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ELIXIR-SI launch & 11th CFGBC Symposium

"Data for Life"

BOOK OF ABSTRACTS

September 20 - 21, 2016
Ljubljana

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Medicinska fakulteta

ELIXIR-SI LAUNCH & 11TH CFGBC SYMPOSIUM:

"DATA FOR LIFE"

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CANKARJEV DOM & FACULTY OF MEDICINE, UNIVERSITY OF LJUBLJANA

LJUBLJANA, SEPTEMBER 20 – SEPTEMBER 21, 2016

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LAUNCH OF ELIXIR SLOVENIA AND 11TH SYMPOSIUM OF CENTRE FOR FUNCTIONAL GENOMICS AND BIO-CHIPS

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FROM SLOVENIAN CONSORTIUM FOR BIO-CHIPS THROUGH CENTRE FOR FUNCTIONAL GENOMICS AND BIO-CHIPS TOWARDS ELIXIR

The Slovenian Consortium for Bio-Chips was initiated by Slovenian researchers in 2001 at the Faculty of Medicine, University Ljubljana. The initial goal of this network of academic, research and clinical institutions joint with pharmaceutical industry was to place Slovenia on the map of European countries in the raising field of genomics. This was the time when the Human Genome Project was close to its completion with huge anticipations for a major immediate impact on medicine. In 2005 the Centre for Functional Genomics and Bio- Chips (CFGBC, http://cfgbc.mf.uni-lj.si/) was formally established and moved to new laboratory space at the Faculty of Medicine. This year the consortium members also signed a formal act "Regulations of the Activities of the Centre for Functional Genomics and Bio-Chips" ("Pravilnik o delovanju Centra za funkcijsko genomiko in bio-čipe") that was signed by 12 directors or deans from the collaborating institutions from UL Faculty of Medicine, Biotechnical Faculty, Faculty of Pharmacy, Lek Pharmaceuticals, National Chemical Institute, Agricultural Institute of Slovenia, National Institute of Biology, Institute of Oncology, University Medical Centre Ljubljana, Institute Jožef Stefan, Blood Transfusion Centre, Institute for Health Protection. The boards of CFGBC, including the Management Board and the Scientific Board, have been established in January 2006. In the same year the members also signed an agreement for the purchase of equipment that was in joint interest of the consortium members "Pogodba o nakupu skupne raziskovalne opreme". These documents represented a crucial step in further development of the consortium and its activities, also in line with further activities to join ELIXIR and become a sustainable infrastructural center. A step forward in this direction has been achieved in 2004 when CFGBC became a part of the Network of Research Infrastructural Centres of UL (MRIC-UL, http://www.uni-lj.si/research_and_development/). Of particular importance and help to Head of CFGBC and the entire Consortium were the Heads of the Management board, prof. dr. Radovan Komel (2006 – 2009), prof. dr. Simon Horvat (2010 –2012) and prof. dr. Kristina Gruden (2013 – present).

The CFGBC Consortium now has 15 members. We still share the equipment, with open access to all partners, and organize common activities. The CFGBC symposia become a traditional scientific networking event on the research activities in the post-genome era in Slovenia, which is in 2016 held for the 11th time.

One of the strengths of the Slovenian Consortium is a bottom-up and timely notion of the post-genome era challenges. The consortium worked continuously with Slovenian Ministries, especially with the Ministry of Science, Education and Sports, presenting urgent needs for Slovenia to stay in line with the novel developments. These efforts resulted in the inclusion of two for CFGBC consortium relevant European ESFRI infrastructures into the "Roadmap of Research Infrastructure Development 2011 – 2020", which is a formal act of the Government of the Republic of Slovenia. The first infrastructure is ELIXIR (Infrastructure for Life-Science Information, https://www.elixir-europe.org/) which is coordinated at the Faculty of Medicine, UL. The majority of CFGBC partners participate actively in the Slovenian Elixir node. The second is EATRIS (European Infrastructure for

Translational Medicine, http://www.eatris.eu/), which is coordinated at the Faculty of Pharmacy, UL. The infrastructure BBMRI Biobanking and Molecular Resources Research Infrastructure, http://bbmri-eric.eu/) is on the waiting list until the first update of the document. ISBE (Infrastructure Systems Biology Europe, http://project.isbe.eu/) is also of great interest for the Slovenian consortium since two of its partners (National Institute of Biology and the Faculty of Medicine, UL) participate in the preparatory phase and is also on the waiting list for the first update of the Slovenian infrastructural roadmap.

Throughout this period CFGBC hosted researchers working on multiple national and FP6, FP7, Marie Curie and Horizon 2020 international functional genomic projects, where scientists from the Consortium coordinated activities or participated as collaborators. In recent years, several CFGBC groups oriented their research towards bioinformatics, systems biology and systems medicine. Examples of successful projects in this area include Marie Curie projects, such as FightingDrugFailure, FP7 research infrastructure project ISBE, CASyM leading to EASyM, Horizon 2020 projects ARTEMIDA, EXCELERATE, EU-PGx, MAESTRA, HBP, ERACoSySMed projects, and COST actions EUPancreas, Open Multiscale Systems Medicine (OpenMultiMed), GREEKC (GRECO), etc.

WHAT IS ELIXIR INFRASTRUCTURE?

ELIXIR is a distributed infrastructure for information in the field of life sciences. It is organized as a network of European nodes, with ELIXIR hub established within the European Bioinformatics Institute in Hinxton, England. Currently 20 ELIXIR nodes from 19 European countries and one observer country are participating in this infrastructure. ELIXIR combines the capabilities of the leading European organizations in order to increase the total capacity for safe and long-term management of data obtained in the context of research financed by public funds. ELIXIR coordinates, integrates and maintains bioinformatics tools and services in the Member States and beyond, and provides academic organizations and industry an easier access to (1) data, (2) tools, (3) standards, (4) information and communication services, and (5) resources for promotion, education and training.

Slovenian ELIXIR node (ELIXIR-SI) is coordinated by the Faculty of Medicine, University of Ljubljana. The initiator of the connection has been the Centre for Functional Genomics and Bio-Chips (CFGBC). The node currently consists of CFGBC members and of associate partners that will become full members after signing the agreement, tentatively in autumn this year. The Vision of ELIXIR-SI is in establishing a national infrastructure for the core national data node. The node will provide optimal standard and long-term management of large-scale data in the field of life sciences on the principles of FAIR and the complementary integration into existing national ICT infrastructures (e-Infrastructures, duplication of data, the use of existing computer cluster). Part of the data nodes are also services to help researchers in the life sciences, particularly in the fields of training, bio (medical) informatics, bioinformatics analysis, data mining, and biostatistics. The data hub will enable a simple and standard data exchange and sharing of tools and services with ELIXIR nodes in other European countries. In addition to the infrastructure for data management, it is necessary to ensure the appropriate infrastructure that generates the data, for example the most powerful equipment of next generation sequencing (NGS). Currently we only have small-scale sequencing capacities in Slovenia and a true high-throughput NGS national infrastructure is lacking at present. So it makes sense to combine activities in building the infrastructure for data management and data acquisition. With ELIXIR-SI data node and the infrastructure of next-generation sequencing, Slovenia will be able to provide the appropriate reference genetic information on the Slovenian human population, as well as plant and animal biodiversity. This will enable better quality of individualized health and nutritional care, concern for environmental conservation, environmental friendly and renewable economy in all fields related to the biosphere.

THE INAUGURATION EVENT ON BEHALF OF SLOVENIA'S FULL MEMBERSHIP IN THE PRIORITY EUROPEAN RESEARCH INFRASTRUCTURE ELIXIR

The vision of ELIXIR is to make better use of information in the data for research in medicine, biology and in other life sciences. With the publication of the consortium agreement ELIXIR on 12.02.2016 Slovenia fulfilled all of the conditions for full membership of the priority European research infrastructures for information in the field of life sciences ELIXIR. The Government of the Republic of Slovenia committed to sustainably support the priority research infrastructures as outlined in the "Research Infrastructures Roadmap 2011 - 2020" dated 28.04.2011. It states that the inclusion of Slovenia in ELIXIR is fundamental for all research in life sciences since it brings many benefits for the Slovenian society, from improvement of the health status of aging population, sustainable production of quality food, increasing the competitiveness of pharmaceutical and biotechnology industry, and better environmental protection.

On behalf of the full association of Slovenia to ELIXIR infrastructure, we organize the gala opening of the Slovenian ELIXIR node Slovenian with the event entitled "Data for Life" in conjunction with the scientific symposium on the 11th anniversary of the Center for Functional Genomics and Bio-Chips, CFGBC. The opening will be attended by representatives of the international infrastructure ELIXIR: Andrew Smith (representative of the ELIXIR hub), prof. dr. Barend Mons (Head of special expert group of the European Commission to open a European research cloud - EOSC), prof. dr. Bengt Persson (leader of ELIXIR node Sweden), prof. dr. Jaap Heringa (Head of ELIXIR node Netherlands) and prof. dr. Jiri Vondrášek (Head of ELIXIR node Czech Republic). They will, each from their perspective, present the role of data and information in life sciences and their importance for research, industry and the general public. Members of the Slovenian ELIXIR node will present their successful national and international projects related to research topics relevant for the post-genomic era. At the scientific symposium of CFGBC 11th anniversary members of the CFGBC Scientific Advisory Board, Dame dr. Janet Thornton (former director of the European bioinformatics institute in England) and dr. Thomas Svensson (HR SciLifeLab from Sweden). The symposium will continue with lectures and posters from members of CFGBC and ELIXIR-SI.

As Heads of the Scientific Committee of ELIXIR-SI launch and the 11th CFGBC symposium "Data 4 Life" we hope that you will enjoy this meeting in Ljubljana both scientifically and socially, and achieve new partnerships and collaborations.

