateri pride do malignega razraščanja plazmatk. Kot napovedni dejavnik za potek bolezni je pomembna tudi citogenetska preiskava celic kostnega mozga. Ponavljajoče kromosomske spremembe pogosto določamo z interfazno FISH preiskavo. Pri plazmacitomu tako rutinsko določamo zlasti delecijo kromosoma 13. Ker se plazmacitomske celice lahko v kostnem mozgu razraščajo zelo neenakomerno, je določanje deleža celic s tarčno kromosomsko spremembo zelo težavno. Dobljeni rezultat je lahko dokaj majhen, pogosto v območju mejnih vrednosti. Zato smo pri pacientih, kjer je bil delež določene del(13) pod 15%, uvedli cIg-FISH preiskavo. Pri tem postopku z uporabo protiteles za težke oziroma lahke verige (κ ali λ) monoklonskih Ig označimo plazmacitomske celice, nato pa na tako označenih celicah določimo prisotnost kromosomske spremembe. V literaturi najdemo različne postopke za izvedbo navedene preiskave. Nas pa je zanimalo zlasti, kako bi uporabili celice kostnega mozga, ki so bile predhodno pripravljene za standardno citogenetsko preiskavo. Preverili smo vse stopnje postopka, tako da smo dosegli dobro obarvanje citoplazemskih lahkih verig ter izrazite signale za kromosom 13 na jedrih teh celic. Omenjeno preiskavo uporabljamo, ko že citološki pregled pokaže zelo majhen delež plazmatk oziroma tedaj, ko je zelo majhen delež celic z določeno del(13).

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A STUDY OF PRO12ALA AND C1431T POLYMORPHISMS IN PPARG GENE IN PATIENTS WITH POLYCYSTIC OVARY SYNDROME

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Insulin resistance affects 50-70% of women with polycystic ovary syndrome (PCOS). Peroxisome proliferator-activated receptor-γ (PPAR-γ) has been shown to influence insulin sensitivity. We studied the relationship of Pro12Ala and C1431T (His477His) polymorphisms of the *PPARG* gene to anthropometric, biochemical and hormonal features of PCOS. The study involved 69 PCOS patients and 16 controls. Both polymorphisms were distributed according to Hardy-Weinberg equilibrium in control and patient group. Genotype and allele frequencies of particular polymorphism did not differ between groups, therefore we analysed the control and the patient group combined.
For Pro12Ala polymorphism 28,7% of heterozygotes and no homozygotes were found. Heterozygotic state showed a trend towards lower C-reactive protein concentration in serum (hsCRP; $p=0,053$) and lower body-mass index (BMI; $p=0,072$, both t-test). For C1431T 24,7% heterozygotes and no homozygotes were found. Heterozygous state was associated with significantly lower hsCRP, BMI, waist circumference and sistolic pressure ($p<0,05$, t-test). Pro12Ala and C1431T polymorphisms were in considerable linkage disequilibrium ($D'=0,77$). Haplotype analysis of the two polymorphisms gave 4 different haplotypes and 4 different diplotypes with the first haplotype always being wild-type Pro-C and the second haplotype being Pro-C or a haplotype with one polymorphism, another polymorphism, or both. Only heterozygous state with both polymorphisms showed borderline significance for lower hsCRP ($p=0,058$ for Kruskal-Wallis test, $p=0,075$ for Mann-Whitney test) compared to wild-type. One reason for lost significance could be splitting into small groups. The results suggest that Pro12Ala and/or C1431T might have beneficial influence on insulin resistance-associated adiposity and pro-inflammatory state in PCOS.

