UDK 616 – 053.2 CODEN PCROE6 ISSN 1330 – 724X
Vol. 60 Suppl 2 2016

PAEDIATRIA CROATICA

Časopis Hrvatskog pedijatrijskog društva i Hrvatskog društva za školsku i sveučilišnu medicinu Hrvatskog liječničkog zbora

The Journal of the Croatian Paediatric Society and the Croatian Society for School and University Medicine of Croatian Medical Association

Paediatr Croat. – Godište 60 Zagreb, svibanj 2016. Suppl 2 (str. 1 – 46)

4th Central Eastern European Symposium on Free Nucleic Acids in Non-Invasive Prenatal Diagnosis

Split
May 25-26, 2016

4th Central Eastern European Symposium on Free Nucleic Acids in Non-Invasive Prenatal Diagnosis

Split, May 25-26, 2016

Organisers:

J. J. Strossmayer University in Osijek, Faculty of Medicine
Croatian Society of Human Genetics

Honorary presidents of Scientific committee:

Dennis Y.M. Lo (Hong Kong)
Howard S. Cuckle (Tel Aviv)
Erik A. Sistermans (Amsterdam)

International Advisory Committee:

Ewa Brojer (National Blood Institute, Warsaw)
Peter Celec (Comenius University, Bratislava)
Ishraq Dhaifallah (Palacky University, Olomouc)
Ilona Hromadnikova (Charles University, Prag)
Zora Lasabova (Comenius University, Martin)
Gordan Lauc (University of Zagreb)
Balint Nagy (University of Debrecen)
Borut Peterlin (University Medical Center Ljubljana)
Hakan Savli (Kocaeli University, Kocaeli)
Marta Szell (University of Szeged)
Jasenka Wagner (J.J.Strossmayer University of Osijek)

Scientific committee:

Ingeborg Barišić (chairman), Aleksandar Včev (co-chairman), Vida Čulić, Tomislav Hafner, Marija Heffer, Sanda Huljev Frković, Ratko Matijević, Oleg Petrović, Silvija Pušeljić, Damir Roje, Snježana Škrablin

Organising committee:

Jasenka Wagner (chairman), Balint Nagy (co-chairman), Ivanka Bekavac Vlatković, Ljubica Boban, Kristina Crkvenac Gornik, Irena Drmić Hofman, Andrijana Muller, Feodora Stipoljev, Ivana Škrlec

GLAVNA UREDNICA / EDITOR-IN-CHIEF

Ingeborg Barišić, Zagreb

POMOĆNICA UREDNICE / ASSISTANT EDITOR

Katja Dumić Kubat, Zagreb

POČASNI UREDNIK / EDITOR EMERITUS

Duško Mardešić, Zagreb

TAJNICA UREDNIŠTVA / SECRETARY

Martina Bošnjak

Slog / Typesetting

DENONA d.o.o., Getaldićeva 1, Zagreb

Tisak / Printed by

DENONA d.o.o., Getaldićeva 1, Zagreb

NAKLADA 200 primjeraka

Izdavač / Editor

KLINIKA ZA DJEČJE BOLESTI ZAGREB, KLAIĆEVA 16, ZAGREB

UDK 616 – 053.2
CODEN PCROE6
ISSN 1330 – 724X

PAEDIATRIA CROATICA

Vol. 60 • Svibanj/*May* 2016. • Suppl 2

SADRŽAJ / *CONTENT*

BOOK OF ABSTRACTS

Časopis Hrvatskog pedijatrijskog društva i Hrvatskog društva za školsku i sveučilišnu medicinu Hrvatskog liječničkog zbora
The Journal the Croatian Pediatric Society and the Croatian Society for School and University Medicine of the Croatian Medical Association
Izdavač/Editor: Klinika za dječje bolesti Zagreb, 10000 Zagreb, Klaićeva 16

Paediatr Croat. Godište 60 ▪ Zagreb, svibanj 2016. ▪ Suppl 2 (str. 1–46)

BOOK OF ABSTRACTS

Guest editor: Jasenka Wagner

PL1

DECIPHERING THE ORIGIN OF DNA IN PLASMA: IMPLICATIONS FOR NON-INVASIVE PRENATAL TESTING

Y. M. Dennis Lo

Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong SAR, China.

Since 2011, non-invasive prenatal testing (NIPT) for chromosomal disorders have been performed over 2 million times in over 90 countries. Recently, there are a flurry of publications reporting the detection of genomic aberrations during the course of NIPT that have originated from cancer of the mother, rather than originating from genomic aberrations in the fetus. Such data have created a demand for new technologies would allow one to trace the source of a genomic aberration observed in plasma DNA to its tissue of origin. In this regard, we have recently developed a new technology, called 'plasma DNA tissue mapping', that would allow one to elucidate the composition of plasma DNA to its various tissues of origin. This new technology is like a 'molecular whole body scan' that allows us to locate the organs or tissues that are likely sources of an observed plasma DNA aberration. As a demonstration of the utility of this technology, we traced genomic aberrations observed in the plasma of a pregnant woman undergoing NIPT to B-lymphocytes. This pregnant woman was found to be follicular lymphoma. Apart from use in NIPT, plasma DNA tissue mapping is expected to have numerous applications in molecular diagnostics.

Keywords: cell free DNA, non-invasive prenatal testing (NIPT)

PL2

NIPT COST REVISITED

Howard Cuckle

Visiting Associate Professor, Faculty of Medicine, Tel Aviv University, Israel;
Adjunct Professor, Department of Obstetrics and Gynecology, Columbia University Medical Center, New York, USA;
Emeritus Professor, School of Medicine, University of Leeds, UK

From a public health perspective there are two broad aspects of cost that need to be considered when deciding whether and how to implement cfDNA screening: (a) total cost of the program or the average cost per screened woman; and (b) average cost per additional benefit achieved compared with or incremental cost effective ratio.

A policy of Secondary cfDNA, whereby testing is restricted to those who would otherwise have invasive prenatal diagnosis, has been examined in eight cost analyses. Seven found it to be of comparable cost, or cheaper than, the current conventional screening policy. In contrast, eight studies have examined a policy of Primary cfDNA, for everyone, and seven concluded that it is too expensive. A single analysis found the strategy to be cost-effective but only from a societal perspective, when the *indirect* as well as direct lifetime care costs for Down's syndrome is included. Indirect costs would include for example, loss of income for the affected individual. Five cost analyses have demonstrated that Contingent cfDNA, with up to 20% selected for testing based on conventional screening, is considerably more cost-effective than Primary cfDNA.

The unit cost of a cfDNA test is falling and may eventually approach that of conventional screening. In anticipation of this process some analyses have considered the lowest unit cost that would make Primary cfDNA testing cost-effective. One study estimated this to be about double the unit cost of conventional screening.

Specific screening policy, test and treatment costs will inevitably differ between localities, and public health providers will need to make their own assessment about cfDNA screening. Of the competing strategies, Secondary cfDNA is the easiest to introduce as it is likely to be a zero-cost option. Primary cfDNA is currently very expensive but under certain circumstances it may be cost-effective. Contingent cfDNA options offer an acceptable compromise as it is inevitably cheaper than Primary cfDNA albeit with a lower detection rate.

Keywords: cell free DNA, non-invasive prenatal testing (NIPT)

PL3

INTRODUCING NIPT ANALYSIS IN THE NETHERLANDS, A STORY OF SCIENCE AND POLITICS

Erik A. Sistermans

VU University Medical Center Amsterdam, the Netherlands

This presentation will be separated in two parts. The first part will be about several different boinformatics tools that were developed at VUmc Amsterdam, such as WISECONDOR and SANEFALCON. WISECONDOR detects smaller chromosomal deletions and duplications without increasing the need for NIPT/NGS data. It is now widely used in many countries for routine diagnostic NIPT analysis, including the Netherlands, Denmark, France and South Korea. SANEFALCON was published very recently and is a tool to determine foetal fraction based on differences in NGS starting positions between foetal and maternal DNA. Both tools are freely available for non-commercial use (https://github.com/rstraver) and can also be used for the detection of tumour profiles in cfDNA from cancer patients.

The second part will be about the introduction of NIPT in the Netherlands. The Netherlands are the first country where NIPT is incorporated into a governmentally supported and health care funded prenatal Down syndrome screening program. In many countries, NIPT has been introduced commercially, without governmental guidance. In the Netherlands the Population Screening Act regulates the introduction of screening programs for untreatable diseases such as Down syndrome. The Dutch NIPT consortium, consisting of all relevant stakeholders, obtained a license for 2 years for a nationwide NIPT implementation study called TRIDENT. Inclusion criteria are an increased risk (>1:200) for trisomy (T) 21, 18 or 13 based on the first trimester combined test, or because of medical history. Data of the first year will be presented, including full clinical follow up of the first five months, and information on findings other than trisomy 21, 13 or 18. The incorporation of the test in a university hospital laboratory and clinical service guarantees appropriate counselling and allows for proper follow up including thorough exploration of biological causes of false positive and false negative findings including detailed placental examination.

Keywords: NIPT, cfDNA, bioinformatics, population screening

L1

THE ROLE OF GENETIC SERVICES IN PROVISION OF NIPT

Ales Maver[1], Alenka Hodzic[1], Borut Peterlin[1]

[1]Clinical institute of Medical Genetics, Department of Obstetrics and Gynecology, University Medical Centre Ljubljana, 3, Šlajmerjeva Street, Ljubljana 1000, Slovenia

Non-invasive prenatal testing (NIPT) using next-generation sequencing of cell-free fetal nucleic acids has rapidly established itself as a powerful method of non-invasive detection of fetal chromosomal aneuplodies. An increasingly large body of evidence supports the performance and clinical utility of NIPT in the clinical setting. Despite the support from large studies and demonstration of clinical utility, the method is still predominantly offered through commercial providers. Due to rapid translation process, the status of NIPT is highly diverse across different countries and the optimal path to incorporation of NIPT into health systems is not yet known.

We will present an analysis of current status of NIPT across countries of eastern Europe, especially in terms of access to NIPT, extent of its integration in health systems and present opinions of relevant stakeholders in the field. We will address possible benefits and pitfalls of commercial provision of genetic tests in health care systems. Finally, we will share our experiences with implementation of NIPT on a regional and national level and our current progress with introducing this powerful approach into standard clinical care.