Prof. Dr. Damjana Rozman Assist. Prof. Dr. Brane Leskošek

COMMITTEES

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Maja Ravnikar (National Institute of Biology, SI)

Thomas Svensson (SciLifeLab, Sweden)

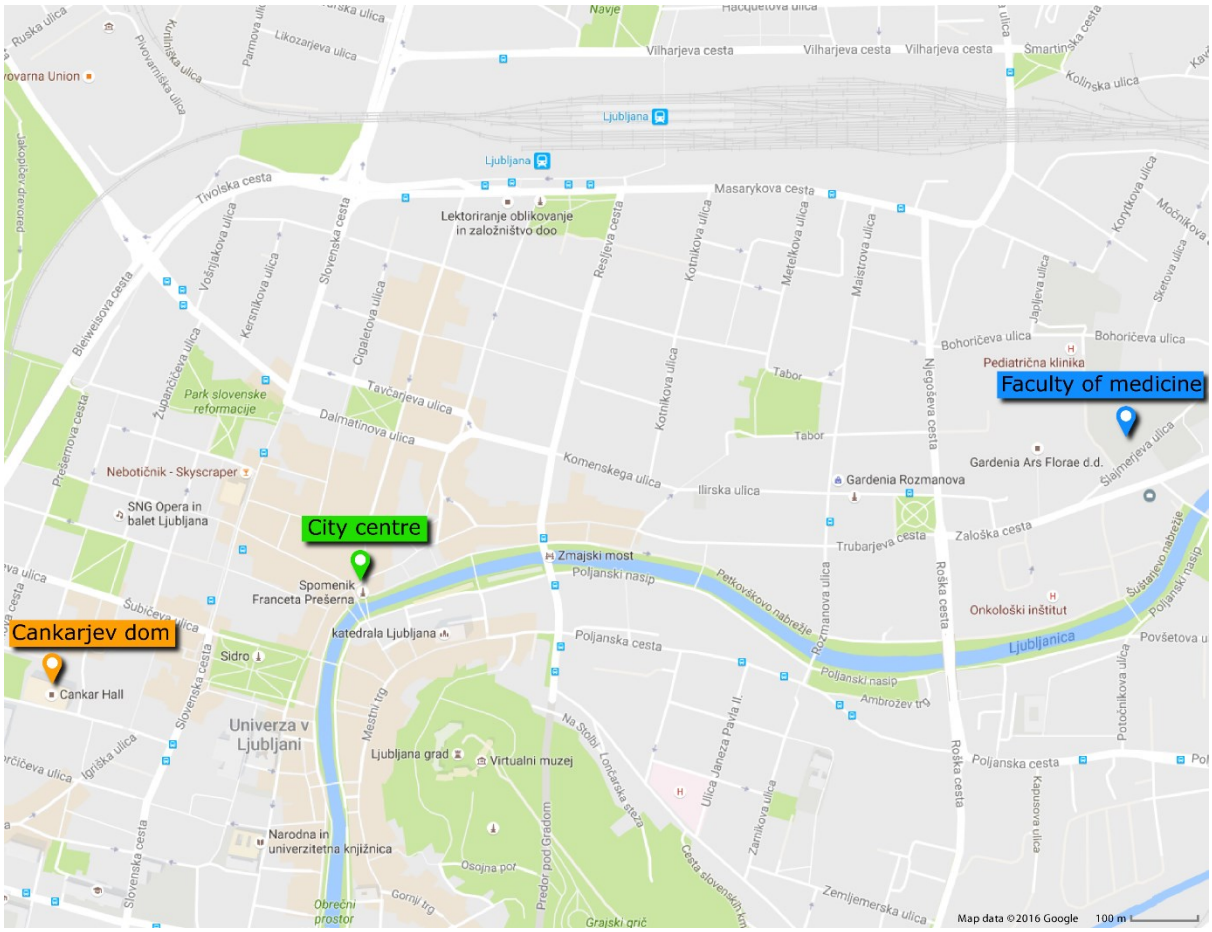
Dame Janet Thornton (European Bioinformatics Institute, UK)

GENERAL INFORMATION

SYMPOSIUM VENUE

The ELIXIR-SI launch on **September 20th** will take place **at Cankarjev dom**, Štihova hall, Prešernova cesta 10, Ljubljana.

The 11th CFGBC symposium on **September 21st** will take place at the **Faculty of Medicine**, University of Ljubljana, Korytkova 2, Ljubljana.



REGISTRATION AND INFORMATION DESK

Tuesday, September 20, 13:00 – 19:00 in front of Štihova hall, at Cankarjev dom, Prešernova 10, Ljubljana.

Wednesday, September 21, 8:00 – 9:00 in the lobby of the Faculty of Medicine, Korytkova 2, Ljubljana.

The certificate of attendance will be issued at the registration desk.

PRESENTATION PREVIEW AND DEPOSITION

Presentation preview point, where speakers can check and load their presentations, will be available in lecture halls. Speakers are kindly requested to bring their presentations in the lecture hall, where the talk will be given, during breaks before sessions.

POSTER SESSION

Poster session will be held in the **lobby of the Faculty of Medicine**, on **Wednesday, September 21**.

Presenters are kindly asked to mount their posters by 11:00 on Wednesday, September 21, and remove them the same day by 18:00.

Presenters are responsible for setting and removing the posters. Material for mounting the posters will be available at the venue. Authors are kindly requested to be present at their poster board for the duration of the poster session.

INTERNET ACCESS

Password for internet access will be provided on Tuesday, September 20, at Cankarjev dom. EDUROAM will be available during the symposium on Wednesday, September 21.

COFFEE BREAKS AND LUNCHES

Coffee breaks and lunches will be arranged in front of Štihova hall at Cankarjev dom on Wednesday, September 20, and in the lobby of the Faculty of Medicine on Wednesday, September 21.

CONTENT

PROGRAMME OUTLINE

TUESDAY, SEPTEMBER 20, 2016 – ELIXIR-SI LAUNCH

13:00 – 13:30 **Registration**

13:30 – 14:00 **Opening ceremony and welcome**
- *Ministry of Education, Science and Sports*
- *ELIXIR Hub*
- *University of Ljubljana*

14:00 – 14:20 **ELIXIR Slovenia – quick overview on history, goals and vision**
Brane Leskošek, Damjana Rozman

14:20 – 14:35 Coffee break

14:35 – 15:00 **Keynote lecture by ELIXIR Hub**
Andrew Smith

15:00 – 15:20 **Keynote lecture by European Commission on ELIXIR and other ESFRI's**
Barend Mons, Chair of High Level Expert Group on "European Open Science Cloud"

15:20 – 15:40 **Keynote lecture by ELIXIR Sweden – Good practices in the Node**
Bengt Persson, Head of Node ELIXIR Sweden

15:40 – 16:00 **Keynote lecture by ELIXIR Netherlands – Good practices in the Node**
Jaap Heringa, Head of Node ELIXIR Netherlands

16:00 – 16:20 **Keynote lecture by ELIXIR Czech Republic – Good practices in the Node**
Jiri Vondrašek, Head of Node ELIXIR Czech Republic

16:20 – 16:55 **ELIXIR Slovenia poster session**

16:55 – 18:30 **International collaborations and projects of ELIXIR Slovenia members (flash talks)**
- *BBMRI-ERIC – gateway for health and data, Markus Pasterk, BBMRI-ERIC*
- *ELIXIR-EXCELERATE, Jure Dimec, Brane Leskošek, University of Ljubljana; Živa Ramšak, NIB*
- *Methods of estimation of key indicators in population cancer survival, Maja Pohar Perme, University of Ljubljana*
- *COST action Systems medicine and European Association for Systems Medicine, Nataša Debeljak, Damjana Rozman, University of Ljubljana*
- *Use of e-infrastructure and ELIXIR-SI services for life science researchers, Avgust Jauk, Arnes; Jan Jona Javoršek, Institute Jožef Stefan*
- *EU-PGx project and EUPancreas COST action – the steps toward the personalized medicine, Vita Dolžan, University of Ljubljana*
- *Synthesis and use of biocompatible materials, Milica Pantić, Željko Knez, University of Maribor*
- *Anaerobic systems biology in the era of multi 'omics approaches: Large-scale methane measurements on individual ruminants for genetic evaluations (COST FA1302), Blaž Stres, University of Ljubljana*
- *Centre of Excellence in Translational Medicine (CETM), Samo Ribarič, University of Ljubljana*

18:30 – 19:00 **Closing ceremony**

19:00 **Welcome reception and cont'd poster session**

WEDNESDAY, SEPTEMBER 21, 2016 – 11TH CFGBC SYMPOSIUM

08:00 – 09:00	**Registration**
09:00 – 09:10	**Opening of the meeting**
09:10 – 09:55	**Keynote lecture:** *Thomas Svensson, SciLifeLab, Sweden* *Bioinformatics support at SciLifeLab - past, present and future*
SESSION 1:	**Data for life in complex human pathologies** ***Chairs: Damjana Rozman & Aleš Maver***
09:55 – 10:20	*Katarina Trebušak Podkrajšek, University Medical Centre Ljubljana, Slovenia* *Next generation sequencing in clinical diagnosis of familial hypercholesterolemia*
10:20 – 10:45	*Aleš Maver, University Medical Centre Ljubljana, Slovenia* *The Slovenian genome variability project*
10:45 – 10:55	*Commercial presentations: Kemomed*
10:55 – 11:05	*Commercial presentations: Mikro+Polo*
11:05 – 11:30	**Coffee break with Poster session 1**
11:30 – 11:55	*Tanja Kunej, Biotechnical Faculty, University of Ljubljana, Slovenia* *Genetic variability of microRNA regulome and its potential for biomarker discovery*
11:55 – 12:20	*Boris Rogelj, Institute Jožef Stefan, Slovenia* *RNA-binding proteins in neurodegeneration*
12:20 – 12:45	*Andraž Šmon, University Medical Centre Ljubljana, Slovenia* *Newborn screening for inherited metabolic disorders*
12:45 – 13:10	*Irena Mlinarič Raščan, Faculty of Pharmacy, University of Ljubljana, Slovenia* *Thiopurine S-methyltransferase (TPMT) pharmacogomics and beyond*
13:10– 14:30	**Lunch**
SESSION 2:	**Systems biology and bioinformatics** ***Chairs: Tadeja Režen & Thomas Svensson***
14:30 – 14:55	*Jure Bordon, Faculty of computer and information science, University of Ljubljana, Slovenia* *Evaluating the kinetic parameters for quantitative models in systems biology*
14:55 – 15:20	*Nada Kraševec, National Institute of Chemistry, Slovenia* *Actinoporin-like proteins through powerful bioinformatics optics*
15:20 – 15:45	*Maja Križnik, Kristina Gruden, National Institute of Biology, Slovenia* *Small RNAs regulatory networks - linking developmental and immune signaling in potato*
15:45 – 16:10	*Uršula Prosenc Zmrzljak, Faculty of Medicine, University of Ljubljana, Slovenia* *Mouse genotypes drive the liver and adrenal gland clocks*
16:10 – 16:35	*Tanja Prunk Zdravković, Faculty of Medicine, University of Maribor, Slovenia* *Influence of selected UV-filters on ABCB5 gene expression in melanoma cells*
16:35 – 17:00	**Coffee break with poster session 2**
17:15 – 18:00	**Keynote lecture:** *Dame Janet Thornton, EBI-EMBL, United Kingdom* *Exploring Human Variation and its impact on proteins and in the Clinic*
18:00 – 18:05	**Closing of the meeting**

ABSTRACTS OF LECTURES

BIOINFORMATICS SUPPORT AT SCILIFELAB - PAST, PRESENT AND FUTURE

Thomas Svensson[1]

[1]DEPARTMENT OF BIOLOGY AND BIOLOGICAL ENGINEERING, CHALMERS UNIVERSITY OF TECHNOLOGY, GÖTEBORG, SWEDEN

Science for Life Laboratory (SciLifeLab) was established during early 2010 with two nodes in Stockholm and Uppsala respectively. The venture was enabled by a strategic grant from the Swedish government and organised as a collaboration between the universities in the respective cities. With regards to bioinformatics support and competence, SciLifeLab has enabled that various initiatives have been co-organised within the same framework.