ANALIZA POLIMORFIZMOV PRO12ALA IN C1431T V GENU PPARG PRI BOLNICAH S SINDROMOM POLICISTIČNIH JAJČNIKOV

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Inzulinska rezistenca se pojavlja pri 50-70% žensk s sindromom policističnih jajčnikov (PCOS). Znano je, da s peroksisomskim proliferatorjem aktiviran receptor γ (PPAR-γ) vpliva na občutljivost na inzulin. V naši študiji smo raziskovali povezavo med polimorfizmomoma Pro12Ala in C1431T (His477His) v genu *PPARG* z antropometričnimi, biokemijskimi in hormonskimi kazalci pri PCOS. Analizirali smo 69 bolnic s PCOS in 16 zdravih preiskovank. Oba polimorfizma sta bila v obeh skupinah porazdeljena v skladu s Hardy-Weinbergovim ravnotežjem. Genotipske in alelne frekvence posameznega genotipa se med bolnicami in kontrolami niso razlikovale, zato smo skupini obravnavali skupaj. Za polimorfizem Pro12Ala smo našli 28,7% heterozigotov in nobenega homozigota. Heterozigotno stanje je bilo mejno značilno povezano z znižano serumsko koncentracijo C-reaktivnega proteina (hsCRP; $p=0,053$) in nižjim indeksom telesne mase (ITM; $p=0,072$, t-test). Pri polimorfizmu C1431T smo našli 24,7% heterozigotov in nobenega homozigota. Heterozigotno stanje je bilo značilno povezano z nižjim hsCRP, ITM, obsegom pasu in sistoličnim tlakom ($p<0,05$, t-test). Polimorfizma sta bila v vezavnem neravnovesju ($D'=0,77$). Pri haplotipski analizi smo dobili 4 različne haplotipe in 4 različne diplotipe, pri čemer je bil prvi haplotip vedno nemutiran Pro-C, drugi haplotip pa je vseboval bodisi prvi polimorfizem, bodisi drugi ali oba. Le heterozigotno stanje z obema polimorfizmoma je bilo mejno značilno povezano z nižjim hsCRP ($p=0,058$, Kruskal-Wallisov test; $p=0,075$ Mann-Whitneyev test) glede na nemutiran diplotip. Izguba signifikantnosti bi lahko bila povezana z razdelitvijo na manjše skupine. Razultati študije kažejo na verjeten pozitiven učinek polimorfizma Pro12Ala in/ali C1431T na debelost in pro-vnetno stanje pri PCOS, ki sta povezana z inzulinsko rezistenco.

GENETIC CHANGES OF THE WNT PATHWAY COMPONENTS FOUND IN BRAIN TUMORS

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Research carried out in this paper deals with the molecular characterisation of E-cadherin (CDH-1), beta-catenin (CTNNB1) and adenomatous polyposis coli (APC) genes in a panel of 50 central nervous system tumors. All genes are involved in the Wnt signalling pathway. Beta-catenin is the main downstream effector of the Wnt signalling. E-cadherin is associated with ß-catenin and plays a role in cell-cell adhesion, while APC protein also plays a signalling role as a negative regulator of the wnt pathway. Our interest in genes of the wnt pathway stemmed principally from the findings that wild-type APC protein is highly expressed in the central nervous system, and upon the finding that it is involved in particular syndromes, among which the brain tumors play a significant role. Brain tumor samples were tested for gene instability of the APC gene by PCR/loss of heterozygosity using RFLP method. PCR amplification of tetranucleotide GATA polymorphism (D16S752) was used to test loss of heterozygosity of the E-cadherin gene. Changes of beta-catenin were tested by heteroduplex method. The results of our analysis showed allelic loss of the APC gene in 33.3% of glioblastomas. Another 23.8% of samples demonstrated allelic imbalance. Altogether, there were 12 samples (57%) demonstrating instability of APC gene confined to glioblastomas. Twenty five percent of meningeomas showd LOH of CDH1 gene. One LOH of this gene was found in germinoma and one in teratoma. Heteroduplex method revealed 3 samples with additional bands indicating possible mutations in exon 3 of the beta-catenin gene. Mutations of ß-catenin were confined to a meningeoma, a glioblastoma and a germinoma. Our findings on genetic changes of the wnt components may contribute to better understanding of brain tumors genetic profile and could be used as prognostic marker of disease evolution and progression.

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FREQUENCY OF SOME RECURRENT CHROMOSOMAL ABERRATIONS IN CLL PATIENTS

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Chronic lymphocytic leukemia is a lymphoprolipherative disease. The time course of the illness can be mild as well as more progressive. In the last few years some new biological drugs for CLL have been developed. To achieve the desired effect of therapy patients should be classified into risk groups on the basis of different diagnostic parameters. Cytogenetics has been for a long period of time of the utmost importance in diagnostics of hematological malignancies. Additionally, it is often an independent prognostic factor for the outcome of a disease. The role of cytogenetics has been well established also in diagnostics of CLL. The most important chromosomal aberrations with negative prognostic impact are deletion of p53 gene on chromosome locus 17p13.1 and deletion of ATM gene on chromosome 11 (11q22.3). Patients with these particular chromosome aberrations should undergo an intensive chemotherapy. On the other hand, deletions on long arm of chromosome 13 as the sole chromosomal change are reported as a good prognostic factor. Although the role of trisomy 12 is still disputed, it is determined in CLL since it is a relatively frequent change. Using commercially available DNA probes (Vysis) for determination of above mentioned aberrations, a group of our CLL patients was studied by interfase FISH. In accordance with literature data the most frequent chromosomal change was del(13) followed by trisomy 12. Both aberrations with a negative prognostic impact, del(17)(p13.1) and del(11)(q22.3), were rarely confirmed among our patients.