Keywords: cell free fetal DNA, next-generation sequencing, non-invasive prenatal testing (NIPT)

L2

NONINVASIVE PRENATAL TESTING AND THE CLINICAL PRACTICE

Ishraq Dhaifalah and Marek Godava

FETMED (centrum of fetal medicine and Genetic) Olomouc, Czech Republic

Cell-free DNA (cfDNA) analysis of maternal blood for detection of trisomies 21, 18 and 13 has been proved to be the most sensitive methods of screening. The objective of the presentation will to be to discuss up to date feasiblity of implementing such screening method in practice. The test could be considered as effective only when the question of being expansive is solved.

Keywords: cell free DNA, non-invasive prenatal testing (NIPT)

L3

NIPT AND THE FUTURE NEED FOR TRADITIONAL MARKERS

Howard Cuckle

Visiting Associate Professor, Faculty of Medicine, Tel Aviv University, Israel;
Adjunct Professor, Department of Obstetrics and Gynecology, Columbia University Medical Center, New York, USA;
Emeritus Professor, School of Medicine, University of Leeds, UK

If the unit cost of a cfDNA test falls substantially, Primary cfDNA will be affordable in public health systems. At that point health planners may consider abandonment of the first trimester ultrasound NT scan, perhaps replacing it by a simpler dating scan. However, a large NT is associated with increased risk of structural abnormalities and genetic syndromes even in the absence of aneuploidy. Among the former are major cardiac defects and this aspect alone may be sufficient to justify retaining the scan. A meta-analysis of 20 studies found a detection rate of 44% for a false-positive rate of 5.5%. A vast range of genetic syndromes have been reported in women with increased NT and among these cases Noonan's syndrome is frequent. This disorder has an incidence about half that of Down's syndrome.

A case may also be made for retaining some first trimester biochemical markers, specifically for use in screening for adverse pregnancy outcomes such as pre-eclampsia and growth restriction. These outcomes are much more common than all aneuploidies combined. A large proportion can be prevented through first trimester screening using maternal serum PAPP-A and PlGF, together with ultrasound uterine artery Doppler and blood pressure measurement followed by daily low dose soluble aspirin in screen-positives. Cost-benefit analysis has indicated that this type of screening is affordable.

Decisions about changes in screening policy for aneuploidy will inevitably have implications for other types of prenatal screening. Such decisions need to be made in the broad context of all prenatal services offered.

Keywords: cell free DNA, next-generation sequencing, non-invasive prenatal testing (NIPT)

L4

NONINVASIVE DIAGNOSTICS OF BLOOD GROUP ANTIGENS IN POLISH PREGNANT WOMEN

Agnieszka Orzińska, Katarzyna Guz, Ewa Brojer

Institute of Hematology and Transfusion Medicine, Warsaw, Poland

Fetal red blood cell or platelet antigens may induce maternal alloimmunization if they are not expressed in the mother. In consequence there may occur such disorders as hemolytic disease of the fetus/newborn (HDF/N) or fetal/neonatal alloimmune thrombocytopenia (FNAIT), the frequency of which is ~1/1000 live newborns. If maternal alloantibodies originate from previous pregnancy or blood transfusion the fetus is not endangered providing the incompatible antigen is not inherited from the father. Non-invasive prenatal diagnostics (NIPD) from cell free fetal DNA (cffDNA) in maternal plasma is used to determine the condition. The diagnosis is not easy since most antigens are based on SNPs and the coding sequence of maternal and fetal allele is difficult to distinguish. In addition the presence of various allelic forms of an antigen must also be considered. NIPD has been performed at IHTM for the last 15 years with certain modifications implemented. These will be presented during the lecture together with our protocols and results based on real-time PCR for determination of 1/ fetal *RHD* in women with anti-RhD antibodies and in women with no antibodies (qualification for antenatal immunoprophylaxis); 2/ *RHCE*c*, *RHCE*E*, *RHCE*C* (with allele-specific primers/probe), *KEL*01* (with LNA-modified primers) or *HPA-1a* (with pre-PCR digestion of *HPA-1b*). We also plan to present our latest achievement, namely the next generation sequencing (NGS) technology which enables high coverage sequencing of target SNP position and is an important alternative to real-time PCR for detection of low-grade cffDNA in maternal plasma in early pregnancy. This is of crucial importance for immunohematology especially for fetal *HPA-1a* diagnostics which must be performed no later than 16 - 22 week of gestation.

Keywords: non-invasive prenatal diagnostics (NIPD), hemolytic disease of the fetus/newborn (HDF/N), fetal/neonatal alloimmune thrombocytopenia (FNAIT), real-time PCR, next generation sequencing (NGS)

L5

NON-INVASIVE SCREENING ALTERNATIVES FOR RARE MONOGENIC DISEASES

Nikoletta Nagy, Márta Széll

Department of Medical Genetics, University of Szeged, Szeged, Hungary

Although the incidence of rare monogenic diseases is lower than 1:2000, their number is estimated to by approximately 6-8000, therefore they affect a significant part of the population worldwide. Developing cell-free DNA based noninvasive prenatal screening methods for these diseases can have huge impact on family planning of the affected families. The aim of this presentation is to review methods applicable for the testing of rare diseases from the maternal serum. Reviewing the literature data demonstrated that in case of cell-free DNA based genetic testing, maternal serum is usually obtained between the 10th and the 20th week of gestation. From the obtained maternal serum, cell-free DNA is isolated and the genetic testing is performed either with next generation based sequencing system and/or with digital PCR system. The reviewed data also demonstrated that both platforms can serve as appropriate screening alternatives for rare monogenic diseases. Overall, the digital PCR platform has smaller capacity, fits for targeted investigations, it is more rapid and provides and economical screening system. The next generation sequencing system has higher capacity, it is suitable for the screening of several different monogenic diseases and/or chromosomal abnormalities at the same time, but it is time consuming and expensive. In general, we concluded that the non-invasive screening of monogenic diseases have huge importance since it can successfully prevent the transmission of these diseases and help the birth of healthy newborns in the affected families.

Keywords: cell free fetal DNA, next generation sequencing, non-invasive prenatal testing (NIPT)

L6

OUR EXPERIENCE WITH NON-INVASIVE PRENATAL TESTING

Zora Lasabová[1], Iveta Švecová[2], Dušan Loderer[1], Marián Grendár[1], Andrea Mendelová[1], Ján Danko[2]

Comenius University in Bratislava, Jessenius Faculty of Medicine in Martin,
[1] Biomedical Center in Martin and [2]Clinic of Obstetrics and Gynecology, Slovakia

The discovery of circulating cell-free fetal DNA (cffDNA) in maternal plasma allowed for the development of alternative methodologies that enabled non-invasive prenatal testing (NIPT). Methods with high sensitivity and precision are required to detect and differentiate fetal DNA from the background of maternal DNA. We have used real-time-PCR approaches to implement noninvasive testing into clinical practice, such as fetal sex assessment and RHD genotyping. Currently, we develop NIPT for the screening of trisomy 21 using next generation sequencing (NGS) platforms.

There were implemented two approaches for fetal sex prediction, using multiple-copy- testing *DYS14* and single-copy *SRY* probes and real-time PCR. *RHD* genotyping was performed using the TaqMan probes for exons 7 and 10 of the RHD gene. Noninvasive trisomy 21 testing is approached by the low-coverage whole-genome sequencing and Z-score calculations.

Since 2011, we try to implement NIPT into clinical settings. In case of RHD testing, our results showed that the real-time PCR approach is sensitive and specific enough to be used in clinical practice, however, we would prefer to test more samples to validate the test.

NIPT for trisomy 21 is in development and the first results from the MiSeq platform are very promising. However, we intend to use HiSeq for final validation of our approach.

Keywords: cell free fetal DNA, next generation sequencing, non-invasive prenatal testing (NIPT)

L7

THE HIDDEN TREASURE, MIRNAS IN THE NIPT

Balint Nagy

Department of Human Genetics, University of Debrecen, Debrecen, Hungary

MicroRNAs (miRNAs) are small, single–stranded, non-coding RNA transcripts that repress gene expression by pairing to mRNAs. They are composed of 18-25 nucleotides and are evolutionarily conserved. The number of described miRNAs exceeds 2000 sequences (www.mirbase.org). They were described by Lee at al. and Wightman et al. in 1993. The genes encoding these molecules account for about 1-2% of the human genes or they located in introns, and they are frequently found in fragile sites. MiRNAs go through a maturation process from pri-microRNAs. MiRNAs are present in all type of biological fluids: serum, plasma, urine, amniotic fluid, milk, semen, etc. They are resistant to RNase, freezing, thawing, and changes in pH. The miRNAs can be found in different forms of "free" miRNA, encapsulated in various vesicles secreted by cells, associated with Ago proteins or high-density lipoproteins (HDL). Different cell types could express 200-600 miRNAs and they have their own expression profiles. The isolation of miRNA can be difficult. Study of miRNAs became popular recently. Different types of methods are used to study them: real-time PCR, Northern blot, in situ hybridization, massive parallel sequencing, array comparative genomic hybridization, etc.

The miRNA profile is different in each cell type and can serve as a biomarker for physiological and pathological conditions. Bioinformatics in systems biology, network analysis, real-time PCR, arrayCGH and massive parallel sequencing are the main tools used to for its identification. "Free" DNA is used to diagnose the most common trisomies, deletions, micro-deletions since 2011. Recently miRNAs have been in the focus of research for the detection of preeclampsia and congenital heart diseases in non-invasive prenatal testing (NIPT).

Keywords: arrayCGH, microRNA, non-invasive prenatal testing (NIPT)

L8

CIRCULATING HEAT SHOCK PROTEIN MRNA PROFILES IN GESTATIONAL HYPERTENSION, PREECLAMPSIA AND FETAL GROWTH RESTRICTION

Ilona Hromadnikova[1], Lenka Dvorakova[1], Katerina Kotlabova[1], Lucie Hympanova[1,2], Ladislav Krofta [2]

[1] Department of Molecular Biology and Cell Pathology, Third Faculty of Medicine, Charles University, Ruska 87, 100 00 Prague, Czech Republic
[2] Institute for the Care of the Mother and Child, Third Faculty of Medicine, Charles University, Podolske nabrezi 157/36, 147 00 Prague, Czech Republic

Background & objectives: Exploration of extracellular heat shock protein mRNA levels in maternal circulation. Quantification of Hsp27, Hsp60, Hsp70, Hsp90 and HspBP1 mRNAs in maternal plasma samples using real-time RT-PCR.

Methods: Pregnancies with gestational hypertension (n=33), preeclampsia w or w/o fetal growth restriction (n=78), and fetal growth restriction (n=25) were involved in the study. Hsp gene expression was analysed in relation to the severity of the disease with respect to the degree of clinical signs, requirements for the delivery and Doppler ultrasound parameters.