When SciLifeLab started the immediate bioinformatics focus was on delivering on the expectations built up by "next-generation sequencing", which was both great and somewhat unrealistic at times. Today, six years later, the expectations are even greater, but the infrastructure for bioinformatics support has matured into an organisation that can deal not only with data management, but also with advanced downstream bioinformatics support.

I will walk you through our struggles, highlight with some success stories, describe our current status och make a personal forecast about the future.

NEXT GENERATION SEQUENCING IN CLINICAL DIAGNOSIS OF FAMILIAL HYPERCHOLESTEROLEMIA

Katarina Trebušak Podkrajšek[1,2], Gašper Klančar[3], Urh Grošelj[1], Jernej Kovač[1], Tadej Battelino[1,2]

[1]UNIVERSITY CHILDREN'S HOSPITAL, UNIVERSITY MEDICAL CENTRE LJUBLJANA, LJUBLJANA, SLOVENIA, [2]FACULTY OF MEDICINE, UNIVERSITY OF LJUBLJANA, LJUBLJANA, SLOVENIA, [3]INSTITUTE OF ONCOLOGY, LJUBLJANA, SLOVENIA

Introduction: Familial hypercholesterolemia (FH) is increasing the risk for developing atherosclerosis and cardiovascular disease in early adulthood up to 100-fold, while the risk can be reduced by early diagnosis and disease management. Elevated total cholesterol level, family history of premature cardiovascular complications, presence of xanthomas and corneal arcus and/or causative variants in genes implicated in FH are representing the criteria for the clinical recognition of the disease. Majority of the patients are heterozygous carriers of disease-causing variants in the gene encoding the LDL receptor (LDLR). Minority have disease-causing variants in genes encoding apolipoprotein B (APOB) or proprotein convertase subtilisin/kexin type 9 (PCSK9). Various screening strategies are proposed to identify children with FH. Slovenia is currently the only country with implemented universal screening for hypercholesterolemia in 5-year-old children enabling identification of patients without known family history (1). Screening began in 1995 and was gradually implemented through the whole country.

Subjects and methods: We aimed to identify individuals with FH from the cohort of children with elevated total cholesterol levels detected in the universal national screening. Children with total cholesterol level of more than 6 mmol/L or more than 5 mmol/L with a positive family history for premature cardiovascular disease were genotyped with next generation sequencing (NGS) for variants in 4 genes associated with FH (LDLR, PCSK9, APOE, APOB) with ADH MASTR v1 (Multiplicon) on the MiSeq (Illumina) platform. The variants were validated using Sanger sequencing.

Results: 38.6% of the patients had disease-causing variants in LDLR, 18.4% in APOB and none in PCSK9 (2). The simulated detection rate of FH in Slovenian universal screening based on an assumed 1 in 500 incidence rate was more than 96%, where analytic sensitivity, specificity and accuracy were 100%, while with the 95% confidence level probability of the false negative was 5% and sensitivity 95%.

Conclusions: Universal national screening for hypercholesterolemia at the age of 5 years genetically confirmed FH in more than a half of referred subjects, and was thus predicted to detect almost all assumed patients in the population. This is proving the NGS based strategy an effective tool in early recognition of FH.

References:

1. Sedej K et al. Decreased prevalence of hypercholesterolaemia and stabilisation of obesity trends in 5-year-old children: possible effects of changed public health policies. Eur J Endocrinol 2014;170:293-300.

2. Klančar G et al. Universal screening for familial hypercholesterolemia in children. J Am Coll Cardiol, 2015;66(11):1250-7.

SLOVENIAN GENOME VARIABILITY PROJECT

Aleš Maver[1], Borut Peterlin[1], Alenka Hodžić[1]

[1]UNIVERSITY MEDICAL CENTRE LJUBLJANA, LJUBLJANA, SLOVENIA

Knowledge of natural and morbid genetic variability in human populations is a key foundation for understanding the relationship between genotype and phenotype, especially as we progress into the genome-wide sequencing era. A majority of information on genetic variability in human populations is either gathered on individuals from larger populations of developed western countries or very specific populations of interest. One the other hand, systematic characterisation of genetic variability for several smaller and fragmented populations, including Slovenian, is currently lacking or virtually inexistent. In effect, genetic diagnostics and research of human diseases are lagging due to this paucity of information.

We have been systematically collecting and organising the population and disease-associated genetic variation obtained using exome and genome sequencing data within the Centre for Mendelian Genomics. Since the time of establishment, we have assembled a database of regional genetic variation that includes information on over 10 million variants in populations of Slovenia and neighbouring regions. We have also established the morbid database with the information on over 600 disease-associated variants we identified and reported to date. In addition to small genetic variation in the nuclear genome, we have also systematically analysed and collected the information on mitochondrial variation, copy-number variation and more complex structural variants.

In our presentation, we will illustrate the significance of capturing the regional genetic variability for improved diagnostics and research of human diseases as defined in the Slovenian genome variability project. We will show that progressively richer population resource results in significant improvement and facilitation of genomic data interpretation. Ultimately, we will present the key role of such a population resource for several aspects of personalised medicine, including presymptomatic identification of medically actionable genetic variants based on population data.

GENETIC VARIABILITY OF MICRORNA REGULOME AND ITS POTENTIAL FOR BIOMARKER DISCOVERY

Tanja Kunej[1]

[1]DEPARTMENT OF ANIMAL SCIENCE. BIOTECHNICAL FACULTY, UNIVERSITY OF LJUBLJANA, SLOVENIA

MicroRNAs (miRNAs) are a class of short non-coding RNAs involved in the regulation of gene expression and it has been estimated that they fine-tune the expression of 30% of protein-coding genes. On average each miRNA is predicted to regulate approximately 200 targets. MicroRNAs are part of the complex regulatory network and are associated with several epigenetics concepts. For example, miRNA silencing is one of the classes of epigenetics mechanisms, additionally, miRNA genes themselves could also be epigenetically regulated like any other protein-coding gene. MicroRNAs have been shown to be involved in numerous physiological processes as well as disease development. They have been shown to have potential for diagnostic and prognostic biomarkers as well as treatment targets. However, prioritization of a miRNA candidate for functional studies still presents a challenge because understanding of complex miRNA related interactions is not yet complete. Additionally, the field lacks central miRNA genomics repository and the data are fragmented through various databases and publications. Additionally, several bioinformatics tools are also missing and many of the existing tools are not regularly updated due to constant updates of the source databases. There are several possible directions for miRNA based biomarker prioritization for functional studies. One of the possible strategies is integrated analysis of heterogeneous gene expression profiles for development of robust disease-specific transcriptional fingerprints. Next, potential biomarkers could be located within miRNA regulatory regions (miR-rSNPs), for example within binding sites for transcription factors, within mature miRNA regions (miR-SNPs) and within miRNA target sites (miR-TS-SNPs). Potential biomarkers also include polymorphisms associated with miRNA silencing machinery (miR-SM-SNPs), which are either located within genes encoding for components of miRNA biogenesis (Drosha, Dicer) or within miRNA genes overlapping Drosha/Dicer cleavage sites. Epigenetic silencing of some miRNAs is cancer specific; therefore, this mechanism could also be used for biomarker development. One of the strategies is to first develop an integrated atlas of miRNA gene regulatory elements, consisting of known upstream regulators, downstream targets and overlapping genomics elements, followed by selection of potential biomarkers. Understanding of complex interplay between miRNAs, other classes of non-coding RNAs and protein-coding genes is not yet complete, but is of importance for development of novel biomarkers and for the design of novel therapeutic strategies.

RNA-BINDING PROTEINS IN NEURODEGENERATION

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Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) are devastating neurodegenerative diseases that form two ends of a complex disease spectrum. Cytoplasmic aggregation of otherwise nuclear RNA binding proteins, such as TDP-43 or FUS, is one of the hallmark pathological features of ALS and FTDL, and suggests perturbance of the RNA metabolism and/or nuclear transport in their aetiology. In 95% of all ALS and 60% of FTLD patients the aggregating protein is TDP-43, thus defining the major part of the disease spectrum as TDP-43 proteinopathies. However, only a very small percent of aggregation is caused by TDP-43 mutations. Therefore, the main questions in the field are what makes wildtype TDP-43 or FUS mislocalize and aggregate in ALS and FTLD and what is the role of perturbed RNA metabolism in these diseases. Recent identification of the disease-associated expansions of the intronic hexanucleotide repeat GGGGCC (G_4C_2) in the C9orf72 gene further substantiates the case for RNA involvement. This hexanucleotide repeat expansion mutation (HREM) has turned out to be the single most common genetic cause of ALS and FTLD and also presents itself as TDP-43 proteinopathy. HREM may enable the formation of complex DNA and RNA structures, changes in RNA transcription and processing and formation of toxic RNA foci, which may sequester and inactivate RNA binding proteins. This complexity is furtherer increased by the fact that expanded repeat is also transcribed in the antisense direction forming the CCCCGG (C_4G_2) repeat RNA. Additionally, the transcribed expanded repeats from both directions can undergo repeat-associated non-ATG-initiated (RAN) translation resulting in accumulation and aggregation of a series of dipeptide repeat proteins.

NEWBORN SCREENING FOR INHERITED METABOLIC DISORDERS

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Introduction: Screening methods are very important in preventive medicine; their goal being the detection of the disease before the development of clinical signs. Tandem mass spectrometry plays a major role in newborn screening of inborn errors of metabolism, as it allows screening for more than one disease simultaneously, short time of analysis and high sensitivity. In one test tandem mass spectrometry allows quantification of many amino acids and acylcarnitines from dried blood spots, which allows detection of disorders in amino acid metabolism, organic acidurias and fatty acid oxidation disorders.

