ABSTRACTS

Symposium "New Diagnostic Trends in Early Detection of Oncological and Rare Diseases"
17 April, 2018
Ruđer Bošković Institute, Zagreb, Croatia

The one-day symposium „New Diagnostic Trends in Early Detection of Oncological and Rare Diseases" was held at the Ruđer Bošković Institute, Zagreb on April 17, 2018. The symposium was organised by the Croatian Society of Biochemistry and Molecular Biology and the Slovenian Biochemistry Society, with the Organising Committee comprising Tatjana Marčac Grahek, Zrinka Kovarik, Tihomir Balog, and Janko Kos. The aim was to inform the scientific community on developments and new perspectives in biomolecular life sciences arising from advancements in methodology. The symposium comprised lectures given by eminent researchers devoted to diagnostic and early detection of oncological and rare diseases. Tremendous advancements in methodology, such as next-generation sequencing technology, have transformed biological and biomedical research, providing a platform that drives genetic analysis at unprecedented rates. The symposium undoubtedly provided an excellent opportunity to exchange ideas and experiences with researchers and professionals, such as physicians and clinicians, whose attendance was recognised by the Croatian Medical Chamber and Croatian Chamber of Medical Biochemists. The symposium was attended by 70 participants, 25 of whom came from Slovenia.

Our special thanks go to the general sponsors Kemomed and Illumina, for providing us with the resources necessary to organize this meeting. Kemomed was established in 1996 and has constantly grown to provide a range of products from the fields of genetics, biotechnology, molecular biology, *in vitro* diagnostics, small lab inventory and equipment, and analytical columns. Kemomed is known for their high degree of proficiency, short delivery times, and excellent customer support. Illumina is the world's leading provider of solutions in next generation sequencing.

We thank *Arhiv* for giving us the opportunity to publish abstracts and short CVs in this issue.

Zrinka Kovarik
on behalf of the Organising Committee

The organisers and participants of the Symposium

ABSTRACTS
Arh Hig Rada Toksikol 2018;69:A18-A23

Five years of NGS in a diagnostic setup: experience from the Unit of Special Laboratory Diagnostics at the University Children's Hospital in Ljubljana

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The rapid technological advancement in the field of DNA/RNA sequencing technology in the last decade has boosted research in genomics and revolutionized the field of medical genetics. With the development of high-throughput DNA sequencers, next-generation sequencing (NGS) technology, and novel analytical algorithms, as well as the release of huge public databases of genetic variant allele frequency in the general population (GnomAD, ExAc, etc.), the paradigm of medical genetics has changed and shifted from diagnosis driven to a phenotype driven analytical approach. Moreover, the price to generate genomic data has significantly dropped and, consequently, the possibility of unbiased whole-genome sequencing to identify the disease-causing variants has become a reality. In the last five years since the introduction of NGS in the routine genetic diagnostic algorithms of the University Children's Hospital in Ljubljana, major changes occurred regarding the analytical process, data handling and storage, as well as the development of novel applications using NGS. Processing more than 500 cases per year, ranging from genetic diagnostics of orphan diseases to the genetic analysis of more common diseases (hypercholesterolemia, obesity, hearing loss, etc.), the analytical burden to establish rapid sequencing, validated data analysis, and strict quality control became our primary concern. The lecture will try to illustrate the path from the sequencing of small targeted panels to whole human genomes and from basic capture technology to nanopore sequencing and the application of the NGS technology in a diagnostic and research setup.

KEY WORDS: *common diseases; genetic diagnostics; orphan diseases; technology*

About the Speaker

Jernej Kovač defended his PhD in biochemistry and molecular biology in 2014 with a thesis researching the genetic background of oxidative stress mechanism genetics in the autism spectrum disorder. He participated in the development and establishment of the laboratory for cytogenetics at the Unit of Special Laboratory Diagnostics at University Children's Hospital in Ljubljana and later switched to next generation sequencing and genetics of orphan diseases at the same institution. Currently, his main research interests are the genetics and epigenetics of obesity, hypercholesterolemia, insulin resistance and type 1 diabetes in the paediatric population, while his everyday work routine is focused on the genetic diagnostics of orphan diseases, using next-generation sequencing techniques.