Results: Upregulation of Hsp70 was observed in patients with mild and severe preeclampsia (p= 0.004, p= 0.005) and in pregnancies complicated with preeclampsia delivering before and after 34 week of gestation regardless of the degree of clinical signs (p= 0.015, p= 0.009). No difference in expression of other hsp genes among studied groups was observed. No association between hsp gene expression and Doppler ultrasonography parameters was found.

Interpretation & conclusions: These data strongly support that maternal circulation is able to reflect both maternal and fetal pathologic conditions. Hsp70 represents the sole plasmatic marker, whose increased mRNA levels reflect maternal and placental stress response to pregnancy related complications such as gestational hypertension and preeclampsia irrespective of the severity of the disease.

Acknowledgements: The work was supported by the Charles University research program PRVOUK P32.

Keywords: Fetal growth restriction, gestational hypertension, heat shock protein, maternal circulation, preeclampsia

L9

IS THERE A ROLE FOR EXTRACELLULAR DNA IN THE PATHOGENESIS OF PREECLAMPSIA?

Peter Celec, Barbora Vlková

Institute of Molecular Biomedicine, Comenius University, Bratislava, Slovakia
petercelec@gmail.com

Higher concentrations of fetal DNA have been found in the plasma of pregnant women suffering from preeclampsia. Despite the well-described pathogenesis the etiology of preeclampsia remains unclear. In the past, we have postulated a hypothesis that the higher fetal DNA is not the consequence of fetal and placental damage in preeclampsia, but might rather be the cause of this common disease dangerous for both, mother and child. This hypothesis was proved in animal experiments showing that fetal DNA is recognized by toll-like receptor 9 and induces preeclampsia-like symptoms in mice. However, we were unable to reproduce these findings. In addition, the association of higher fetal DNA and preeclampsia has been questioned as it was found that total extracellular DNA in plasma and not only DNA of fetal origin is higher in patients with preeclampsia. One of the potential explanations could be a lower activity of deoxyribonuclease, but our analyses showed that plasma from preeclamptic patients has significantly higher, not lower deoxyribonuclease activity. In a large animal experiment we tested the effects of fetal and adult DNA from mice or humans on pregnant mice. However, in our hands none of them induced preeclampsia-like symptoms. Fetal and placental hypotrophy and some indices of maternal renal involvement were induced only by lipopolysaccharide as a model of bacterial infection. This model of sepsis includes activation of neutrophils and production neutrophil extracellular traps that leads to an increase in extracellular DNA bound to histones. One of the inducers of NETosis are damage-associated molecular patterns such as mitochondria. Mitochondria and their DNA have been shown to induce inflammation as they are recognized by the immune system as bacteria. To prove whether this mechanism could be of relevance to preeclampsia, we have quantified mitochondrial DNA in plasma from women with preeclampsia, but found no difference in comparison to healthy pregnant women. In addition, we are now conducting an animal experiment focused on the effects of mitochondria/mitochondrial DNA injections on pregnant mice and their pups. Hopefully, the results will be presented at the meeting. But it is clear that other NETosis inducers have to be taken into account.

Keywords: cell-free DNA, mitochoondrial DNA, preeclampsia, DNase

L10

FIRST TRIMESTER SCREENING OF CIRCULATING C19MC MICRORNAS CAN PREDICT SUBSEQUENT ONSET OF GESTATIONAL HYPERTENSION

Ilona Hromadnikova[1], Katerina Kotlabova[1], Lucie Hympanova[1,2], Ladislav Krofta[2]

[1] Department of Molecular Biology and Cell Pathology, Third Faculty of Medicine, Charles University, Ruska 87, 100 00 Prague, Czech Republic
[2] Institute for the Care of the Mother and Child, Third Faculty of Medicine, Charles University, Podolske nabrezi 157/36, 147 00 Prague, Czech Republic

Objective: The objective of the study was to evaluate risk assessment for gestational hypertension based on the profile of circulating placental specific C19MC microRNAs in early pregnancy.

Study design: The prospective longitudinal cohort study of women enrolled at first trimester screening at 10 to 13 weeks was carried out (n=267). Relative quantification of placental specific C19MC microRNAs (miR-516-5p, miR-517*, miR-518b, miR-520a*, miR-520h, miR-525 and miR-526a) was determined in 28 normal pregnancies and 18 pregnancies which developed gestational hypertension using real-time PCR and a comparative Ct method relative to synthetic C. elegans microRNA (cel-miR-39).

Results: Increased extracellular C19MC microRNA plasmatic levels (miR-516-5p, $p< 0.001$; miR-517*, $p= 0.007$; miR-520h, $p< 0.001$; miR-518b, $p= 0.002$) were detected in patients destined to develop gestational hypertension. MiR-520h had the best predictive performance with a PPV of 84.6% at a 7.1% false positive rate. The combination of miR-520h and miR-518b was able to predict 82.6% of women at the same false positive rate. The overall predictive capacity of single miR-518b (73.3% at 14.3% FPR), miR-516-5p (70.6% at 17.9% FPR) and miR-517* (57.9% at 28.6% FPR) biomarkers was lower.

Conclusion: The study brought interesting finding that the up-regulation of miR-516-5p, miR-517*, miR-520h and miR-518b is associated with a risk of later development of gestational hypertension. First trimester screening of extracellular miR-520h alone or in combination with miR-518b identified a significant proportion of women with subsequent gestational hypertension.

Acknowledgements: The work was supported by the Charles University research program PRVOUK P32.

Keywords: circulating C19MC microRNA, first trimester screening, plasma, gestational hypertension, prediction

S1

CLINICAL EXPERIENCE RESULTS ON NON-INVASIVE PRENATAL TESTING: IMPLICATIONS FOR COUNSELING AND CHOICES

Lieve Page-Christiaens, Patricia A Taneja, Holly L Snyder

Reproductive & Genetic Health, Illumina, Inc.

The results of non-invasive prenatal testing for trisomy 21, 18 and 13 in a clinical setting in two interdependent cohorts of women will be presented. Cohort A (n=85,298) was women pregnant with a singleton who either had a normal result or a result indicative for trisomy 21, 18 or 13. Technical test failure occurred in 0.1 %. Mean time to result was 3.3 working days. False positive rate was 0.3 % and false negative rate 0.02 %. The combined aneuploidy rate was 2.5%. Clinical outcome was available in 41%. Observed PPV in cases with known clinical outcomes was 83.5 %. Cohort B (n=113,415), of which Cohort A is a subset, also contained women with singleton pregnancies, and describes the occurrence of multiple aneuploidies and single monosomies. In about half the identified cases, fetal or neonatal karyotypes were available. The clinical significance of these additional findings of NIPT for fetal and maternal health will be discussed.

Keywords: aneuploidy, monosomy, non-invasive prenatal testing (NIPT)

S2

TWO YEARS OF HARMONY PRENATAL TEST IN CROATIA

Maja Barbalić

Genom d. o. o. Zagreb, Croatia

Harmony Prenatal test was one of the first offered non-invasive prenatal tests in Croatia, started in November 2013. From November 2013 to April 2016, 990 Harmony Prenatal tests were performed in more than 30 clinics in Croatia. The average maternal age was 36.3±5.1, gestational age 13.0±2.2 weeks and fetal fraction 12.2 % with a range of 4.0 % to 30.0 %. The rate of successful analyses was 99.6 %. Unsuccessful analyses were due to low fetal cfDNA (2 cases) and laboratory processing/high variance in cfDNA counts (2 cases). Altogether, there were 19 positive results for analyzed aberrations. Number of false positive results was 1 for Down syndrome, 1 for Edwards and 1 for Turner syndrome. Two positive trisomy 21 cases showed mosaicism after invasive procedure. We will present the data and our experience in introducing and offering Harmony Prenatal test in Croatia with the emphasis on several interesting cases. We will also emphasize the need for appropriate genetic education and counseling in the introduction and application of new genetic tests to medical practice.

Keywords: cell free fetal DNA, mosaicism, non-invasive prenatal testing (NIPT)

S3

IMPLEMENTATION OF A DECENTRALIZED LAB SOLUTION FOR NIPT TESTING

Jessie Theuns

Multiplicom N.V., Niel, Belgium.

Clarigo™ is a CE marked non-invasive prenatal test (NIPT), specifically designed for implementation in local clinical laboratories to enable NIPT for all. This simple, robust and highly reliable test screens for the aneuploidy status of chromosome 21, 18 and 13 early in pregnancy using standard lab equipment. Since its launch in October last year, labs all over Europe are implementing the Clarigo test rapidly and efficiently.

Keywords: NIPT, Lab solution, CE-IVD, chromosome 21, 18 and 13, CLarigo

S4

THE UTILIZATION OF MISEQ PLATFORM FOR NONINVASIVE PRENATAL TESTING OF TRISOMY 21 AND EVALUATION OF SIZE SELECTION METHODS ON ANALYTICAL PERFORMANCE

Tomas Szemes[1,2], Michaela Hyblova[1,2], Barbora Vlkova-Izrael[6], Jaroslav Budis[2], Frantisek Duris[2], Orsolya Biro[7], Balint Nagy[7,8], Gabriel Minarik[1,2,6]

[1]Dept. of Molecular Biology, Faculty of Natural Sciences, Comenius University in Bratislava, Bratislava, Slovakia
[2]Geneton Ltd., Bratislava, Slovakia
[6]Institute of Molecular Biomedicine, Faculty of Medicine, Comenius University in Bratislava, Bratislava, Slovakia
[7]1st Dept. of Obstetrics and Gynecology, Semmelweis University, Budapest, Hungary
[8]Dept. of Human Genetics, University of Debrecen, Hungary

Background: The majority of currently available NIPT tests is based on whole genome sequencing by ultrahigh throughput NGS platforms. The lack of validation studies performed on low/middle throughput benchtop NGS systems limits their use. The aims of our study were to test the MiSeq platform for NIPT of trisomy 21 applying different z-score calculation methods and to optimize the sample preparation and data analysis.

Methods: Samples from 130 pregnant women were analyzed by whole genome sequencing on benchtop NGS system, MiSeq. The targeted yield of 3 million raw reads was used for z-score calculation. We evaluated the impact of in silico and physical size selection on analytical performance of the test.

Results: Using a z score value of 3 as the cut-off, 99.06-100% (105-106/106) specificity and 100% (24/24) sensitivity were observed. After in silico size selection the test reached 100% specificity and sensitivity. Following the physical size selection, z-scores of tested trisomic samples increased significantly (p=0.025).