Slovenia currently screens newborns only for phenylketonuria and congenital hypothyroidism. Last year a pilot study of expanded newborn screening for inborn errors of metabolism using tandem mass spectrometry started. It will contribute to the development of optimal strategy of newborn screening for inherited errors of metabolism in Slovenia and determination analyte cut-off values. 10000 dried blood spots from newborns were analysed retrospectively for the following disorders, which are included in most screening programmes worldwide: MCAD (medium-chain acyl-CoA dehydrogenase deficiency), GA 1 (glutaric aciduria type 1), GA 2 (glutaric aciduria type 2), 3-MCC (3-methylcrotonyl-CoA carboxylase deficiency), MSUD (maple syrup urine disease), VLCAD (very long-chain acyl-CoA dehydrogenase deficiency), LCHAD, IVA, PA/MMA (isovaleric aciduria / methylmalonic aciduria), CUD (carnitine uptake deficienc), CPT 1, CPT 2. We also included phenylketonuria, so we could compare our results with the results of the current method for phenylketonuria screening (fluorimetric detection of phenylalanine).

Results: The study is still ongoing; final confirmation tests are in progress. 5 cases of inborn errors of metabolism were identified so far. First case was a VLCAD deficiency, which is a fatty acid oxidation disorder (the case was confirmed with sequencing, enzymatic activity analysis and palmitate loading test). 4 cases of organic acidurias were found; three cases of 3-MCC deficiencies (confirmed with organic acid analysis in urine) and one GA 1 (confirmed with enzymatic activity analysis). In our study we also detected two already known patients with phenylketonuria.

Conclusions: Based on the preliminary results from the pilot study the cumulative incidence of inborn errors of metabolism (7 cases in 10000 newborns) is high in Slovenia. We are currently doing follow-up tests on selected newborns with the highest disease possibility to set the cut-off values for the chosen disorders.

THIOPURINE S-METHYLTRANSFERASE (TPMT) PHARMACOGOMICS AND BEYOND

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The enzyme thiopurine S-methyltransferase (TPMT) plays a major role in the deactivation of thiopurines and is to a large extent responsible for inter-individual differences in response to treatment. Although polymorphisms in the TPMT gene are the major cause for reduced enzyme activity, the genotype and enzyme activity is incomplete.

The challenge in this field is thus to identify novel biochemical and genetic factors, which either by influencing TPMT activity or, independently of TPMT, influence the efficacy and safety of acute limfoblastic leukemia (ALL) treatment with thiopurines.

We have shown that mutated MTHFR gene augmented the effect of mutated TPMT gene, to 6-MP related toxicities in childhood ALL patients.

We have reported on the impact of the cofactor and methyl donor S-adenosyl methionine (SAM) on TPMT activity and on the cytotoxic effects of 6-MP. SAM may modulate TPMT activity in the intercellular setting, possibly by post-translational stabilization. Consequently, the endogenous availability of SAM may influence TPMT activity, the formation of 6-MP metabolites, and the toxicity of thiopurine drugs.

Further we showed protective role of inosine triphosphate pyrophosphatase (ITPA) polymorphisms in relation to event-free survival and relapse rate in pediatric ALL.

The influence of PACSIN2 (rs2413739) on appearance of side effects in pediatric acute leukemic patients was demonstrated.

The genome-wide association study (GWAS) approach has also been used to comprehensively investigate the relationship between constitutional genotype and TPMT activity.

EVALUATING THE KINETIC PARAMETERS FOR QUANTITATIVE MODELS IN SYSTEMS BIOLOGY

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Introduction: Modelling of biological systems has become an indispensable tool in designing of novel and analysis of existing biological systems. It can significantly reduce the time and cost of experimental work and is an integral part of interdisciplinary fields such as computational biology, bioinformatics, systems and synthetic biology, and systems medicine, each with their own goals and each tackling different aspects of understanding life as it is [1,2]. The choice of modelling technique depends on the complexity of the system we are observing, desired accuracy of simulation results or the topology, and the availability of data, which are required for model construction. Existing quantitative methods are mostly based on the numerical simulations of the system of ordinary differential equations or chemical master equation. In order to quantitatively model processes using these approaches we require accurate kinetic data, which govern the system's dynamics. However, these are often unavailable and are most of the time hard or even impossible to measure experimentally.

Results: We present the possibility of using expert knowledge to construct a model which produces quantitatively relevant results, even when some kinetic data is missing or is only vaguely defined. This approach is based on fuzzy logic, which uses linguistics to describe biological processes. In addition, deep mathematical knowledge is not needed to construct a model using fuzzy logic. Using this approach, we constructed a model of the circadian rhythm of *Neurospora* [3]. Simulation results show that in most cases using linguistic approach retains the quantitative aspect of the conventional model.

Conclusion: Processes in a model based on the proposed approach are described linguistically and an expert from any field of life science with minimal mathematical background could use an existing fuzzy logic framework to define the model of a biological system he desires to simulate and still obtain quantitatively relevant simulation results.

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ACTINOPORIN-LIKE PROTEINS THROUGH POWERFUL BIOINFORMATICS OPTICS

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Introduction: Beside the need to understand the functioning of human being in details, it is also necessary to study (fungal) pathogens if we want to treat emerging diseases successfully. When mining fungal genomes several challenges limit the reliability of data sets comparisons (Meyer et al 2016). Superfamily of actinoporin-like proteins (ALP) comprises diverse protein families sharing structural similarity (a rigid b-sandwich flanked by two a-helices) but low sequence similarity. Aegerolysins have been described to exhibit pleiotropic functions; some of them are hemolytic in the presence of another MACPF-domain containing protein or they are expressed during formation of fungal primordia and fruiting bodies. The mushroom lectin XCL after sugar binding induces changes in the target cytoskeleton and has insecticidal activity. Infiltration of oomycetal NPP1 into plant leaves results in accumulation of PR genes, production of ROS, ethylene, callose apposition and cell death.

Results: We performed genome mining in fungal kingdom and comparison of lifestyles, analysis of loci, promoters, transcription, secretion and protein signatures. Genome datasets analyzed are hosted at different resources, offering benefits and limitations: NCBI - limited fungal hits, several Blast options; Mycocosm – lots of fungi, limited tools; AspGD – Aspergilli, lots of tools & data; E-Fungi - limited in fungi, useful search tools. Other applied tools were: PFAM - search by domain, limited in fungal proteins; SecretomeP - prediction of secretion, mammals based; for PHYRE2 - protein structure prediction, and CLUSTALW - multiple sequence alignment, identity threshold is empirically set. Distribution of fungal fruit body lectins, necrosis inducing proteins and aegerolysins was heterogeneous; FB lectins were rare, NPP1 and aegerolysins overrepresented. Without at least one member of the protein family per species, we consider ALP as noncore proteins. Some of aegerolysins co-distribute with MACPF. No correlation to taxonomy or pathogenic lifestyle was observed. We ascribe a part of ALP as small secreted proteins, also without recognizable signal peptide.

Conclusions: The quality of early genome sequences is limited due to the sequencing technology but data were often manually curated. Recent genomes suffer from propagation of errors due to automated annotation. The accuracy of gene calling is complex due to numbers of introns. If a genome is included in more resources, it is not always the same version of sequence or annotation. A single genome sequence is available for most species, usually from a lab pet. Variation in experimental conditions between datasets makes comparability difficult. Omics data are deposited in raw formats making them usable for researchers with bioinformatics skills. When collecting proteins from more sources it is difficult to define a pull of screened species. Fungal lifestyle is not univocally described.

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SMALL RNAS REGULATORY NETWORKS - LINKING DEVELOPMENTAL AND IMMUNE SIGNALING IN POTATO

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Plants respond to pathogen infections by activating variety of defence mechanisms, which can be detected as a broad spectrum of physiological and histological changes. One of such changes is the induction of changes in small RNA (sRNA) level. The major classes of plant sRNA types include microRNAs (miRNAs) and small interfering RNAs (siRNAs); one specific type of the latter are secondary phased siRNAs [1]. sRNAs have emerged as key post-transcriptional regulators of genes. They can directly regulate gene expression by targeting mRNAs through complementary base pairing. In plants, sRNAs are involved in various biological processes, including growth and development, hormone signalling and defense responses against several pathogens [2]. The growing body of evidence suggests that sRNAs are important in plant defense immunity, albeit none of the studies performed so far investigated sRNA regulatory level in potato. Therefore, we aimed at unraveling the role of sRNAs in a complex immune signaling network controlling defense responses that render the plant tolerant to the viral infection. We have identified and quantified miRNAs as well as phasiRNAs and viral sRNAs in the PVYNTN tolerant cv. Désirée and its susceptible transgenic counterpart impaired in accumulation of salicylic acid (NahG-Désirée). We have identified more than 93 differentially expressed miRNAs/phasiRNAs at the onset of viral replication in cv. Désirée. The miRNA response was however strongly attenuated in salicylic acid deficient plants suggesting that salicylic acid plays an important role in enhancing the miRNA regulatory network response to PVYNTN infection. Next, a miRNA regulatory network was constructed using in silico prediction as well as degradome sequencing and gene expression data to link our response on sRNA level to the physiological response. As already described for some other pathosystems, regulation of immune receptor transcripts is under control of this network. In cv. Désirée however the NBS-LRR targeting miRNAs were not down-regulated, but up-regulated, resembling the regulation of these genes in symbiotic interactions. We have additionally discovered an interesting novel connection between sRNAs and gibberellin biosynthesis. Increased levels of miRNA167 and several phasiRNAs were reflected in decreased levels of the target transcripts involved in gibberellin biosynthesis in the genotype Désirée only. We have functionally confirmed this interaction as a reduced level of biologically active gibberellin was measured in cv. Désirée. The intertwining of sRNA and hormonal networks revealed here, sheds novel insights into regulation of developmental signalling, symptoms development and stress signalling.

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MOUSE GENOTYPES DRIVE THE LIVER AND ADRENAL GLAND CLOCKS

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Background: Circadian rhythms regulate a plethora of physiological processes. Perturbations of the rhythm can result in pathologies which are frequently studied in inbred mouse strains. Genotype of different mouse strains effects circadian physiology: free-running activity rhythms, feeding cycles, phase-shifting effects etc.
Methods: We compared expression patterns of two mouse strains: C57BL/6JOlaHsd and 129S2/SvPasxC57BL/J6 in dark – dark (DD) and light – dark (LD) experimental conditions. With exome sequencing and data mining we searched for possible single nucleotide variants (SNVs) that could explain the difference in expression patterns. With the use of Imputed Mouse SNP Resource (http://csbio.unc.edu/imputation/) and data from ChIP seq experiments in circadian settings 1 we evaluated if SNVs in clock protein binding regions are more frequent than in open chromatin regions 2 that are considered as transcription active places.
Results: Expression of the majority of core clock and output metabolic genes is phase delayed in the C56BL/6J line compared to 129S2 in the adrenal glands and the liver. Circadian amplitudes are generally higher in the 129S2 line. Exome sequencing data proposed that mouse lines differ in SNVs in the binding regions of clock related transcription factors in open chromatin regions. One of the possible mechanisms of differential circadian expression could be the entrainment and transmission of the light signal to the peripheral organs. This is supported by the genotype effect in adrenal glands that is largest under LD, and by the high number of single nucleotide variants in the Receptor, Kinase and G-protein coupled receptor Panther molecular function categories. Different phenotype of these two mouse strains and changed amino acid sequence of the Period 2 protein possibly contributes further to the measured differences in circadian gene expression. 3
Conclusions: Genotype of mouse lines defines the circadian gene expression patterns.
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INFLUENCE OF SELECTED UV-FILTERS ON ABCB5 GENE EXPRESSION IN MELANOMA CELLS

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Introduction: There is no clear evidence on whether sunscreens and personal care products containing UV filters like Octocrylene (OCT) and Titanium dioxide (TiO2) are protective against or may be a contributing factor in melanoma development. A transmembrane protein ABCB5 is involved in tumor progression, disease recurrence and in melanoma clinical drug resistance. The aim of the present study was to investigate the influence of OCT and TiO2 on the proliferation activity of melanoma cells and on their ABCB5 mRNA expression.