POJAVNOST NEKATERIH PONAVLJAJOČIH KROMOSOMSKIH SPREMEMB PRI SLOVENSKIH BOLNIKIH S KLL

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Kronična limfatična levkemija (KLL) je limfoproliferativna bolezen. Lahko poteka počasi in v blagi obliki, ki ne zahteva posebnih terapevtskih posegov, pri določenih bolnikih pa je njen potek mnogo agresivnejši. V zadnjem času je razvoj novih bioloških zdravil sprožil potrebo po intenzivnejši in poglobljeni diagnostični opredelitvi sicer dokaj heterogene skupine bolnikov s KLL. Zaradi včasih težke odločitve o intenzivnosti zdravljenja (uporaba kemoterapije) je potrebno bolnike opredeliti glede na stopnjo rizičnosti. Pri tem nastopajo citogenetske spremembe kot eden temeljnih kriterijev. Kot posebno negativen dejavnik na potek in izid bolezni so raziskave potrdile delecije gena za protein p53 na kromosomu 17 (17p13.1) ter delecije gena ATM na kromosomu 11 (11q22.3). Bolniki z navedenima spremembama naj bi bili takoj deležni intenzivnega zdravljenja. Nasprotno pa naj bi bili bolniki z delecijami na q kraku kromosoma 13 kot edinimi kromosomskimi spremembami relativno ugodna skupina, pri kateri takojšnje intenzivno zdravljenje ni nujno. Nekoliko nasprotujoče so si literaturne navedbe o pomembnosti trisomije 12 pri KLL. Ker pa gre za pri tej bolezni pogosto kromosomsko spremembo, jo navadno tudi določamo. Pri naših bolnikih s KLL smo s pomočjo FISH postopka ugotavljali zgoraj navedene kromosomske spremembe. V skladu z literaturnimi navedbami smo ugotovili, da je tudi pri slovenskih bolnikih s KLL najpogostejša kromosomska sprememba del(13), ki ji tesno sledi trisomija 12. Obe izrazito negativni kromosomski spremembi, del(17)(p13.1) kot tudi del(11)(q22.3), se pojavljata relativno redko, kar zlasti velja za spremembe na genu za p53.

5P DUPLICATION SYNDROME: A RARE MULTIPLE CONGENITAL ANOMALY-RETARDATION SYNDROME CAUSED BY SO FAR UNPUBLISHED PARTIAL DUPLICATION (5) (P15.2-P12) COMBINED WITH PARTIAL DELETION (5) (PTER-P15.31)

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We report on an infant with multiple congenital anomalies, developmental delay, abnormal neurological and dysmorphic signs. He is the first and only child of a healthy nonconsanquinous parents with no positive family history regarding congenital diseases. He's phenotype is characterised by failure to thrive, developmental retardation, severe muscular hypotonia, congenital heart anomaly, agenesis of corpus calosum, pronounced macrodolichocephaly, unusual face with hypertelorism and bulbous nose with flat bridge, full lips, long fingers, limb abnormalities and hearing loss. With combination of banding studies and FISH analyses the karyotype 46,XY, der (5)dup(5) (p15.2-p12) del(5) (pter-p15.31) was identified. This so far unpublished partial duplication 5p combined with partial deletion 5p of de novo origin is the cause of described clinical picture that is typical for 5p duplication syndrome since the critical 5p13 region is also included in the duplication.