Conclusions: The utilization of benchtop NGS platform for NIPT of trisomy 21 led to results equivalent to previously published studies performed on high/ultrahigh throughput NGS systems. Our work could represent a basis for increasing the cost-effectiveness of the test and thus facilitate its penetration worldwide.

Keywords: NIPT, trisomy 21, benchtop NGS platform

S5

HOW TO OVERCOME THE CHALLENGES IN CIRCULATING NUCLEIC ACID WORKFLOWS

Burcu Dartan-Karagozler

QIAGEN Germany

Liquid biopsy is a new, minimally invasive technology for detecting disease biomarkers without the need for costly or invasive procedures. It can sidestep stressful, costly surgical procedures and holds benefits for patients, physicians and pathologists once the challenges are overcome. By studying elusive, low-abundance DNA fragments, evaluating valuable biomarkers from cellular vesicles, or even analyzing a whole genome or transcriptome from a single cell, serious diseases can be detected, studied and monitored. This requires overcoming the challenges associated with isolating and analyzing circulating cell-free nucleic acids, exosomes and circulating tumor cells from blood and other biofluids with high sensitivity, specificity and speed, along with interpreting the relevant data from high noise-to-signal results.

We believe that liquid biopsies have the potential to transform research and healthcare. By delivering solutions for each step that can help scientists conduct, analyze and interpret liquid biopsy studies with greater precision and reliability, we can overcome the challenges in circulating nucleic acid workflows.

Keywords: cell free nucleic acid, liquid biopsy

S6

MYHEALTHYSTART: A NEW PARADIGM IN REPRODUCTIVE GENOMIC HEALTH

Simen BB[1], Russell H[1], Martinez D[1], Fortin H[1], Callahan M[1], Fernandes Z[1], Thakuria JV[1,2,3]

Veritas Genetics, Danvers, MA, USA
Massachusetts General Hospital, Boston, MA
Harvard Medical School, Boston, MA

Enabled by advances in massively parallel next generation sequencing (NGS), genomic testing in reproductive health is changing current standards in clinical care. Veritas Genetics has developed a comprehensive genomic health offering that starts at preconception (with carrier testing of Mendelian disorders in parents), followed by NIPT (non-invasive prenatal testing for aneuploidies) and concluding with a first of its kind molecular newborn test that combines both genetic and biochemical testing for serious neonatal and pediatric conditions.

Veritas Genetics is founded by leaders in genomics from Harvard Medical School including Professor George Church. Veritas Genetics is dedicated to making genomic testing accessible and affordable - offering valuable reproductive genomic health information to individuals and their healthcare providers globally.

Keywords: Circulating cell free fetal DNA (ccffDNA), NIPT (non-invasive prenatal testing), NGS (next generation DNA sequencing), expanded carrier screening, NBS (newborn screening)

Paediatr Croat. 2016;60 (suppl 2):1-44

S7

PERSPECTIVES ON THE FUTURE OF NIPT

Maximilian Schmid

Roche

Not provided.

O1

NIPD AND IMPORTANCE OF GENETIC COUNSELING

Vida Čulić

Medical Genetics, Paediatrics Clinics, UHC Split

The 1997 discovery of free fetal DNA in maternal plasma launched clinical researchers' efforts to establish a reliable method for non-invasive prenatal testing for fetal genetic conditions. Cell free fetal DNA analysis from maternal blood is reliable from the early gestation and it has potential for routine diagnostic practice, so that invasive procedures could be avoided. It is an option for patients whose pregnancies are considered to be at an increased risk for certain chromosome abnormalities and carriers of single gene disorders.

NIPT/NIPD can only be offered in the context of informed consent, education, and counseling by a qualified provider, such as a certified genetic counselor. Patients whose NIPT/NIPD results are abnormal should receive genetic counseling and be given the option of standard confirmatory diagnostic testing.

Keywords: cell free fetal DNA, genetics counselling, non-invasive prenatal diagnosis (NIPD)

O2

SEVEN FALSE-POSITIVE CASES FROM NONINVASIVE CELL-FREE FETAL DNA TESTING: DATA OBTAINED FROM THREE REFERRING CENTRES IN CROATIA

Feodora Stipoljev[1,6], Kristina Crkvenac-Gornik[2], Sanda Huljev Frković[3], Romana Gjergja-Juraški[4,6], Bojana Brajenović-Milić[5]

[1]Cytogenetic laboratory, Clinic of Obstetrics and Gynecology, Clinical Hospital „Sveti Duh", Zagreb;
[2]Cytogenetic laboratory, University Hospital Centre Zagreb,
[3]Division for genetics, Department of Pediatrics, University Hospital Centre Zagreb;
[4]Children's Hospital Srebrnjak Zagreb,
[5]Department of Biology and Medical Genetics, School of Medicine, University of Rijeka;
[6]Faculty of Medicine, University of Osijek, Osijek, Croatia

Noninvasive prenatal testing (NIPT) using cell-free fetal DNA for screening of chromosomal aneuploidies offers high sensibility and specificity in the routine clinical praxis. However, there is still insufficient data regarding false positive and false negative cases. Both, false positive and negative results are mainly due to the confined placental mosaicism, or vanishing twin phenomenom. A possible mosaic maternal aneuploidy can be due to false positive result for fetal sex aneuploidy.

We report seven cases with positive NIPT evaluated by chorionic villus biopsy or amniocentesis. The results of NIPT showed high risk for Turner syndrome in two cases, in two cases for Edwards syndrome, one triple X, one Down syndrome and one trisomy 16. All evaluated fetuses have normal karyotypes.

Positive results from NIPT must always be confirmed by invasive prenatal diagnosis. In cases where aneuploidy for X chromosome was detected, maternal cytogenetic evaluation should be considered, and offered through genetic counselling. Full information about advantages and limitation of this method is mandatory.

Keywords: aneuploidy, cell free fetal DNA, genetic counselling, non-invasive prenatal testing (NIPT)

O3

CONTINGENT CELL-FREE DNA TEST IN ROUTINE PRENATAL ANEUPLOIDY SCREENING

Martin Hynek, Filip Zembol, Ivona Marešová, Svatava Horáčková, Martina Bittóová, Monika Koudová, David Stejskal

Gennet, Centre for Fetal Medicine and Reproductive Genetics, Prague, Czech Republic

Objectives: To assess an incorporation of cell-free DNA (cfDNA) test into routine prenatal screening for fetal aneuploidies. Our in-house cfDNA test was integrated in a contingent way as a part of first-trimester combined test on non-commercial basis.

Methods: This was a prospective study in which cfDNA analysis was performed in pregnant women with the risk for trisomies from first-trimester combined test between 1/100 and 1/500. Moreover, samples from pregnancies with trisomies confirmed by karyotyping were processed in order to further assess cfDNA test performance. For each sample, cfDNA was isolated from maternal plasma using *QIAamp Circulating Nucleic Acid Kit (Qiagen)* and the whole-genome sequencing using Ion Proton (Life Technologies) was performed. The results were assessed using MoM-based approach with chromosomal fingerprint and exponentially weigthed moving average charts (EWMA). Cut-off ^{3} 15 % of EWMA curve above upper limit was considered a high risk for trisomy. In case of positive cfDNA test amniocentesis was performed to confirm the diagnosis.

Results: A total of 1356 prenatal samples were processed since January 2015. 1298 samples had test results evaluable, 58 samples (4.9%) were re-sampled and re-processed. Out of 79 trisomic cases there was one false negative result for trisomy 21. One sample was false positive for trisomy 13, six samples for trisomy 18 and 11 samples for trisomy 21. The cfDNA test had a detection rate of 100% for trisomy 13 (n=8), 100% for trisomy 18 (n=18) and 98.1% for trisomy 21 (n=53). The false positive rates were 0.1 %, 0.5 % and 0.8% for trisomy 13, 18 and 21, respectively. The overall detection rate for all trisomies was 98.7% and the overall false positivity was 1.4 %.

Conclusion: We presented that cfDNA test can be successfully incorporated into fetal aneuploidy screening on non-commercial basis. Our cfDNA test showed high sensitivity and low false positivity.

Keywords: non-invasive prenatal testing, chromosomal aneuploidies, prenatal screening, cell-free DNA, exponentially weighted moving average chart

O4

RELATIONSHIPS BETWEEN TOTAL AND CELL FREE DNA, FETAL CHARACTERISTICS AND SERUM ANALYTES IN THE FIRST TRIMESTER OF PREGNANCY

Ivanka Bekavac Vlatkovic [1], Jasenka Wagner [2], Ana Vicic [1], Feodora Stipoljev [1,2]

[1]University Hospital „Sveti Duh"; Department of Obstetrics and Gynecology, Zagreb
[2]Faculty of Medicine, Josip Juraj Strossmayer University of Osijek, Department of Medical biology and genetics

We aimed to compare the levels of fetal and total cell free DNA in maternal plasma between 11 and 13+6 weeks gestation on prospectively colllected samples to assess the relationship with first trimester maternal biochemical screening markers and fetal characteristics in euploid and aneuploid fetuses.

DNA from pre CVS maternal plasma was extracted from 56 controls and 56 aneuploid first trimester pregnancies and quantified using real time PCR. A coding region of the male specific SRY gene was used to monitor the presence of free fetal DNA and a coding region of the human telomerase reverse transcriptase gene to quantify total free DNA.

There was no significant association between maternal serum first trimester biochemical markers and crown-rump lenght in both groups with levels of fetal or total cell free DNA. In the group of aneuploid fetuses, there was no correlation with increase of nuchal translucency tickness (total cell free DNA p=0,173, fetal cell free DNA =0,082). Furthermore, there was no association between cell free total DNA (p= 0,440) and fetal (p=0,246) and NT irrespective of morphological apperance / septated or not/.

The levels of both fetal and maternal cell free DNA were not significantly altered by fetal biological factors in first trimester of pregnancy. As the release of fetal cell free DNA is closely tied to placental morphogenesis, condition that alter the placenta may directly impact its level on maternal concentration an prediciton of adverse pregnancy outcome irrespective of fetal characteristics. Furhtermore, because PAPP-a, βHCG and total and fetal cell free DNA are not statisticaly correlated and cell-free fetal DNA can be reliably detected in the first trimester, the addition of total and cell-free DNA to serum screening strategies may be helpful in prediciting adverse pregnancy outcome.