Methods: Metastatic melanoma cell line was used and treated with different concentrations (from 1 to 250 µg/mL) of OCT or TiO2 (in the form of nanoparticles: nano-TiO2 or with the average particle size of ≤ 5micron: micro-TiO2) and incubated for up to 144 hours. We used the MTT and LDH assays to measure cells' proliferation activity and cytotoxicity, respectively. Quantitative real-time PCR using TaqMan chemistry was performed and relative gene expression ratios were calculated for the target (ABCB5) and the reference - endogenous control (LDHA) gene. We used ANOVA and post-hoc Bonferroni tests for statistical analysis.

Results and conclusions: OCT group resulted in increased ABCB5 mRNA expression at 24 and 48h of exposure when compared to 2h ($p<0.01$). The increase was 2-fold at 250 µg/mL and 5-6 fold at lower OCT concentrations after 48h. Concomitantly, reduced cell number for 1.3% to 11.6% at 48h, increased proliferation activity at 8h and thereafter decreased, and morphological changes (including cannibalistic activity) were observed.

On the other hand, our results suggest that TiO2 might open a new window in the treatment modalities of melanoma. Micro-TiO2 is progressively decreasing the ABCB5 mRNA expression, however nano-TiO2 has a rebound increase at 48h of exposure at all but one concentrations ($p<0.05$) and then a significant decrease after 120 hours of exposure ($p<0.01$). This increase raises questions which should be answered before any potential use in medicine.

EXPLORING HUMAN VARIATION AND ITS IMPACT ON PROTEINS AND IN THE CLINIC

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Genome sequencing has opened up the possibility of exploring the genetic basis of human evolution and the differences between individuals. Using computational methods and 3D protein structures, we compared disease associated variants with 'natural' variants observed in 1000 human genomes, showing significant differences in their distributions. Many of the mutations that most often because diseases were the least frequently observed 'natural' variants. Recently we have been exploring how protein domain information can help in the interpretation of the effects of non-synonymous mutations, especially from a structural perspective. We observe that the 'equivalent' mutation in the same domain family but in different proteins, can have very different consequences, depending on the context in which it occurs.

The 100,000 genomes project, funded by the UK government through the NHS, provides a stimulus to bring this new technology into the clinic. This brings both challenges and great opportunities. One of the major challenges is handling the scale and interpreting the complexity of genomic data. This will require a close collaboration between the basic biological and clinical sciences if it is to make a major impact. These challenges will be discussed.

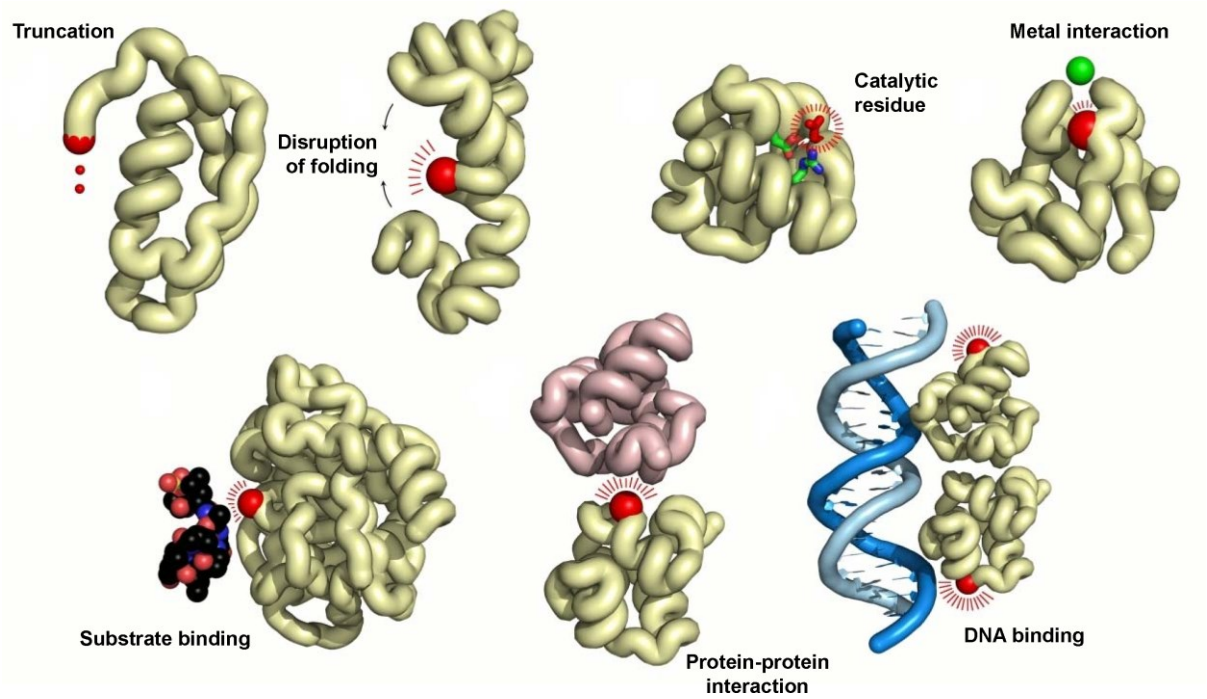

FIGURE 1: Figure Legend: Inherited diseases and causative variants. Structure can help explain a variant's disruption of protein function (by Roman Laskowski)

ABSTRACTS OF POSTERS

IDENTIFICATION OF MOLECULAR AND METABOLIC DIFFERENCES BETWEEN NASH AND HCC OF *CYP51* KNOCKOUT MICE

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Introduction: Nonalcoholic fatty liver disease (NAFLD) encompasses numerous histopathological features, ranging from simple steatosis to steatohepatitis (NASH), which is characterized by a more serious form of liver damage and fibrosis. NASH can progress to inflammation- and oxidative stress-induced cirrhosis and even hepatocellular carcinoma (HCC) [1,2]. Based on the literature, up to 29% of HCC cases could result from NAFLD [3]. The purpose of this study is to identify the differences between NASH and HCC with regard to gene expression and metabolism that may provide deeper insight into the progression of disease.

Methods: Experiments were performed on the hepatocyte knockout mouse model of lanosterol 14α-demethylase ($H^{Cyp51-/-}$) to evaluate the age-related changes due to cholesterol imbalance in the liver. Since liver is a sexually dimorphic organ we performed experiments on animals from both sexes aged 1, 1.5 and 2 years. Special focus was paid to molecular mechanisms which are important for the initiation and progression from NASH to HCC. Histopathology was performed on H&E liver sections. Biochemical parameters were measured in plasma. Gene expression profiling was conducted by qPCR. The data was processed and analysed using two-way ANOVA test.

Results: At the ages of 1 year and older, the $H^{Cyp51-/-}$ mice exhibit advanced chronic inflammation and fibrosis, which are characteristic for NASH. In our model NASH progresses to HCC with an age-dependent liver pathology and sexual dimorphism, with a HCC female to male ratio of 2 to 1. Liver is a sexually dimorphic organ with crucial metabolic pathway differing between females and males. Special focus is paid to hepatocyte cholesterol synthesis in the liver, where fundamentally up-regulated cholesterogenic genes (*Sqle*, *Lss*, *Nsdhl*, *Tm7sf2*) in 1.5 year old males were observed. Lipid plasma parameters were mostly unchanged, however, elevated ALT and AST ratio in KO mice pointed to liver injury.

Conclusions: Mechanisms involved in HCC development in $H^{Cyp51-/-}$ mice include a combination of chronic complementary effects: the block of the hepatocyte *Cyp51* reaction resulted in deregulation of the sterol network, which promotes inflammation and ER stress. At the same time, it leads to metabolic deregulation due to deficiency in post-lanosterol RORC ligands. Our future perspective is to assess how this chronic inflammatory environment with leukocyte infiltration and activated cytokine signalling influences on RORC, WNT and HGF signalling and find major hallmarks in transition from NASH to HCC.

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USE OF STEATONET AS A PREDICTIVE TOOL TO DISCLOSE THE COMPLEXITY OF GENDER-BASED LIVER-RELATED DISEASES

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Introduction: Almost 200 reactions are used by computational model *SteatoNet* to describe complex interactions of the liver with peripheral tissues. Metabolic, gene regulatory and signal transduction pathways are all included within the model. Due to its object-oriented nature, it can be easily adapted to liver-associated pathologies such as non-alcoholic fatty liver disease. Furthermore, it can be used to perform dynamical simulations even in the case of sparse experimental data (1).

Problem: Liver is one of the most sexually dimorphic organs, surpassed only by testes and ovaries. Gender-based differences were discovered also in the livers of mice with hepatocyte deletion of *Cyp51*, which have and shown how disrupted hepatic cholesterol synthesis reflects differently on the whole body homeostasis in males and females (2).

Results: Current modifications of *SteatoNet* include simulations of sex hormone levels in the blood and their networking with liver and peripheral tissues. To our knowledge *SteatoNet (F/M)* represents the first gender-based liver metabolic model. To set one's sight on the predictive nature of SteatoNet, consequences of the disrupted hepatic cholesterol synthesis in adipose tissue will give an excellent starting point for further experimental testing.

Conclusion: *SteatoNet* has the potential to be developed further into a diagnostic, predictive or analytical tool geared towards personalized treatment of patients. It additionally shows large potential to predict the network effects of polymorphisms associated with liver-related pathologies.