How next-generation sequencing improves care of patients with genetic diseases

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The identification of a specific genetic cause in patients with suspected genetic diseases is essential to confirm the diagnosis and guide their medical management. It also enables the prediction of disease prognosis and occurrence in family members. Although the primary purpose of genetic testing in the past was in confirming a specific suspected diagnosis, recent advancements in sequencing technologies now permit a much broader survey of genetic causes in patients with suspected rare diseases. This is of particular importance, because a considerable fraction of patients does not have a specific diagnosis established prior to genetic testing and because the suspected diagnosis may be incorrect due to atypical or misleading presentation. At the Clinical Institute of Medical Genetics in Ljubljana, we have been performing the analysis of either the mendeliome, whole exome, or whole genome sequencing for diagnosing patients with suspected monogenic etiology since 2013. So far, we performed genetic testing using these approaches in over 2,000 patients, referred with a wide variety of disease categories. In all of these, we routinely examine the final diagnostic outcomes in their relation to the original diagnosis of referral and made a systematic follow-up on the diagnostic significance of untargeted approaches in a clinical setting. In the group of patients with a wide variety of disease categories, we reached a high overall diagnostic rate of 40.1 %. We have noted the emergence of new significant diagnostic outcomes of genetic testing in a considerable proportion of cases. This included the establishment of a specific diagnosis in a previously undiagnosed patient, which accounted for over 20 % of all positive findings. Furthermore, we have observed that in numerous positive cases (4.3 %), the original diagnosis was reclassified based on the result of genetic testing using exome or genome sequencing. In selected cases, we were also able to identify new disease genes and novel genotype to phenotype associations using these approaches. We will present a selection of cases, where the establishment or reclassification of a diagnosis proved to be of direct medical significance for patients. In conclusion, we present novel diagnostic outcomes associated with the implementation of exome and genome sequencing in routine genetic diagnostics. These outcomes shift the role of genetic testing from a confirmatory role to its utility in establishing diagnosis, reclassification, and expansion of existent gene-phenotype associations, with direct implications for medical management of patients. These observations also support early (albeit rational) use of genetic testing in diagnosing patients with a wide variety of disease categories.

KEY WORDS: *exome; genetic disease diagnostics; mendeliome; rare diseases*

About the Speaker

Aleš Maver is currently leading the Centre for Mendelian Genomics (CMG) at the Clinical Institute of Medical Genetics in Ljubljana, in Professor Borut Peterlin's team. CMG is one of the leading institutions in the region, providing next-generation sequencing based diagnostics and research, predominantly using exome and genome sequencing, as well as RNA sequencing. Their team has over five years of experience in diagnostic NGS with over 2000 exome/genome-based analyses performed so far. They reported the benefits of diagnostic exome sequencing in multiple disease categories and we published innovative approaches to analysing exome sequencing for maximum diagnostic yield. Recently, they reported identifications of several new genes for rare and complex human disorders, including novel findings in premature aging, epileptic encephalopathies, and multiple sclerosis.

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Arh Hig Rada Toksikol 2018;69:A18-A23

Genomics enabling the latest cancer breakthroughs in personalised medicine

Agnieszka Grybos-Gajniak

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An expanding next-generation sequencing (NGS) oncology portfolio is helping Illumina drive the revolution in cancer genomics. Our sample-to-data solutions deliver high-quality, reproducible results to speed the discovery and analysis of cancer-related variants and potentially transform the cancer care cycle. Immuno-oncology is an emerging field that has taken great strides in the fight against cancer, bolstered by a refined understanding of how tumours evade the natural immune response. Leading immuno-oncology researchers are leveraging next-generation sequencing to discover biomarkers and apply genomics to personalized immunotherapy. Research into the mechanisms tumours use to evade the immune response have led to promising therapeutic targets. These therapies boost the ability of the immune system to target cancer or limit the tumour's ability to evade the natural immune response. Analysis of cell free DNA (cfDNA) is increasingly used to obtain information about the genetic state of normal and tumour tissues. Combining liquid biopsy with NGS technology provides a non-invasive method to analyse numerous cancer-related genes in a single assay. Find out how our products are helping propel progress in personalized oncology.

KEY WORDS: *genetic diseases; immune response; immuno-oncology; next-generation sequencing*

About the Speaker

Agnieszka Grybos-Gajniak is currently a Senior Clinical Specialist Field Application Scientist at Illumina. She has been responsible for supporting oncology applications across EMEA for the past two years. The products she supports include AmpliSeq™ for Illumina® as well as TruSight® Oncology portfolio. She has received her master's degree in biotechnology from the Jagiellonian University in Cracow, Poland (Faculty of Biochemistry, Biophysics and Biotechnology). Her research focused on the influence of oxidative stress on etoposide-induced DNA damage in human leukaemia cells. In her previous commercial roles she has been involved in development and application support of various assays used in oncology field including fluorescence in situ hybridisation (FISH), microarrays (aCGH), and next generation sequencing.