Keywords: aneuploidy, cell free fetal DNA, first trimester

O5

QUANTITATIVE MPS-BASED NIPT METHOD USING INTERNAL AMPLIFICATION CONTROL DURING LIBRARY PREPARATION

Priskin K[1], Pinter L[1,2], Jaksa G[1], Balogh D[1], Farkas K[4], Nagy N[3], Csűrös M[5], Széll M[3], Haracska L[1,6]

[1]Delta Bio 2000 Ltd. Szeged, Temesvari krt. 62, H-6726
[2]Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Temesvari krt. 62, H-6726, Hungary
[3]Department of Medical Genetics, University of Szeged, 4 Somogyi B., H-6720, Szeged, Hungary.
[4] MTA-SZTE Dermatological Research Group, University of Szeged, Szeged, Hungary
[5]Department of Computer Science and Operations Research, University of Montréal, C.P. 6128 succursale Centre-Ville, Montréal, Québec H3C 3J7, Canada.
[6]Institute of Genetics, Biological Research Centre, Hungarian Academy of Sciences, Szeged, Temesvari krt. 62, H-6726, Hungary haracska@brc.hu

There is a permanent need for a cost-effective NIPT process since high costs impede extensive application of this type of screening. Even targeted NGS-based tests require a huge amount of sequence data to achieve the expected reliability. Sequencing large numbers of genomic regions provides us with information about microdeletions, but the clinical relevance of these abnormalities is, in most cases, unclear. Furthermore, the exhibition of genetic data with all the potentially clinically relevant information leads to ethical issues regarding the personality rights of the fetus.

The NGS-based NIPT technique identifies large numbers of genomic regions by sequencing mixed DNA circulating in the maternal plasma. After defining the chromosomal origin of the sequencing reads, the relative amount of cfDNA from the aneuploid chromosome found in the maternal plasma is counted by comparing the number of reads mapping to the chromosomes of interest with the number of reads mapping to one or more, presumably normal, reference chromosomes. Since the compared genomic regions are sequentially different, this difference results in technical bias in direct comparism, even the quantitative targeting method requires a high number of checking points in the genome. To reduce costs, but to maintain the expected reliability, our technical innovation focuses on a NGS-based NIPT method that uses a limited number of specific regions for the detection of chromosomal aneuploidy.

Keywords: cost-effective, quantitative, internal amplification control

O6

IDENTIFYING SUB-CHROMOSOMAL DELETIONS AND DUPLICATIONS BY NIPT IN CONGENITAL HEART DISEASE CASES

Orsolya Biro[1], Balint Nagy[1,2], Lucia Strieskova[3,4], Michaela Hyblova[3,4], Tomas Szemes[3,4]

[1]Dept. of Obstetrics and Gynecology, Semmelweis University, Budapest, Hungary,
[2]Dept. of Human Genetics, University of Debrecen, Hungary,
[3]Dept. of Molecular Biology, Faculty of Natural Sciences, Comenius University in Bratislava, Bratislava, Slovakia,
[4]Geneton Ltd., Bratislava, Slovakia

Background: Clinically relevant micro-deletions and -duplications occur in up to 1 in 60 pregnancies, and can be present in cases lacking ultrasound anomalies. Congenital heart defects (CHD) are the most common fetal anomalies and often are associated with chromosomal abnormalities. Our aims were to search for sub-chromosomal aberrations in CHD cases and to assess the feasibility of in house NIPT to detect these aberrations.

Methods: 19 maternal blood samples were analyzed by the Illumina NextSeq whole-genome sequencing system. A combined in silico and physical size-selection was performed to enrich fetal DNA in maternal plasma samples. WISECONDOR algorithm was used for data analysis which allows the detection of small aberrations by shallow sequencing.

Results: 9 samples of total 19 met the criteria for WISECONDOR lowest threshold sensitivity which is at least 8 million mappable reads. Sub-chromosomal aberrations were detected in 2 CHD cases: In the first one we found probable microduplication within region 138-139 Mb which is a part of genomic position 8q24.23. In the second one we found probable microdeletion within region 104-105 Mb which is a part of 5q22.3.

Conclusions: The clinical problem that is most often diagnosed in children with 8q duplication is a conotruncal heart defect, and 5q22 deletions are also associated with minor heart conditions. According to our results, the detection of sub-chromosomal aberrations may be assisted using a standard NIPT, however further developments are needed to improve the methodology.

Keywords: NIPT, sub-chromosomal aberrations, congenital heart disease

O7

EXTRACELLULAR DNA IN PREGNANCIES WITH INTRAHEPATIC CHOLESTASIS

Barbora Vlková[1], Marta Kalousová[2], Peter Celec[1]

[1]Institute of Molecular Biomedicine, Comenius University, Bratislava, Slovakia
[2]Institute of Medical Biochemistry and Laboratory Diagnostics, General University Hospital and The First Faculty of Medicine of Charles University, Prague, Czech Republic
barboravlk@gmail.com

Although extracellular DNA (ecDNA) is widely used for noninvasive prenatal diagnosis, its biological fate, especially its clearance is everything but clear. Previous studies have shown that kidneys excrete only a small part of plasma DNA fragments in the form of urinary DNA. Most of the ecDNA protected against deoxyribonucleases is bound to histones in the form of nucleosomes. These particles can be catched and cleared by the liver, very likely via phagocytosis by the Kupffer cells. To analyze the role of liver damage in the clearance of ecDNA we have quantified total extracellular DNA using Qubit, as well as nuclear and mitochondrial DNA using real time PCR in plasma samples from pregnant women suffering from liver damage due to intrahepatic cholestasis (n=13). In comparison to age and gestation age matched healthy pregnancies (n=19) plasma samples from patients with increased liver enzymes contain more than two times more nuclear ecDNA (336 ± 259 GE/ml vs 722 ± 551 GE/m; $p<0,05$). No significant differences were found between the groups in mitochondrial DNA. Our results indicate that liver damage leads to higher nuclear ecDNA in contrast to free mitochondrial DNA that is sensitive to the effects of plasma deoxyrboonucleases. This is in line with the hypothesis that liver clears ecDNA bound to histones. An alternative hypothesis would be that the found higher concentrations of nuclear ecDNA in plasma might be due to the damage to hepatocytes. Whether the observed association is causative can only be proved through interventional experiments and detailed analysis of the origin of plasma ecDNA. Animal experiments focusing on the liver and its role in the clearance of ecDNA are ongoing and will hopefully shed more light on this fascinating topic.

Keywords: cell-free DNA, liver damage, pregnancy-related diseases

O8

IDENTIFYING POTENTIAL MIRNA BIOMARKERS FOR PREECLAMPSIA BY SYSTEMS BIOLOGY APPROACHES

Orsolya Biró[1], Bálint Nagy[1,2], János Rigó Jr.[1]

[1]1st Dept. of Gynecology and Obstetrics, Semmelweis University, Budapest, Hungary
[2]Dept. of Human Genetics, University of Debrecen, Hungary

Background: Preeclampsia is the major cause of maternal and fetal morbidity and mortality, affecting 3-8% of all pregnancies worldwide. miRNAs are small, non-coding RNA molecules, which negatively regulate gene expression. Recent findings suggest that miRNAs contribute to pregnancy complications, including preeclampsia. The aims of our study were to find possible regulatory mechanisms, which are connected to the pathogenesis of preeclampsia, and to find a candidate miRNA biomarker in a Hungarian cohort.

Methods: We integrated publicly available placental miRNA and mRNA expression profiles from preeclamptic and normal pregnancies. A bipartite network was created from the significant miRNA-mRNA pairs using MAGIA and Cytoscape softwares. We analyzed miRNAs and their targets by different bioinformatics tools and through literature research. In the second part of the study, we collected plasma samples from woman affected by preeclampsia and from healthy controls. At first, exosomes were isolated using a specific precipitation method and then miRNA expression analysis was performed by RT-PCR.

Results: We created a network, which consists of 85 nodes and 80 edges signalizing the connections between 52 regulated genes and 33 miRNAs. 11 of the regulated genes are preeclampsia related and 9 of them were targeted by multiple miRNAs. 8 miRNAs were associated with preeclampsia before, and 13 miRNAs regulated more than one mRNA. We identified several miRNA-mRNA regulatory mechanisms which may contribute to the pathogenesis of preeclampsia. Hsa-mir-210 was the highest degree node in the network and its role in preeclampsia is well-established. According to our preliminary results, the miRNA is a potential biomarker in the Hungarian cohort.

Keywords: preeclampsia, miRNA, network analysis, biomarker

O9

THE CITY OF FLOWERS, DEBRECEN
INVITATION TO THE 5TH CEE SYMPOSIUM ON FREE NUCLEIC ACIDS IN NON-INVASIVE PRENATAL DIAGNOSIS IN 2018

Balint Nagy

Medical Genetics Department, University of Debrecen, Hungary

It is our great honor to invite you to the 5th Central-Eastern European Symposium on Free Nucleic Acids in Non-Invasive Prenatal Diagnosis Congress in Debrecen, Hungary in 2018.

The city of Debrecen is situated in the eastern part of Hungary, with around 204,000 inhabitants. As the nation's second largest city, it enjoys some of the best concerts, entertainment events, and cultural gatherings to be found in Hungary. Since 1966 the city organizes the Flower Carnival, which is a very popular event not only in Hungary, but in the neighboring countries too.

Debrecen is also known as a student city; the University of Debrecen is the most popular higher educational institute in Hungary based on the number of international students. Founded in 1538, it is Hungary's oldest continuously running higher education institution.

Today the 14 faculties situated on 7 campuses have over 27,000 students. The international programs were launched in 1987 and all of them are delivered in English. At present, the number of international students is about 4,000 and they represent countries from all the major regions of the world, including Europe, the Middle and Far East, Africa, and North America.

The research groups of medicine-related sciences were organized into the Research Center for Molecular Medicine (RCMM), dedicated to clinically oriented and cutting edge biomedical research. In 2002, the research, training, and networking activities of the RCMM were recognized by the European Commission, receiving the "Centre of Excellence" title.

The knowledge on free nucleic acids and associated technologies have advanced substantially since the first meeting in Budapest in 2010, the second in Olomouc in 2012, and the third in Martin in 2014. I hope following the well organized and successful symposium in Split in 2016, you will avidly look forward to participating in the fifth symposium in Debrecen, Hungary in 2018.