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DISCOVERING BIOMARKERS OF ALZHEIMER DISEASE

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It is estimated that there were 46.8 million cases of dementia in the world in 2015 [1]. The most frequent cause of dementia is Alzheimer disease (AD), which is associated with extracellular amyloid plaques and intracellular neurofibrillary tangles [2]. 95% of cases with AD can be classified as late onset AD (LOAD), of which the biggest genetic risk factor is Apolipoprotein E (*APOE*) E4 allele. The carrier of this allele does not necessarily get LOAD, but E4/- heterozygotes have two to three-fold increased risk of LOAD, while E4/E4 homozygotes have five-fold increased risk of LOAD. Carrying this allele is also associated with earlier onset of the disease [2,3]. The frequency of *APOE* E4 allele varies by region and its estimate is the highest in Northern Europe and the lowest in Asia and Southern Europe [3]. The aims of our research are (i) to assess the frequency of *APOE* alleles in Slovene AD and non-AD population, as such estimate was not done yet, and (ii) to enable more accurate assessment of risk of AD in Slovenia with incorporation of genetic biomarkers into diagnosis protocol. Test subjects will be recruited in clinic of Centre for neurodegenerative diseases at Neurological clinic from patients with diagnosed AD by established clinical criteria. Controls will be recruited from patients, relatives and companions without diagnosed AD and with comparable demographics (sex and age), as the test group. We estimate to have at least 200 subjects in each group. The peripheral blood will be taken from subjects and from it the DNA will be isolated and used for *APOE* genotyping. Our project started with application for ethical approval from the Commission of the Republic of Slovenia for medical ethics. At the same time, we assembled a database which could be used routinely in diagnostic laboratories for verification of genetic biomarkers. By doing this we expect to achieve traceability of the patients' tissue samples and their anonymity. After verifying and improving both the database and the protocol, the ApoE genotyping will be performed.

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FACILITATING TREATMENT SELECTION IN MALIGNANT MESOTHELIOMA USING CLINICAL-PHARMACOGENETIC MODELS

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Introduction: Malignant mesothelioma (MM) is an aggressive tumor with poor prognosis. Large interindividual differences in response to standard gemcitabine/cisplatin (GEM/CIS) or pemetrexed/cisplatin (PMX/CIS) chemotherapy present an important issue in MM treatment. Because both clinical characteristics and genetic variability may affect treatment outcome, our aim was to construct and validate clinical-pharmacogenetic prediction models of treatment outcome in MM for both chemotherapy combinations and to develop an algorithm for genotype-based treatment recommendations.

Methods: Clinical-pharmacogenetic models were built on 71 GEM/CIS-treated and 57 PMX/CIS-treated MM patients. Pharmacogenetic scores were assigned by rounding the regression coefficients. GEM/CIS model was validated on 66 independent MM patients.

Results: The model for predicting response to GEM/CIS chemotherapy included CRP level, histological type, performance status, *RRM1* rs1042927, *ERCC2* rs13181, *ERCC1* rs3212986, and *XRCC1* rs25487 with scores ranging between 0 and 3.4. Cutoff value of 0.75 had sensitivity of 0.62 and specificity of 0.81. Patients with higher score had significantly shorter progression-free and overall survival ($P<0.001$). In the validation group, positive predictive value was 0.74 and negative predictive value was 0.56. The model for predicting response to PMX/CIS chemotherapy included CRP level, *MTHFD1* rs2236225, and *ABCC2* rs2273697 with scores ranging between 0 and 3.9. Cutoff value of 2.7 had sensitivity of 0.75 and specificity of 0.61. Patients with higher score had significantly lower probability of good response and shorter progression-free survival ($P<0.001$).

Conclusions: Clinical-pharmacogenetic models could enable stratification of MM patients based on their probability of response to GEM/CIS or PMX/CIS, thus facilitating treatment selection. This approach could therefore improve treatment outcome and also enable translation of pharmacogenetic testing to clinical practice.

NITROSATIVE STRESS, TELOMERE LENGTH AND TELOMERE DYNAMICS IN JUVENILE PATIENTS WITH TYPE 1 DIABETES

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Type 1 diabetes (T1D) is a disease caused by autoimmune destruction of pancreatic β-cells. Lack of insulin is associated with the chronic state of hyperglycemia, oxidative stress and inflammation and leads to development of diabetic complications. The aim was to evaluate telomere length (TL) and TL dynamics as biomarkers in juvenile patients with T1D in relation to glycemic control and nitrosative stress.

44 juvenile participants with T1D, 17 with poor (HbA1c>9%) and 27 matched patients with good glycemic control (HbA1c<7.5%) were included in the study. 237 chronological DNA samples were assessed for relative TL telomere dynamics. Additionally, nitrosative stress was measured with ELISA assay using 8-Nitroguanine (8-NO2-Gua) as a biomarker in plasma samples of 10 patients with poor glycemic controls and their matched good glycemic controls.

Our study indicates increased nitrosative stress in patients with poor glycemic control (Fig. A), but TL association with glycemic control is not seen probably due to the short investigated period of time. The linear regression of all TL measurements indicates the impact of T1D duration, even after adjustment to the age of participants (Fig. B).

A larger number of chronological samples over longer period of disease duration would be required to confirm telomere dynamics and nitrosative stress in association with glycemic control.

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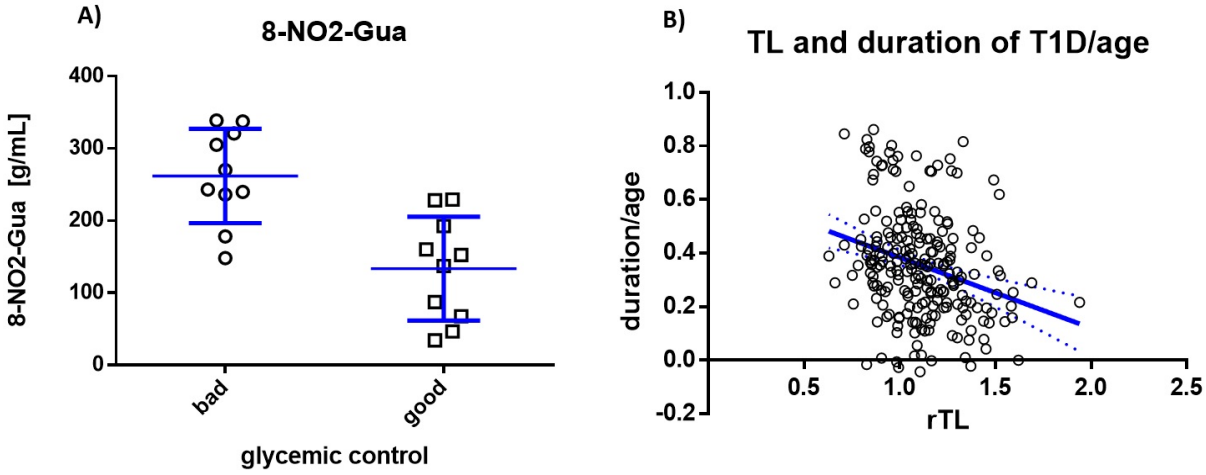

FIGURE 1: Fig. A: The difference in 8-NO2-Gua concentration between T1D patients with poor (c=256.7 ng/mL) compared to good glycemic control (c= 144.8 ng/mL; p=0.0007). Fig. B: The correlation between rTL and duration of T1D adjusted for age (slope= -0.264, r2= 0.077, p<0.0001)

ASSOCIATION OF POLYMORPHISMS IN AURKA AND POLO KINASES WITH GASTRIC CANCER RISK

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Introduction: Gastric cancer is in decline in most developed countries; however, it still accounts for a notable amount of global mortality and morbidity related to cancer. It is associated with *H. pylori* infection, EBV virus, and diet, as well as with genetic and epigenetic factors. In addition, it has been established that chromosomal instability (CIN) plays an important role in the development of gastric cancer. It was assumed that CIN could develop due to aberrant segregation of chromosomes during mitosis. Polymorphisms in genes implicated in chromosome segregation could affect their expression and could therefore increase the risk of developing cancer.

Aim: The aim of our research was to define the association between polymorphisms in segregation genes, including rs911160 (gene *AURKA*), rs2289590 (gene *AURKB*), rs11084490 (gene *AURKC*) and rs42873 (gene *PLK1*), and risk of developing gastric cancer. We also evaluated the association between polymorphisms and clinicopathological characteristics of gastric cancer.

Methods: We performed a retrospective case-control study. Genotyping was performed using real-time polymerase chain reaction. (RT-PCR). The results were statistically evaluated.

Results: Polymorphism rs11084490 in gene *AURKC* was associated with invasion of cancerous cells in lymphatic tissue (F=13,550; p=0,001). This association was most significant for patients with genotype CC. Genotype GG was associated with diffuse type of gastric cancer (F=5,837; p=0,041) and the poorest survival rates (Mantel-Cox test; $\chi 2$ =6,557; p=0,038), whereas genotype CG was found commonly in the group of patients with intestinal type of cancer. We also confirmed a strong association between survival of patients with gastric cancer and Lauren classification and perineural invasion.

Conclusions: We confirmed that alleles of polymorphism rs11084490 in gene AURKC were associated with key histopathological features of gastric adenocarcinomas and survival rate of the patients. Polymorphisms, like rs11084490, could be therefore used as prognostic biomarkers.

NORMALIZATION OF ERYTHROPOIETIN AND ESTROGEN RECEPTORS DETECTION IN BREAST CANCER CELL LINES

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Introduction: Hormone dependent breast cancer is characterized by estrogen receptors (ER), enabling treatment with an estrogen receptor antagonist tamoxifen. Signalization of ER with other membrane receptors, including erythropoietin receptor (EPOR), presumably plays a role in resistance to tamoxifen [1, 2]. In aim to characterize the level of ER and EPOR receptors on multiple breast cancer cell lines via Western blot, appropriate normalization and validation procedure is necessary.

The most common normalization controls include housekeeping proteins, such as Glyceraldehyde 3-phosphate dehydrogenase (GAPDH), beta-actin and tubulin. However, in cancer cells the expression of many proteins including housekeeping proteins may be altered [3]. Therefore, it is important to validate with appropriate normalization control. Furthermore, antibody validation is crucial to confirm antibody specific and reduce batch-to- batch variations [4].

The aim of our study is to determine appropriate normalisation process for selected breast cancer cell lines and to test different primary and secondary antibodies to ensure specificity and reproducibility of the results.

Results: Total proteins were isolated from breast cancer cell lines (human MCF-10A, MCF-7, T-47D, MDA-MB 361, MDA-MB 231, Hs578T). EPOR, ERa, ERb, membrane G protein-coupled estrogen receptor (GPER) and beta-common receptor (bcR) were analyzed and quantified by standard Western blot analysis. GAPDH was tested as a normalization body. Different quantities of secondary antibodies were tested.

The expression of EPOR was confirmed in all analyzed cell lines, specificity of other receptors varied upon antibodies. The normalization of results using GAPDH is not optimal, because of uneven GAPDH expression among analyzed cell lines. Normalization to total amount of protein was also applied. Normalization with other antibodies is in progress.