NGS-based BRCA1,2 mutation testing of high- grade serous ovarian carcinoma

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Technological advances in the development of sequencing techniques have led to the increasing application of next-generation sequencing (NGS) in understanding the nature of many diseases and their improved treatment, especially for malignant diseases. An increasing amount of data shows that high-grade (HG) serous ovarian cancer patients carrying *BRCA1* and *BRCA2* mutations – both somatic and germline – experience a survival advantage and are more sensitive to agents such as platinum and PARP inhibitors. In the period 2016-2018 we analysed mutations of *BRCA1,2* genes in patients with HG- serous ovarian cancer, treated at the University Hospital Centre Split (n=80). Genomic DNA was isolated from blood, a library was prepared using the TruSeq Custom Amplicon Low Input Library Prep Kit and analysed on a MiniSeq Instrument (both Illumina, San Diego, CA, USA). Deleterious germline mutations were identified in 21 patients (26 %), 13 in the *BRCA1* and 8 in the *BRCA2* gene. The most common mutations in the *BRCA1* gene were c.5266dupC (p.Gln1756ProfsV74) and c.843_846delCTCA (p.Ser282Tyrfs), found in 9 (42.85 %) and 2 patients, respectively. The most common mutation in the *BRCA2* gene was c.9371A>T (p.Asn3124Ile), found in 2 patients (9.5 %). Numerous advantages of the NGS approach have been demonstrated, since it has proven to be highly sensitive, as have specific methods for *BRCA* mutation analysis in patients with HG-serous ovarian cancer, since it allows the simultaneous screening of multiple genes, reduces turnaround time of analysis, and requires a very low input of DNA.

KEY WORDS: *genomics; malignant diseases; next generation sequencing; sensitivity of method*

About the Speaker

Irena Drmić Hofman is a full Professor of Biochemistry and Molecular Biology at the University of Split (School of Medicine) and a member of the Laboratory for Molecular Diagnostics at the University Hospital Centre Split. She received her undergraduate education from the University of Split and her master's and doctoral degrees from the University of Zagreb. Study periods abroad include University of Verona, Italy, and University of Münster, Germany. She started working as a forensic geneticist at the Department of Pathology, Forensic Medicine and Cytology of the University Hospital Centre Split in 1992. Since 2000, she has been a senior researcher at the Laboratory for clinical molecular genetics. Professor Drmić Hofman's main research interests are the genomics and proteomics of tumours and risk factors. She (co)authored more than 40 papers in peer reviewed journals, as well as several textbook chapters and is a reviewer for several international journals. She is a member of The Board of Croatian Society for Human Genetics and Chair of the Split Branch of the HDBMB.

ABSTRACTS
Arh Hig Rada Toksikol 2018;69:A18-A23

Identification of Lowe syndrome in a child with a novel mutation in the OCRL gene, using whole exome sequencing

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Lowe syndrome is a rare X-linked recessive hereditary disease caused by mutations of the *OCRL* gene, which encodes an inositol polyphosphate- 5-phosphatase. Congenital cataracts, psychomotor retardation, and proximal tubulopathy clinically characterize the disease. At 12 days of age, the patient presented hypotonia and congenital bilateral cataracts. Metabolic analysis appeared normal and the child was released with recommendations for neurodevelopmental habilitation. At the age of 12 months, abdominal ultrasonography showed morphology of the patient's kidneys, urine, and blood levels of creatinine were within regular limits. However, over the next 36 months, the clinical picture deteriorated and the patient presented highly elevated values for creatine kinase, aminoaciduria, and severe psychomotor retardation. Lowe syndrome was confirmed at the age of 52 months using Whole-Exome-Sequencing (WES), showing a novel mutation in the *OCRL* gene, introducing a nonsense mutation in exon 10 at amino acid position 215 (c.643C>T / p.Gln215*). The mother of the patient was a heterozygous carrier of the respective mutation. In view of the clinical data, in particular kidney morphology and metabolic values, our findings indicate phenotypic variability in patients with Lowe syndrome, which made a precise diagnosis challenging and time consuming.

KEY WORDS: *bilateral cataract; Fanconi syndrome; genomics; glaucoma; hypophosphataemia; psychomotor retardation; WES*

About the Speaker

Oliver Vugrek is a molecular biologist with many years of experience in genetics of rare diseases. He graduated in Biology at the Albert Einstein University in Ulm, Germany. He was a PhD fellow at the Max Planck Institute for Cell Biology from 1992 to 1995 and received his PhD in 1995 at the Karl Ruprechts University in Heidelberg, Germany. He spent two years at Australia's National University (ANU) in Canberra as a postdoctoral fellow. He has been employed at the Ruđer Bošković Institute since 1999 and is currently head of the Laboratory for Advanced Genomics. Dr Vugrek has led significant EU projects, including the FP7 project InnoMol, the largest Natural Sciences project ever conducted in Croatia, bringing genomics research at the Ruđer Bošković Institute to a new level. His research interests are devoted to the introduction of new technologies for advanced DNA sequencing and analysis of genetic disorders. Oliver Vugrek is an active member of the HDBMB and member of the Federation of European Biochemistry Societies (FEBS) Fellowships Committee (2017-2010).