SINDROM DUPLIKACIJE 5P: REDEK MALFORMACIJSKO-RETARDACIJSKI SINDROM POVZROČEN Z DOSLEJ ŠE NEOPISANO DELNO DUPLIKACIJO (5) (P15.2-P12) IN SOČASNO DELECIJO (5) (PTER-P15.31)

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Predstavljamo dojenčka s številnimi prirojenimi anomalijami, abnormnimi nevrološkimi in displastičnimi znaki ter razvojnim zaostankom. Deček je prvorojenec zdravih staršev, ki nista v sorodu. Tudi v širši družini ni prirojenih bolezni ali anomalij. Že prenatalno so pri dečku ugotovili defekt ventrikularnega septuma. Zaradi krvavitve je bil porod induciran v 37. tednu gestacijske starosti in zaradi zastoja ter bradikardije ploda dokončan z vakuumsko ekstrakcijo. Ob rojstvu je bil zahiran, imel je opisano srčno hibo, izrazito mišično hipotonijo centralnega tipa, ekvinovarusno deformacijo obeh stopal in številna kraniofacialna displastična stigmata z diskranijo. Izstopala je zlasti makrodolihocefalija, hipertelorizem, širok nos z vdrtim nosnim korenom, izrazite ustnice, visoko obokano nebo, displastični posteriorno rotirani uhlji in dolgi tanki prsti s širšimi distalnimi falangami. Že v prvih tednih življenja so se pojavili znaki dihalne stiske s potrebo po dodatku kisika v vdihanem zraku, neuspevanje in znaki zaostanka v motoričnem in umskem razvoju z odsotnim očesnim kontaktom in reakcijo na zvok. Slikovne preiskave glave so pokazale agenezijo korpus kalozuma, preiskave sluha pa potrdile obojestransko prevodno-zaznavno okvaro sluha. Glede na klinično sliko so bile poleg nevrologih in metabolnih preiskav opravljene tudi citogenetske analize. Kariotipizacija s tehniko visoke ločljivosti in GTG proganjem je pokazala, da gre pri dečku za de novo nastalo strukturno aberacijo kromosoma 5. Z dopolnilno FISH diagnostiko (sonde za subtelomerne regije, Cri du chat regijo in WCP 5) smo potrdili, da ima deček doslej v literaturi še neopisano kombinacijo delne duplikacije (5) (p15.2-p12) in sočasne delecije (5) (pter-p15.31). Dokazana strukturna kromosomska aberacija tako zajema 5p13 regijo, ki je kritična regija za pojav pri dečku vidnih tipičnih kliničnih značilnosti Sindroma duplikacije 5p.

CONTEMPORARY METHODS IN PRENATAL GENETIC DIAGNOSTICS

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Karyotyping of cultivated cells is a basic method for detection of chromosome aberrations in prenatal diagnostics for at least more than 30 years. It is used for detection of structural and numerical chromosome changes after amniocentesis and chorionic villus sampling. For rapid detection of chromosome aberrations some new methods have developed. Fluorescent in situ hybridization (FISH) and Quantitative fluorescent-polymerase chain reaction (QF-PCR) are methods used in prenatal diagnostics for detecting of most common aneuploidies in a short time. 10-15% of all pregnancies finished as spontaneous miscarriages. In the first trimester most of them (50%) are the result of chromosomal aberrations – mostly numerical chromosome changes (86%). Bed quality of chromosomes and the culture failure is often a problem in these cases. In these cases we perform Multiplex ligation-dependent probe amplification (MLPA), a fast method that can provide information about chromosome number even on the uncultivated material. The methods used for whole karyotyping and other possible rapid methods for detection of specific chromosome rearrangements are demonstrated. The advantages and disadvantages of available methods for rapid and successful prenatal diagnosis are discussed.

SODOBNE METODE PRENATALNE GENETSKE DIAGNOSTIKE

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Mikroskopska analiza kariotipa kultiviranih celic je zlati standard prenatalne diagnostike že več kot 30 let. Od prvih aplikacij v 70. letih prejšnjega stoletja se je metoda uporabljala za ugotavljanje številčnih in strukturnih kromosomskih sprememb po amniocentezi oziroma biopsiji horionskih resic. Zaradi potrebe po hitrejši genetski diagnostiki pa sta se vpeljali sodobnejši metodi fluorescentne in situ hibridizacije (FISH) in QF-PCR (Quantitative fluorescent-polimerase chain reaction), metodi, ki omogočata hitro in natančno analizo specifičnih kromosomskih sprememb, predvsem pogostih aneuploidij. Posebno poglavje so spontani splavi, ki se pojavljajo v približno 10-15% prepoznavnih nosečnosti. V prvem trimesečju jih je 50% posledica kromosomskih napak, v večini primerov (86%) so to spremembe v številu kromosomov. Citogenetska analiza zgodnjih spontanih splavov je težavna zaradi pogoste odsotnosti celične rasti in slabe kvalitete kromosomov. V teh primerih smo v našem laboratoriju uvedli metodo MLPA (Multiplex ligation-dependent probe amplification). Metoda je hitra, poceni in da popolno informacijo o številu kromosomov tudi na nekultiviranem materialu. V prispevku prikazujemo metode, ki se uporabljajo pri analizi kromosomov, jih primerjamo in razpravljamo o prednostih in pomanjkljivostih posameznih postopkov za uspešno, hitro in kvalitetno prenatalno diagnostiko.