P1

PRENATAL SCREENING OF ANEUPLOIDIES USING THE FIRST TRIMESTER QUAD TEST HAS HIGHER DETECTION RATE AND POTENTIALLY DECREASES INVASIVE PRENATAL PROCEDURES WHEN COMBINED WITH NIPT: A CZECH PILOT STUDY

Vlckova M[1], Tesner P[1], Peskova M[1], Vlk R[2], Matecha J[2], Hanulikova P[2], Zimmermann P[3], Geryk J[1], Havlovicova M[1], Macek M. Jr[1], Stambergova A[1], Macek M. Sr[1]

[1] Department of Biology and Medical Genetics, Charles University 2nd Faculty of Medicine and University Hospital Motol, Prague, Czech Republic
[2] Department of Obstetrics and Gynaecology, Charles University 2nd Faculty of Medicine and University Hospital Motol, Prague, Czech Republic
[3] Department of Statistics and Probability, Faculty of Informatics and Statistics, University of Economics, Prague, Czech Republic

Trisomy 21 (T21), as the most common genetic cause of intellectual disability, has been the focus of prenatal screening since 1980s. Since then multiple alternative protocols for T21 prenatal screening have been developed, concurrently with the gradually increasing maternal age in Europe, and beyond.

Here we present our first experience with the first trimester combined QUAD prenatal test (measurement of PAPP-A, fβ-hCG, PlGF, AFP together with ultrasound nuchal translucency /NT/ and nasal bone /NB/ assessement) in comparison to the current "dual screening" protocol (measurement of PAPP-A, fβ-hCG with NT and NB). The main aim of the study was to optimize the indications for invasive prenatal diagnosis and non-invasive prenatal testing (NIPT). We compared the results of QUAD screening with those generated by the "dual protocol" in 41 singleton pregnancies with proven T21 and 433 controls.

Biochemical analyses were performed by B.R.A.H.M.S Kryptor for dual test and Perkin Elmer Delfia® Xpress for QUAD test, and the LifeCycle™ software was used for the risk calculations.

Examined cases of pregnant women were stratified into 3 study cohorts: high risk (> 1:100), intermediate risk (1:101 – 1:500) and low risk (< 1:500) for data assessment and re-evaluation. Overall, we detected 97.6 % (for QUAD) and 85.4 % (for the "dual protocol") of all T21 pregnancies, respectively. In controls, 16.9 % of pregnant women were at high or intermediate risk (for the QUAD test), compared to 18.2 % (for the "dual protocol"). In conclusion, the QUAD test has higher detection rate of T21 than currently utilised prenatal screening protocols at our centre. Furthermore, if the QUAD testing strategy is combined with NIPT for the intermediate group, indications for invasive testing could be markedly reduced.

Supported by FNM 00064203, IGA-NT13770, LN14073, NF-CZ11-PDP-3-003-201 and CZ.2.16/3.1.00/24022.

Keywords: first trimester screening, trisomy 21, QUAD test, NIPT

P2

NON-INVASIVE PRENATAL TESTING FOR FETAL ANEUPLODIES: A REPORT OF THREE ATYPICAL CASES

Ivona Maresova, Svatava Horackova, Jana Vavrova, Filip Zembol, Vera Krutilkova, Marie Trkova, Martin Hynek, David Stejskal, Martina Bittoova, Monika Koudova

Center for Fetal Medicine and Reproductive Genetics, Prague, Czech Republic

We are presenting three cases of false negative or inconclusive results of non-invasive prenatal testing (NIPT).

The first case was a mother with the negative first trimester screening result for trisomy 21 (1/1800) and low risk of trisomy 21, 18 and 13 assessed by NIPT. However, a fetus with Down syndrome was born. The mother's karyotype showed 10% gonosomal mosaic of chromosome X. Her partner's chromosome 21 specific FISH revealed an inclination to nondisjunction of chromosome 21 (21 monosomy/trisomy clones). False negative NIPT result was probably due to low fetal fraction or placental mosaicism.

The second case was a mother with positive first trimester screening result for trisomy 21 (1/70). The NIPT result showed an increased risk of trisomy of chromosome X. Surprisingly, amniocentesis revealed trisomy of chromosome 18. The pregnancy was terminated and cytogenetic examination of the placenta detected various representations of cell lines with trisomies of chromosomes 18 and X, with a predominance of the line XXX. NIPT method did not reveal trisomy of chromosome 18, most likely due to placental mosaicism with a predominance of the line XXX.

The third case was a mother with positive first trimester screening result for trisomy 21 (1/20). CVS proved de novo translocation of trisomy 21. Follow-up NIPT performed as a part of validation NIPT study showed a borderline risk of trisomy 21 most likely due to loss of part of 21 genome during translocation event.

The aim of these case studies is to present biological limits of NIPT which should be taken into consideration. The most frequently occurring NIPT limits include low fetal fraction, higher body weight, placental mosaicism, and rare chromosomal aberrations.

Keywords: trisomy 21, non-invasive prenatal testing, placental mosaicism

P3

MOM-BASED APPROACH TO NON-INVASIVE PRENATAL TESTING USING EXPONENTIALLY WEIGHTED MOVING AVERAGE CHART AND CHROMOSOMAL FINGERPRINT

Martin Hynek, Filip Zembol, Ivona Marešová, Svatava Horáčková, David Stejskal

Gennet, Centre for Fetal Medicine and Reproductive Genetics, Prague, Czech Republic

Objectives: The aim of this study was to develop a new method of screening for fetal aneuploidies based on the detection of deviation in the distribution of cell-free fetal DNA (cfDNA) in maternal plasma. We combined three innovative approaches: the number of reads expressed in multiples of median (MoM), chromosomal fingerprint and exponentially weighted moving average chart (EWMA).

Methods: In singleton pregnancies cfDNA was isolated from maternal plasma using *QIAamp Circulating Nucleic Acid Kit (Qiagen)* and the whole-genome sequencing using Ion Proton (Life Technologies) was performed. The numbers of reads in 60 kb bins were expressed in MoMs. Revealing that all fragments are not replicated and sequenced identically, we constructed „chromosomal fingerprints", a kind of chromosomal map, where every bin is characterized by the average and standard deviation of the number of reads in MoMs. When testing an unknown sample, the deviation in the distribution of MoMs in every bin from the expected values of the fingerprint is expressed as Z-score. To assess the distribution of Z-scores along the chromosome the EWMA chart is used, where the deviation of the curve above the upper limit indicates a high risk of trisomy. The approach was assessed using 179 prenatal samples.

Results: Fingerprints for chromosomes 13, 18 and 21 were constructed using 93 samples from euploid pregnancies. 179 samples with known fetal karyotype (145 euploid, 3 with T13, 9 with T18 and 22 with T21) were sequenced and EWMA charts produced. More than 15% of EWMA curve was above the limit in all 34 samples from trisomic pregnancies (100% detection rate for T13, T18 and T21). In 145 euploid samples there were only 2 cases in which chromosome 21 showed more than 15% of EWMA above the limit.

Conclusion: The new MoM-based approach using chromosomal fingerprint and EWMA has a high performance in non-invasive aneuploidy screening with high sensitivity and low false positivity. Further information will be from currently ongoing clinical phase.

Keywords: non-invasive prenatal testing, chromosomal aneuploidies, prenatal screening, exponentially weighted moving average chart

P4

A NON-INVASIVE PRENATAL DIAGNOSTIC (NIPD) SERVICE FOR PATERNAL MUTATION AND DE NOVO RECURRENCE EXCLUSION

Suzanne Drury, Sarah Mason, Sandra Moore, Fiona McKay, Lucy Jenkins, Lyn S Chitty

NE Thames Regional Genetics, Great Ormond Street Hospital, London, UK

Analysis of cell free DNA (cfDNA) in maternal plasma for the detection of fetal aneuploidy is available world-wide, but there is very limited availability of NIPD for women with pregnancies at increased risk of monogenic disorders. We offer a NIPD service for detection of paternal or *de-novo* alleles for the diagnosis of *FGFR3 and FGFR2*- related conditions and for cystic fibrosis in pregnancies where parents are heterozygous for different mutations. Here we describe expansion of our NIPD service to families at risk of a range of rare monogenic conditions as a safe, earlier alternative to invasive testing.

Primers were designed to target relevant mutations and included P5 and P7 Illumina sequencing adapters along with incorporation of a 6bp unique index. For cfDNA, in addition to the mutation specific primer, primers for *HLA* and *ZFX/ZFY* were included for each patient enabling us to confirm the presence of fetal DNA. Amplicons were sequenced on an Illumina MiSeq and FASTQ files analysed to count the number of wild-type and mutant reads.

In our accredited public-sector genetics laboratory we have developed bespoke definitive testing for tuberous sclerosis (3), neurofibromatosis type 1 (2), Rhabdoid tumour predisposition (1), early infantile epileptic encephalopathy (1), osteogenesis imperfecta (5), Fraser syndrome (2) and intellectual disability (*ARID1B*; 1). Additionally we have delivered NIPD in pregnancies at risk of achondroplasia (133), thanataphoric dysplasia (80), Apert syndrome (13), cystic fibrosis (12) and Crouzon syndrome (7). In our laboratory 32% of monogenic prenatal diagnosis now employs NIPD.

Mutation exclusion or detection by NIPD is a feasible approach for routine diagnostics, including bespoke assay design. NIPD is of particular benefit for parents with a low recurrence risk who do not wish to put a likely unaffected pregnancy at risk. Extension of the service to include recessive and X-linked disorders is underway.

Keywords: aneuploidy, cell free fetal DNA, non-invasive prenatal diagnostic (NIPD)

P5

LET-7C MIRNA, A NEW CIRCULATING BIOMARKER OF CONGENITAL HEART DISEASE

Orsolya Biró[1], Gilda Cobellis[2], János Rigó Jr.[1], Bálint Nagy[1,3]

[1]Dept. of Gynecology and Obstetrics, Semmelweis University, Budapest, Hungary
[2]Dept. of Biophysics, Biochemistry and General Pathology, Second University of Naples, Naples, Italy
[3]Dept. of Human Genetics, University of Debrecen, Hungary

Background: Congenital heart defects (CHD) are the most common fetal malformations. Biomarker research could facilitate early prenatal diagnosis. miRNAs are short, non-coding RNA molecules which regulate eukaryotic gene expression. Recent findings suggest that miRNAs contribute to heart development and can be found in maternal circulation. In this study, our aim was to analyze miRNA expression in maternal plasma and fetal heart samples affected by CHD for the purpose of identifying potential biomarkers.

Materials and methods: Peripheral blood samples were collected from 79 pregnant women, 38 had fetus with CHD and 41 without CHD. In the affected cases, we detected 12 aneuploidies by cytogenetic analysis. Total-miRNA was extracted from the plasma samples. miRNA PCR Array analysis was carried out on 22 affected and 12 normal samples, by using cardiovascular disease specific panel. The miRNA samples were pooled considering heart- and chromosomal abnormalities, and differentially expressed miRNAs were identified. We validated the most significantly overexpressed miRNA, let-7c expression on 14 diseased and 14 control cases. Finally, we analyzed heart samples from fetuses with CHD and/or trisomy 21 and healthy controls to confirm the miRNA's importance in pathogenesis of CHD.