Conclusions: Lack of specificity and reproducibility are common problems among commercial antibodies, therefore antibodies that show multiple nonspecific signals should be carefully validated before use. In our further work we will choose appropriate positive and negative controls in order to determine whether the detected proteins correspond to our target of interest.

To ensure accurate quantitative data from Western blot analysis we need to establish an appropriate normalization procedure. We found that GAPDH is not a suitable normalization control. To overcome this problem, we will analyze the expression of some other housekeeping proteins and also attempt normalization to total amount of protein in a sample using a protein stain such as Coomassie brilliant blue.

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DESIGN OF SYNCHRONOUS TRANSCRIPTION BASED BIOLOGICAL MEMORY

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Introduction: Attention toward the implementation of robust and scalable biological memory has been increasing in recent years. Despite significant progress in the field of synthetic biological memory, the implementation of scalable synchronous sequential structures in biological systems has yet to be achieved. This type of structures is essential for the implementation of more complex biological information processing structures, since they provide synchronisation between the logic elements, which results in a robust behaviour of the system.

Results: We present a computational design of an edge-triggered D flip-flop based on transcriptional logic. The edge-triggered flip-flop was implemented with a master-slave configuration of the basic clocked D flip-flop. We applied a genetic algorithm to tune the response of the topology with the calibration of kinetic parameter values. We also confirmed the robustness of the topology using a global sensitivity analysis framework developed specifically for the purpose of dealing with high-dimensional and poorly connected parameter spaces, as is the case in the proposed system. The designed structure was then applied to a robust implementation of a Johnson counter, which can count up to 2n events using a sequence of n flip-flops.

Conclusions: The described memory elements are robust and therefore satisfy the requirements of many applications of synthetic biology. The key advantage of our approach is the use of biologically relevant data in the optimization process. This enables the potential construction of biological parts used in the proposed counter topology and, with that, paves the path for an in vivo implementation of biological storage and synchronisation devices.

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TIME-DEPENDENT STOCHASTIC GLOBAL SENSITIVITY ANALYSIS OF GENE REGULATORY NETWORKS WITH MULTIPLE TRANSCRIPTION FACTOR BINDING SITES

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Introduction: Sensitivity analysis methods are widely applied to the assessment of system's robustness and can be used to efficiently tune the modelled response with the experimental results. The Morris sensitivity analysis is a screening method that has been extensively used for performing the global sensitivity of chemical reaction network models in systems biology [1].

Results: We present an adaptation of the Morris technique based on a One-Step-At-A-Time (OAT) screening experiment for investigating the time-dependent sensitivity of quantitative parameters in the stochastic models of gene regulatory networks (GRNs) with multiple transcription factor binding sites. Models describing these networks can quickly become intractable, because of the exponential number of binding reactions. We efficiently reduced their numbers with the application of the multiscale stochastic simulation algorithm (mSSA) which is able to perform the simulations in the observed systems in a feasible time. Moreover, we further reduced the analysis time with the coarse grained parallelization of the mSSA introducing the parallel mSSA algorithm (ParMSSA). ParMSSA is used by the screening experiment to speed-up the simulation time of multiple mSSAs required to obtain statistically relevant results. We demonstrate the proposed approach by performing the sensitivity analysis on different GRNs with multiple binding sites.

Conclusion: The proposed adaptation of the Morris sensitivity analysis method allows us to efficiently perform the analysis of the complex GRNs additionally taking into account the stochasticity of their dynamic response. Its parallelisation allows us to confront the computational complexity of the Morris sensitivity analysis. This allows us to more accurately identify the most relevant parameters governing the dynamics of the system. Furthermore, it enables us to perform additional model reduction and simplification, and to accurately tune its response with the experimental results taking into account the stochasticity of the model response even in complex GRNs.

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GLOBAL SENSITIVITY ANALYSIS OF BIOLOGICAL MODELS WITH HIGH-DIMENSIONAL AND POORLY CONNECTED PARAMETER SPACES

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Introduction: Sensitivity analysis methods have been widely applied to systems as well as synthetic biology. They are able to assess the system's robustness, can guide parameter estimation, experimental design and can serve as model validation tool. Sensitivity analysis methods can be divided in local and global methods. While local methods analyse system sensitivity in the local neighbourhood of the nominal parameter value for single parameter at a time, global methods tend to investigate whole space of possible parameter space applying different sampling techniques. Local sensitivity analysis methods have proven to be inefficient when parameter values are either missing, partially known or exhibits large variation. On the other hand, global sensitivity methods fail when viable solution space is small in comparison to the whole space, which is often the case when dealing with high dimensional models.

Results: We present a computational framework that is able to perform the global sensitivity of biological models having high-dimensional and poorly connected viable parameter regions. The framework can be decomposed into the following steps: (1) generating the solutions that describe viable parameter regions using optimisation meta-heuristics, (2) clustering the solutions based on their connectivity into viable parameter regions, (3) parameter sampling within the clusters, (4) assessing the global sensitivity values using generated samples, and (5) merging the sensitivity values for each parameter from each of the clusters into sensitivity values describing parameter influence on broad system dynamic. Viable solutions are obtained with genetic algorithms. The solutions are then clustered together by k-means method. Orthogonal sampling is then performed on each of the clusters and for every sample Morris sensitivity analysis is applied. Finally average means and standard deviations of partial effects are combined for all clusters.

Conclusions: Described computational framework can be efficiently applied to the sensitivity assessment especially when dealing with poorly connected parameter space. It has already been efficiently applied to the analysis of genetic master-slave D flip-flop, for which several unconnected viable parameters regions were found. We were able to apply the results of the framework to identify the most robust topology and determine the parameter regions for which the flip-flop dynamics were optimal.

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GOMAPMAN: PLANT SPECIFIC ONTOLOGY TO EASE ANALYSES OF HIGH-THROUGHPUT DATA

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Introduction: Understanding the different aspects of plant biology using systems biology tools can contribute to crop plant breeding and development of efficient agricultural practices. However, the approach is hindered by lacking or dispersed crop-specific experimental data, functional annotations and visualization tools.

Results: We have developed GoMapMan (http://www.gomapman.org), an open web-accessible resource for gene functional annotations in the plant sciences. It was developed to facilitate improvement, consolidation and visualisation of gene annotations across several plant species. GoMapMan is based on the MapMan ontology, organized in the form of a hierarchical tree of biological concepts, which describe gene functions. Currently, genes of the model species Arabidopsis and six crop species (potato, tomato, rice, tobacco, beet and cacao tree) are included. The main features of GoMapMan are 1) dynamic and interactive gene product annotation through various curation options, 2) consolidation of gene annotations for different plant species through the integration of orthologue group information, 3) traceability of gene ontology changes and annotations, 4) integration of external knowledge about genes from different public resources, and 5) providing gathered information to high-throughput analysis tools via dynamically generated export files.

Conclusions: Using GoMapMan, the knowledge on plant biology can be improved by translating existing knowledge from model to crop species and by easier data interpretation of crop experimental data.

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GENETIC AND CLINICAL DETERMINANTS OF RESPONSE TO TREATMENT IN PARKINSON'S DISEASE: CAN THEY HELP TO PERSONALIZE THE TREATMENT?

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Introduction: Parkinson's disease (PD) is a complex progressive neurodegenerative brain disorder with increasing prevalence in aging population. Genetic and clinical factors play an important role in the etiopathogenesis of PD and its response to treatment. As the preparative step for the clinical pharmacogenetic study of treatment response in PD we have performed a review of the current literature to decide which clinical and genetic factors should be included in our analysis.

Methods: We performed a PubMed search on factors related to etiopathogenesis of PD and on factors, especially genetic polymorphisms, related to response to anti-parkinsonian drugs.

Results: Regarding etiopathogenesis of PD, several cellular pathways were found to be compromised due to genetic mutations: protein aggregation, protein and membrane trafficking, lysosomal autophagy, immune system, neurodevelopment, neuron cell differentiation and survival, mitochondrial homeostasis and other processes, which may modify the disease course and response to treatment depending on the damaged pathway [1]. In addition, response to treatment may be influenced by genetic polymorphisms in dopamine, neurotransmitter and drug metabolism and transport, dopamine receptors, signalling pathways, inflammation, antioxidative defense and synaptic transmission. Genetic variability could be the reason for occurrence of adverse drug reactions (dyskinesias, motor fluctuations, hallucinations, sleep attacks) and may also affect the appropriate dosing [2]. Beside genetic determinants also several other factors may influence treatment response and disease course: gender, age, age at onset, symptoms before and at the treatment initiation, family history of brain diseases, history of head injuries, accompanying diseases and treatments, living in rural or urban areas, environmental exposures (pesticide exposure, well water drinking) and patient's lifestyle (coffee and alcohol consumption, smoking, physical activity) [1]. Usually, the disease severity at the start of the treatment turns out to be the most significant factor influencing treatment response.

Conclusion: Based on the acquired data we designed a clinical pharmacogenetic study in which we will evaluate the impact of each mentioned factor on the efficacy of anti-parkinsonian drugs as well as the occurrence and time to occurrence of adverse drug reactions. We will analyze the combined effects of clinical and genetic data using up-to-date statistical approaches to identify biomarkers of different treatment outcomes. If such prediction models could be established they would enable personalized treatment approach of PD.

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MICROBES CALL FOR ELIXIR-SI

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Introduction: There are 10 times more microbial cells present within or on the surface of our body than there are our own cells. Microbial counterpart contains 150 times more genes than our genome and hence interact in complex metabolic relationship with the host.

A number of ongoing projects has focused on a more systematic and structured exploration of microbial communities (metagenomes), their gene transcripts (metatranscriptomes) and resulting metabolites (metabolomes) in various types of mammalian intestinal tracts in relation to (i) human physiology or disease. (e.g. Humans - PlanHab (IJS); PreTerm (Faculty of Medicine); IBD- Children's Hospital); (ii) animal physiology or disease or biotechnological applications -Microbial Enzymes (Biotechnical Faculty)) and (iii) environment - WaterCaves (Biotechnical Faculty)).

The resulting datasets from these projects in the range of 20 GB -1 TB are currently being processed either on smaller dedicated servers locally or HPCCs outside ELIXIR.

Results: To comparatively analyze on a large scale the metagenomes, metatranscriptomes, assemble draft genomes of microorganisms from metagenome sequences, analyze draft genomes within the context of already closed genomes and integrate metadata data within Bayesian Networks we call for a more systematic access to ELIXIR-SI based HPC resources in the range of e.g. 500-1000 CPU, 4-8TB RAM per single job. A more systematically dedicated training tailored to the specificities of the datasets and needs of increasing number of biologists and students analyzing the data would boost the development of the microbial systems biology, from the perspective of human health, biotech and environment, fostering discoveries of additional novel enzymes, antibiotics, directed evolution of proteins or transcripts coded in metagenomic or metatranscriptomic DNA for industrial applications.