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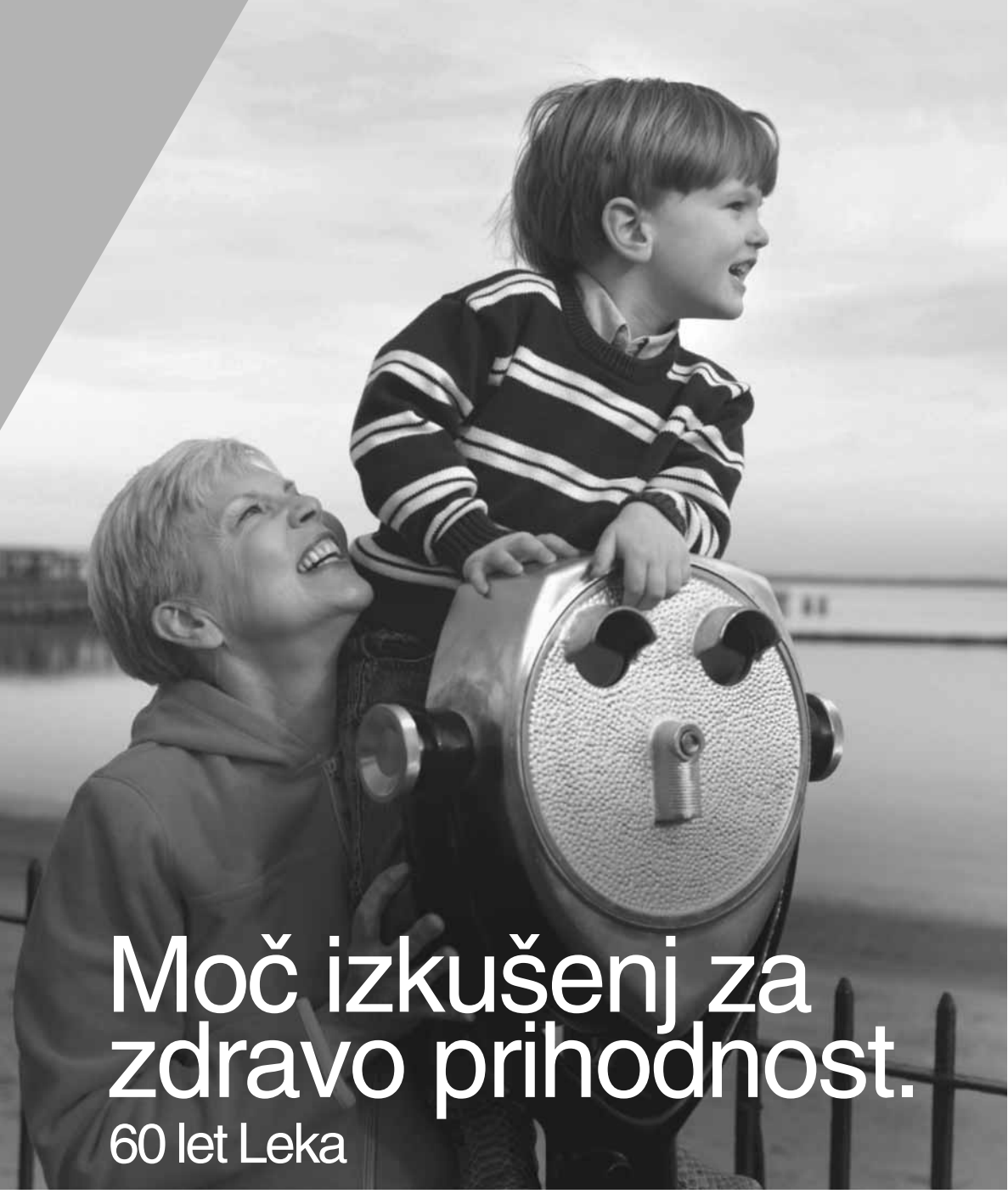

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