Results: We found significant differences in let-7c concentrations between the two groups both in maternal plasma samples, and in fetal heart samples. The highest expression was observed in the cases of trisomy 21 with accompanying CHD. According to our studies elevated let-7c expression is associated with CHD and is a potential biomarker of the disease.

Keywords: congenital heart disease, biomarker, miRNA

P6

THE NUMBER OF INVASIVE DIAGNOSTIC PROCEDURES FOR DETECTION OF DOWN SYNDROME AND OTHER CHROMOSOMAL ABNORMALITIES DUE TO IMPLEMENTATION OF CF-DNA TESTING IN SLOVENIA DECLINES

Uršula Reš Muravec[1], Darija Strah[2], Petra Ovniček[3]

[1] Zdravstveni center Dravlje, Ljubljana, Slovenija
[2] Diagnostični center Strah, Domžale, Slovenija
[3] Univerza v Mariboru, MF, Maribor, Slovenija

Objectives: Currently amniocentesis represents a gold standard for prenatal diagnosis of chromosomal abnormalities. The method is invasive and leads to a miscarriage in less than 1% of the cases. On the contrary, cf-DNA prenatal testing (NIPT) analyses free fetal DNA from maternal blood and represent a highly accurate screening method for most common trisomies.

Methods: In this study we present the results of cf-DNA testing from private outpatient clinics in Slovenia over the last 3 years. In our study, 464 pregnant women from 10th to 33th week of pregnancy were included.

Results: Of total 464 pregnant women on which cf-DNA testing was performed, 325 (51.3 %) were included as advanced maternal age, 112 (17.7 %) were at high risk for T21, T18 or T13, based on prior screening test results. 14 cases out of total 17 high risk NIPT results (including 10 cases of T21, 2 cases of XXY, one case of T13 and one case of Turner syndrome) were confirmed by fetal karyotyping. 2 cases (T18 and Turner syndrome) turned out to be false positive, 1 pregnancy ended with spontaneous abortion with no karyotyping performed in spite of high risk result for T13. There were no false negative cases reported. In general, NIPT testing had 99.6 % sensitivity and 100 % specificity. Regarding only T21, specificity and sensitivity turned out to be 100 %. Repeat blood sampling was required in 2.44 %.

Conclusions: Our results confirmed that NIPT represents safe and highly accurate approach for advanced screening of most common aneuploidies. NIPT in routine clinical practice would significantly decrease the number of unnecessary invasive procedures and thus also the number of fetal loss, caused by invasive diagnostics.

Keywords: NIPT, aneuploidies, amniocentesis, pregnancy

P7

OPTIMISED DIGESTION PROTOCOL FOR RASSF1A DETECTION AS FETAL MARKER IN CFDNA

Ana B. Rodríguez-Martínez, Esther Sarasola Díez, Estíbaliz Achalandabaso, María J. García-Barcina

Basurto Universitary Hospital-Osakidetza, Bilbao, Spain

Circulating free fetal DNA (cffDNA) in maternal plasma is a significant tool which most relevant applications are involved in the management of pregnancies, non-invasive prenatal testing and early identification of pregnancy-associated disorders. It comprises, on average, 10-20% of total cell free DNA in maternal plasma which varies between individuals and generally increases with gestational age. Currently, differentiating fetal from maternal DNA relies on Y chromosome sequences (SRY, DYS14) but also on differentially methylated regions. RASSF1A gene is, at the moment, one of the most reliable fetal markers due to its differential methylation status from maternal DNA. For fetal RASSF1A sequence detection and in order to eliminate maternal RASSF1A sequences, digestion with methylation sensitive restriction enzymes needs to be performed. The EU/ANGELAB ("A New GEnetic LABoratory for non-invasive prenatal diagnosis") project is focused on the development of miniaturized microfluidic devices based on lab-on-a-chip technology for the detection of fetal disorders by the analysis of maternal circulating free DNA extracted from plasma. On this basis, we focused on the optimisation of the digestion protocol for fetal RASSF1A detection in cfDNA samples from plasma of pregnant woman in order to adapt it to a lab-on-a chip format. For the optimization of the digestion protocol, several factors needed to be considered, such as the extraction yield, the extraction method and the starting plasma volume. We optimised a 3.5 hour one-step incubation digestion protocol using a combination of different enzymes which allows the digestion of up to 95.6 RASSF1A gene copies extracted from 800uL of plasma and 130,4 gene copies extracted from 3mL plasma. In summary, this work provides a rather short digestion protocol suitable for cfDNA samples which is able to digest up to 130,4 RASSF1A gene copies. It has already been transferred to a lab-on a chip format whose verification results will be shown elsewhere.

Keywords: fetal marker, cfDNA, MSRE, RASSF1A, digestion

P8

GOOD PERFORMANCE OF FREEZE DRIED REAGENTS FOR THE DETECTION OF CIRCULATING FREE FETAL DNA IN LAB-ON-A-CHIP DEVICES

Ana B. Rodríguez-Martínez, Esther Sarasola Díez, Estíbaliz Achalandabaso, María J. García-Barcina

Basurto Universitary Hospital-Osakidetza, Bilbao, Spain

Circulating free fetal DNA (cffDNA) in maternal plasma is an important tool which most relevant applications are involved in the management of pregnancies, in non-invasive prenatal testing and in the early identification of pregnancy-associated disorders. The determination of fetal rhesus status and the detection of fetal RASSF1A sequences are two more specific applications of cffDNA analysis. The EU/ANGELAB, ("A New GEnetic LABoratory for non-invasive prenatal diagnosis") project is focused on the development of miniaturized microfluidic devices based on lab-on-a-chip technology, for the detection of fetal disorders analysing the maternal circulating free DNA extracted from plasma. On this basis and in order to be transferred to a lab-on-a-chip format, enzyme digestion and qPCR reactions need to be adapted to low reaction volumes as well as the use of freeze dried reagents. In this context, we optimised recipes for enzyme digestion and triplex qPCR reactions for freeze drying. Freeze dried cakes for digestion were synthesised and further resuspended in a final volume of 25uL of cfDNA. Freeze dried cakes for triplex qPCR were also synthesised and further resuspended in a final volume of 10uL of cfDNA. Results showed good performance with both enzyme and qPCR freeze dried cakes allowing, on one hand, the digestion of maternal RASSF1A sequences and, on the other, the detection of fetal rhesus genotypes in Rh negative maternal cfDNA. In summary, this work confirms that protocols in tube can generally be transferred and adapted to lab-on-a-chip formats and that the performance of reactions with freeze dried reagents does not constitute a handicap for their application to miniaturized microfluidic devices.

Keywords: cfDNA, freeze dry, qPCR, digestion, lab-on-a-chip

P9

PLACENTAL INSUFFICIENCY RELATED COMPLICATIONS ASSOCIATED CIRCULATING FREE FETAL DNA LEVELS CHANGES IN PREGNANCY

Svecova I[1], Mendelova A[2], Janusicova V[2], Dokus K[1], Biskupska Bodova K[1], Lasabova Z[2], Danko J[1]

[1]Department of Obstetrics and Gynecology, Kollarova 2, Jessenius Faculty of Medicine in Martin, Comenius University, Martin, Slovakia
[2]Department of Molecular Biology, Malá Hora 10701/4A, Martin, Jessenius Faculty of Medicine in Martin, Comenius University, Martin, Slovakia

Introduction and background: Placental insufficiency related complications (PIRC – preeclampsia and intrauterine growth restriction) are one of the leading causes of the maternal and perinatal morbidity and mortality. The cffDNA originates from apoptotically destroyed trophoblastic cells, cffDNA detection opened new possibilities for non invasive prenatal testing. The aim of this study was to evaluate levels of cffDNA in the first trimester of pregnancy before onset of clinical symptoms and compare them with physiological pregnancies with matched gestational week.

Patients and methods: Pathological PIRC male bearing pregnancies from the 8th to 12th g.w. were selected from the prospective cross-sectional case control longitudinal study cohort (270 patients). PIRC cohort was compared with appropriate gestational week control group physiological pregnancy samples. The samples (venous blood, EDTA tubes) were processed in maximally 6 hours and stored at - 80 ° C. Fetal DNA was isolated according to SAFE protocol using QIAamp DSP VirusKit (Qiagen, Hilden, Germany). SRY sequence was used to detect cffDNA; beta–globine *HBB* sequence was used as the housekeeping gene. Real time PCR analysis was performed on AB 7500 Fast Real-Time PCR System using Taq Man Gene expression MasterMix (AppliedBiosystems). Mann-Whitney U test and the MedCalc statistical software were used for data analyses.

Results: CffDNA plasmatic level medians were significantly increased in first trimester PIRC group (median 64.5845 genome equivalents/ml; interquartile range 47.5268 to 111.8144 genome equivalents/ml; p = 0.0096) in comparison to control group.

Conclusion: Using the method of absolute quantification a significant increase in cffDNA plasma levels both in HDP (p = 0.0209) and PIRC (p = 0.0096) group was observed in first trimester of pregnancy before the onset of clinical symptoms.

Acknowledgement: This work was supported by project "Centre of Excellence for Perinatology Research (CEPV II)", ITMS code: 26220120036 co-financed by EU sources.

Keywords: free fetal DNA, PIRC, placental insufficiency, first trimester

P10

HONESTLY ABOUT PRENATAL SCREENING TESTS IN HUNGARY

Karina Kató[1], Bálint Tobiás[1], Andrea Kövesdi[1], János Pál Kósa[1,2]

[1]PentaCore Laboratory, Budapest, Hungary
[2]Semmelweis University, Budapest, Hungary

Several new methods of prenatal screening and diagnostic tests are available, however, their effectivity is varying on a wide spectrum. SNP sequencing-based fetal cell-free DNA analysis represents the newest generation of NIPTs showing extremely high sensitivity and specificity, however also having limits.

Prenatal care usually begins with ultrasonography during the first trimester. It is often supplemented with different maternal blood tests. The trend is that gynecologists prefer the combined test, despite the fact that it has 90-93% sensitivity with 5% rate of false positive value. The timing of the sample taking can highly influence the test's sensitivity. As a next step of fetal chromosomal screening, the integrated test is often chosen during the second trimester. A sensitivity for T21 lies between 85-95% with a 3-5% FPV. NIPTs are regularly used as a second or third line screening, however their sensitivity and specificity exceeds 99%. The newest technology uses SNP-based fetal cell-free DNA analysis. According to international studies the combined positive predictive value of T21 and T18 of this test is 96,8% in contrast with the MPS method's 43,8%. Technology provides extremely high sensitivity (>99%) and specificity (>99.9%). The most common limit of NIPTs is the price, as these are not financed by the state.