Conclusions: We call for a more focused discussion on the needs of this part of scientific research faced with large-scale data, complex metabolic and co-occurrence networks, multifactorial responses and multivariate datasets with the idea to make ELIXIR-SI functionally and operationally more accessible to researchers performing the analyses in microbial 'omics areas.

REANALYSIS OF BACTERIAL 16S RRNA IN RUMEN OF WILD ANIMALS FOR MICROBIAL PATHOGEN SIGNATURES: ELIXIR-SI IMPLEMENTATION OF MOTHUR AND R

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Introduction: Ruminants are one of the most successful groups of herbivorous mammals on the planet with around 200 species represented by 75 mio wild and 3.5 bil domesticated animals worldwide. The ruminal microbial assemblages are remarkably diverse, containing hundreds of different bacterial, archaeal, protozoal and fungal species capable of plant residues degradation and conversion into plethora of valuable products. Bacterial microbial communities represent the most diverse and functionally resilient part of communities in rumen due to interaction with highly diverse plant residues in feed.

Results: C++ program MOTHUR (Schloss et al., 2009) and its accompanying bacterial, archaeal, protozoal (SILVA (http://www.arb-silva.de/)) and fungal (UNITE (http://www.mothur.org/wiki/File:Unite_ITS_02.zip)) databases were installed next to the relevant R routines (vegan) for streamlined statistical analyses of the training set composed of 1.5 mio sequences.

Conclusions: The implementation enabled (i) testing the established routines for subsequent high-throughput of analyses of additional datasets derived from deep amplicon sequencing, (ii) provided grounds for testing for the presence of microbial pathogens of medical importance for humans (e.g. animal related disease(s), harbouring multiple antibiotic resistance genes) and (iii) assessing the effects of systematic errors related to the use of different sequencing technologies.

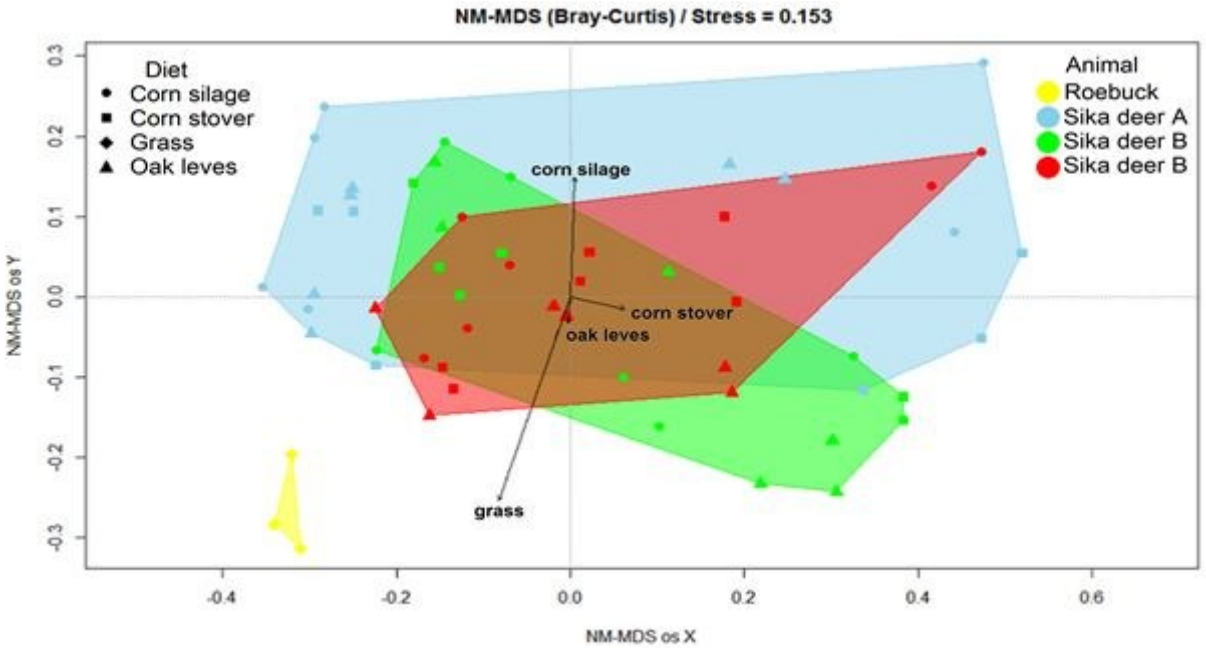

FIGURE 1: Figure 1: An example of NM-MDS visual representation of factors affecting the structure of bacterial microbial communities obtained from two different sequencing platforms and corrected for non-overlapping stretches of sequenced genes over time.

DEFICIENT RORC ACTIVITY CONTRIBUTES TO METABOLIC INSUFFICIENCY IN A SUBSET OF *CYP51* KNOCKOUT MICE

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Background: Initially identified as an orphan nuclear receptor, RORC has in recent years been shown to exhibit pleiotropic effects, ranging from fine-tuning of the circadian clock, immune system development and hepatic metabolic control. It was recently discovered that cholesterol biosynthesis intermediates (CBI) after *Cyp51* mediated demethylation serve as natural RORC ligands in vitro. By applying the hepatocyte *Cyp51* knockout mice ($H^{Cyp51-/-}$) we aimed to examine whether sterols modulate RORC transcriptional activity also in vivo.

Methods: CBIs were measured by GC/MC. Expression profiles of $H^{Cyp51-/-}$ and control mice livers were examined by Affymetrix microarrays. The RORC transcriptional profiles were assessed by RNA sequencing of the hepatocyte *Rorc* knockout mice ($H^{Rorc-/-}$). Comparative transcriptome analysis was conducted in R, using Bioconductor packages limma and pgsea.

Results: We found decreased post-CYP51 CBIs in 6- and 19-week $H^{Cyp51-/-}$mice, particularly evident in a subgroup of mice with the worst observable phenotypes (runts). Gene set enrichment analysis exhibited decreased RORC transcriptional activity in runts and 19-week $H^{Cyp51-/-}$ mice. To improve the selection of RORC targets, transcriptional profiles of $H^{Cyp51-/-}$ mice were compared to those of $H^{Rorc-/-}$ mice. The circadian nature of Rorc expression permitted the identification of the proposed *Rorc* target genes, approximately 30% of which were deregulated also in $H^{Cyp51-/-}$ mice. These genes are involved mainly in amino acid metabolism as shown by GO and KEGG pathway enrichment.

Conclusion: The comparative gene expression analysis shows that ablation of *Cyp51* from cholesterol synthesis reduced the hepatic RORC transcriptional activity stemming from insufficient availability of the natural sterol ligands *in vivo*.

SEARCH OF NEW GLIOBLASTOMA STEM CELL MARKERS BY ANALYSIS OF NCBI GEO DATASETS, AND EXPERIMENTAL VALIDATION OF THOSE MARKERS IN GLIOMA CELL LINES

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Glioblastoma multiforme (GBM) is the most lethal brain tumor. Glioblastoma stem cells are believed to be the reason for the tumor's malignancy and tendency to reappear after a surgical removal. Identification of those stem cells among other cells in the tumor mass would therefore facilitate GBM therapy. Most likely there are different groups of stem cells within a single tumor, each such group expressing its own set of markers. Our goal is to find new markers that would enable targeting of stem cell populations not expressing any of already known markers.

Gene expression profiles of glioblastoma cell lines will be compared with the profiles of non-malignant brain cells from the same dataset. All profiles were obtained by analyzing microarrays and uploaded to NCBI GEO database. The goal is to find genes that are significantly overexpressed in malignant cells in most or all the analyzed datasets. The proteins coded by these genes will be assessed by their function, intracellular location and expression frequency in brain and other tissues. Most interesting candidates, i.e. surface proteins that are not too commonly expressed outside the brain and whose function is related to growth and proliferation, will have their expression investigated in the U87 glioma cell line and the NCH CD133+ purported glioblastoma stem cell line, as well as in a new cell line derived from the U87. Compared to the original U87 line, this new cell line is believed to be enriched in stem-cell like cells, including CD133- stem cells not present in the NCH line.

MUTATION ANALYSIS OF *EPO* AND *EPOR* GENES IN TWO PATIENTS WITH FAMILIAL ERYTHROCYTOSIS: A NOVEL MOLECULAR-GENETIC DIAGNOSTIC TEST FOR *JAK2* NEGATIVE ERYTHROCYTOSIS

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Introduction: Erythrocytosis is heterogeneous group of disorders characterized by the expansion of the erythrocyte compartment including elevated red blood cell (RBC) number, haematocrit, and haemoglobin content in the peripheral blood. Familial erythrocytosis (FE) is a group of rare congenital disorders with various genetic background. Erythropoietin receptor gene (*EPOR*) mutations are the indicator for primary familial erythrocytosis. Secondary erythrocytosis syndromes are typically associated with a defect in various genes included in oxygen sensing pathway that leads to the increased erythropoietin production [1]. The hormone erythropoietin (EPO) and its receptor (EPOR) are the main regulator of RBC production in the bone marrow. Current diagnostic procedure in Slovenia enables exclusion of *JAK2* gene mutations, the cause of polycythaemia vera (PV). Aim of our study was the introduction of new molecular-genetic test for *EPO* and *EPOR* genes in *JAK2* negative erythrocytosis.

Results: Two related patients with erythrocytosis were negative for *JAK2* V617F and *JAK2* exon 12 mutations, indicating exclusion of PV. The level of serum erythropoietin was in the reference intervals. Secondary reasons for increased RBC mass (cardiac, pulmonary and endocrine) were excluded. Ensembl database and literature search was performed to detect the most common mutations in *EPO* and *EPOR* linked with previously described clinical cases of familial erythrocytosis. Sequence analysis of *EPO* promoter and 3' enhancer and *EPOR* exon 8 was performed.

Primary familial erythrocytosis due to *EPOR* mutation was excluded by sequence analysis in both patients. So far, 24 mutations in *EPOR*, located in exon 8, have been associated with erythrocytosis. Exon 8 encodes the C-terminal negative regulatory domain of the protein. Mutations are leading to cytoplasmic truncation of the receptor and loss of the C-terminal negative regulatory domain [2].

However sequence analysis revealed mutation in 3' enhancer region of the *EPO* gene in both patients, previously described in blood donors with upper limit haematocrit. The role of erythropoietin in erythrocytosis is indirect and previously had not been linked to the disease.

Conclusions: We have successfully introduced new molecular-genetic test for analysis of the *EPO* (promoter and 3' enhancer) and *EPOR* (exon 8) mutations and implemented it in clinical use. Future recommendation is to complement the diagnostic algorithm with mutational analysis of other genes involved in disease development.

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