Prenatal care requires personalized multidisciplinary group work. Ultrasonography is a crucial pillar of the nursing, but it is needed to be supplemented with further tests. Regarding the specificity and sensitivity of NIPTs, fetal cell-free DNA analysis seems to be the best choice, however it still has limits. Consensus is urgently needed in order to provide the most accurate health care for pregnant women. Nonetheless, it is needed to be considered that these are screening tests with extremely rare false negative and positive rates and therefore, the role of diagnostic tests should not be underestimated.

Keywords: NIPT, SNP-based sequencing

P11

RAD51 DNA REPAIR GENE POLYMORPHISMS AND GASTRIC CANCER OF PATIENTS IN TURKEY

Ilhami Gok[1] and Süleyman Cetinkunar[2]*

[1*]Department of Laboratory medicine, Faculty of Medicine, Ahkmet Yasawi University Turkestan, Kazakhstan
[2] Departments of Surgery, Adana Numune Training and Research Hospital, Adana, Turkey
Address for Correspondence:
Department of Laboratory medicine, Faculty of Medicine, Ahkmet Yasawi University Turkestan, Kazakhstan,
Phone: + 77027590161,e-mail:dnzgoki@gmail.com

Several studies have reported that the genes involved in DNA repair and in the maintenance of genome integrity play a crucial role in protecting against mutations that lead to gastric cancer. Epidemiologic evidence has shown that the inheritance of genetic variants at one or more loci results in a reduced DNA repair capacity and in an increased risk all of cancers. Polymorphisms have been identified in several DNA repair genes, *RAD51*, but the influence of specific genetic variants on repair phenotype and cancer risk has not yet been clarified. This was a case-control study design with two case groups: 62 cases with gastric cancer and control group included 80 cases with no gastric cancer. Bloods samples were taken from these patients and health controls and DNA is isolated. Interest regions in genome are amplified using Polymerase Chain Reaction method. PCR products were digested with MvaI (Micrococcus variants) restriction enzyme. We observed G135C polymorphisms of RAD51 genes by 52 % percent as shown in 22 out of 62 cancer patients. Only 10 out of 80 control group showed this polymorphism 10%. Statistically significant differences were found between the case groups and the control group for this of the polymorphisms Sample sizes of cases with gastric cancer, whether familial or sporadic, were insufficient to show any small true differences between the groups, but we have to consider that currently there is no clear consensus with respect to the association of these polymorphisms with gastric cancer risk. The greater the risk associated with cancer, the smaller the sample size required to demonstrate this association, and the data of different studies are usually, therefore, more concordant.

Keywords: Gastric cancer, RAD51 Gene, Genetic Polymorphisms, RFLP-PCR and Turkey

P12

RAD51 GENE POLYMORPHISMS AND BREAST CANCER OF PATIENTS IN EAST NORTHERN OF TURKEY

Ilhami Gok[1] and Süleyman Cetinkunar[2]*

[1*]Department of Laboratory medicine, Faculty of Medicine, Ahkmet Yasawi University Turkestan, Kazakhstan
[2] Departments of Surgery, Adana Numune Training and Research Hospital, Adana, Turkey
Address for Correspondence:
Department of Laboratory medicine, Faculty of Medicine, Ahkmet Yasawi University Turkestan, Kazakhstan,
Phone: + 77027590161,e-mail:dnzgoki@gmail.com

The gene RAD51 encodes proteins that are important for the repair of double-strand DNA breaks by recombination. Therefore, genetic variability in these genes may contribute to the occurrence and progression of breast carcinoma. We investigated the association of polymorphisms in the DNA repair genes RAD51-135G/C with the breast cancer risk. Genotypes were determined by PCR-RFLP assays in 55 women patients with breast cancers and 80 age-matched healthy controls. Bloods samples were taken from these patients and health controls and DNA is isolated. Interest regions in genome are amplyified using Polymerase Chain Reaction method. After amplification, we used a restriction enzyme (RAD51; Mval) and digested the PCR product. Then, this DNA fragments were passed through gel electrophoresis. By examining these images, we identified changes in the nucleotides in these specific regions. To clarify fragments polymorphisms, the PCR products were sequenced with an Applied Biosystems Automated Sequencer. We observed the 12 of the 55 patients (22%) carried the RAD51135G/C polymorphism of this gene. The same polymorphism was observed in 6 of the 80 controls (0.07%; $p < 0.05$). The obtained results indicate that the polymorphism of *RAD51* genes may be associated with the incidence of breast cancer in the Turkish population. We hypothesized that common polymorphisms in DNA repair and cell cycle regulator genes modify DNA repair and proliferation capacity, which contribute to breast cancer susceptibility. Further studies, including those on a larger group of patients, are required to further clarify this point.

Keywords: Breast cancer, RAD51 genes, Polymorphism, Turkey

P13

BIOMARKER POTENTIAL OF RASSF1A GENE PROMOTER CPG METHYLATION IN PERIPHERAL BLOOD OF TESTICULAR CANCER PATIENTS

Aleksandar Vojta[1], Dora Markulin[1], Ivana Samaržija[1], Marija Gamulin[2], Aleksandra Fučić[3], Irena Jukić[4], Čedomir Maglov[4], Vlatka Zoldoš[1]

[1]University of Zagreb, Faculty of Science, Department of Biology, Division of Molecular Biology, Horvatovac 102a, HR 10000 Zagreb, Croatia
[2]University Hospital Centre Zagreb, Department of Oncology, Kišpatićeva 12, HR 10000 Zagreb, Croatia
[3]Institute for Medical Research and Occupational Health, Ksaverska cesta 2, HR 10001 Zagreb, Croatia
[4]Croatian Institute of Transfusion Medicine, Petrova 3, HR 10000 Zagreb, Croatia

The RASSF1A gene is a putative tumor suppressor frequently downregulated by promoter methylation in different types of cancer. Its promoter methylation has also been studied in the context of testicular cancer, but the results were not consistent due to the small number of cases investigated. Therefore, we first conducted a meta-analysis to confirm the association between RASSF1A promoter methylation and testicular cancer. We analyzed 7 eligible studies describing in total 339 testicular cancer cases and 72 controls. The calculated odds ratio (OR) identified RASSF1A promoter methylation as a risk factor for testicular cancer. To investigate whether peripheral blood can provide diagnostic or prognostic markers for testicular cancer, we analyzed changes in DNA methylation in promoter regions the RASSF1A gene in 32 testicular cancer patients before and after cisplatin/bleomycin/etoposide (BEP) chemotherapy and in the same number of matched healthy controls. We found statistically significant differences at five CpG sites in the RASSF1A gene promoter. Methylation level at these sites was higher in patients and decreased after chemotherapy returning to levels similar to those of controls. Another meta-analysis of expression data corroborated our findings regarding gene promoter region methylation. This identifies methylation levels in the RASSF1A gene as a potential biomarker from blood for early diagnosis of testicular cancer. Further study on a larger group of testicular cancer patients will clarify reliability and predictive value of the method.

Keywords: testicular cancer, RASSF1A, methylation, biomarker

Author index

illumina®

The reassurance of knowing—

simply, safely, sooner.

**Noninvasive Prenatal Testing (NIPT)—
reliable answers about fetal chromosomal status**

- Detects the most common fetal aneuploidies as early as 10 weeks gestation
- More accurate than traditional screening—even in a general obstetrical population[1]
- A single blood draw reduces the need for and risks of more invasive procedures

Visit www.illumina.com/NIPT

illumina®
AUTHORIZED DISTRIBUTOR

KEMOMED
BRINGING SOLUTIONS

1. Bianchi DW, Parker RL, Wentworth J, et al. DNA sequencing versus standard prenatal aneuploidy screening. *N Engl J Med*. 2014;370(9):799–808.

A reliable non-invasive test designed to exclude the risk of Down's syndrome and other foetal trisomy types

TRISOMY*test*

TEST TODAY FOR A PEACEFUL PREGNANCY

BREAK THE CODE TO YOUR BABY'S HEALTH BEFORE IT'S BORN

TRISOMY*test*

EXCLUDES	the presence of trisomy 21, 18 and 13
DETECTS	potential false positive results of biochemical prenatal screening
MINIMISES	the need for invasive amniotic fluid sampling, or amniocentesis
DETERMINES	the sex of your unborn child, if you wish to know
RELIABLY	thanks to high sensitivity
SAFELY	no sampling risks
PAINLESSLY	using only maternal blood
TIMELY	from the 11th week of pregnancy

www.trisomytest.com

harmony®
PRENATAL TEST

Cell-free DNA testing / NIPT services
and system offerings from Roche

Roche

prenatal

Clarigo™

Enabling NIPT for all

Non-Invasive Prenatal Test

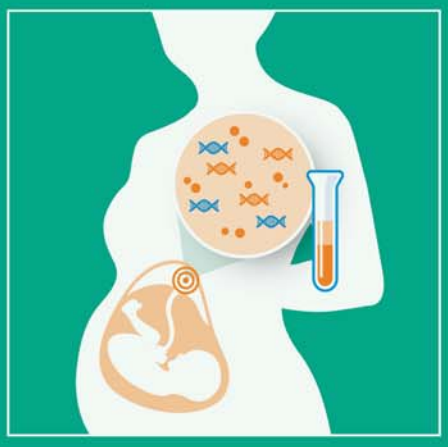
CE

Clarigo™ is an advanced and affordable CE-marked non-invasive prenatal test (NIPT). The test enables a rapid analysis of fetal cell-free DNA in the venous blood of the mother for the most common fetal trisomies (21, 18 and 13) early in pregnancy.

Clarigo has a superior accuracy and significantly lower false positive rate compared to the conventional prenatal screening methods, empowering improved confidence in the outcome for all risk groups and as a result a decrease in unnecessary invasive procedures and associated stress for the pregnant couple. It allows healthcare providers to offer a safe, fast and reliable screening to all pregnant women.

Thanks to implementing Multiplicom's proven MASTR technology in the Clarigo test, a highly reliable and safe screening test for Down syndrome and other common aneuploidies is available to enable NIPT for all pregnant women.

Clarigo screens for trisomies 21, 18 and 13 using a single maternal blood draw from as early as 8 weeks of gestation.



Multiplicom Enabling personalized medicine

For more information: **www.clarigo.com**