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33 Vazha Pshavela ave. 0177 Tbilisi Georgia
Tel.: +995 32 254 24 87
email: hgh.conference@gmail.com
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Oral Presentations

Plenary Lectures (PL 01-15)

PL01 Epigenetics and pain: the present and prospect views of the problems

E. Abzianidze[1,2]*; E. Kvaratskhelia*[1]*, M. Zarandia*[1]*, K.Dzagoevi*[1]*, N. Kraveishvili*[1]*, N. Tsiklauri, G. Gurtskaia*[2]*, M. Tsagareli*[1,2]

[1]*Tbilisi State Medical University, Dept. of Molecular and Medical Genetics, Tbilisi, Georgia,*

[2]*Ivane Beritashvili Centre of Experimental Biomedicine, Dept of Pain and Analgesia, Tbilisi, Georgia.*

Introduction: The persistent nature of pain suggests that epigenetic modification, mainly DNA methylationand histone modification may be a critical factor driving chronic pain. It has been established that the rostral ventromedial medulla (RVM) is one of the important parts of antinociceptive system of CNS. The main goal of the present study was to investigate epigenetic mechanisms of **RVM** and study of epigenetic agents as potentially therapeutic drugs for management of chronic inflammatory pain.

Methods: Adult rats receiving intraplantar formalin were tested for antinociception followinginjection of NSAIDs or/and5-Azacitidine (5-AzaC), or/and HDAC inhibitor (SAHA) in thermal and mechanical tests. In nuclear extracts of RVM neurons the levels of DNMT1, DNMT3a/b, HDAC were measured using ELISA based assay kits (Abcam) and levels of 5mC were measured using Methylated DNA Quantification Kit (Abcam).

Results: Received data demonstrated increased levels of DNMT3a/b andHDACin RVM neurons in formalin induced pain compared with controls. In addition, dose-dependent reduction ofnociceptive mechanical and thermal responses was shown in formalin test by treatment with either NSAIDs or pretreatment with 5-AzaC and SAHA independently.Delay establishment of hyperalgesia and significantly reduction of its intensitywerealso demonstrated in correlation with HDAC amountusing NSAIDs in combination with SAHA.Nociceptiveresponses werereduced as well as both DNMT3b and global DNA methylation levels were decreased using NSAIDs in combination with 5-AzaC in formalin tests.

Conclusions: Our results suggest that DNA methylation and histone acetylation altered in RVM in chronic pain states and manipulation of these processes by **certain substances** influence chronic pain-related behaviors. In summary, treatment strategies that target the epigenetic modifications such as DNA methylation and histone modification may potentially be used as a therapeutic strategy to treat certain inflammatory pain conditions.

PL02 Ethical challenges of reporting following next generation sequencing technologies

Pascal Borry[1]

[1] *Centre for Biomedical Ethics and Law, Department of Public Health and Primary Care, KU Leuven, Leuven, Belgium.*

Whole genome/exome sequencing is revolutionizing clinical practice. By identifying the genetic cause of disease in previously undiagnosable patients, these next generation sequencing technologies have the potential to influence treatment and provide valuable information for genetic counseling purposes. However, these technologies can also identify additional genomic information that is unrelated to the patient's current illness, such as such as variants in genes where the function is unknown or mutations causing phenotypes extraneous to the clinical question. Despite considerable debate, the question of

whether, and to what extent, unsolicited findings (also known as incidental findings) should be returned to patients following next-generation sequencing (NGS) remains unanswered. This is likely partially exacerbated by confusion in the terminology used to describe both disease-causing variants unrelated to the original rationale for testing identified inadvertently (unsolicited findings; UF) and those that are actively searched for (secondary findings; SF). In light of these complexities, we will discuss guidelines and recommendations; the practices of laboratories; informed consent procedures and points to consider in dealing with return of results.

PL03 Enhancing reproductive healthcare through carrier screening for recessive disorders: lessons for a successful implementation in the Georgian context

Chokoshvili D.
Centre for Biomedical Ethics and Law, Department of Public Health and Primary Care, KU Leuven, Leuven, Belgium.

Carrier screening is a powerful tool for identifying healthy couples, prior or during pregnancy, who may be at risk of having children with recessive genetic disorders, such as cystic fibrosis or spinal muscular atrophy. Carrier screening can be performed for a single disorder, or a large number of disorders simultaneously. Due to the substantial progress in molecular diagnostics over the last decade, combined with the diminishing costs of DNA sequencing, nowadays carrier screening is often performed for more than 100 disorders, also known as expanded carrier screening (ECS). Since children with recessive disorders are typically born to parents with no personal and family histories suggestive of these disorders, ECS has been widely recommended for all couples considering having children. However, recent experience with various ECS initiatives and commercial offers shows that the implementation of ECS in the context of reproductive healthcare is a challenging task. This presentation will focus on the main issues associated with organizing population-wide ECS offers and provide recommendations for its successful implementation in the Georgian context

PL04 Limits to the applications of genomics to human health

Angus Clarke
Cardiff University, Cardiff, Wales, UK

There are major potential benefits from the application of genomics to medicine andhealthcare. However, the very power of the technology makes it important to consider thelimits and constraints to these applications.
There are limits to what is possible and limits to what applications of genomics can beresourced. In a social model of health care, costly developments should not be introducedwithout good evidence of utility. While genetic and genomic approaches to diagnosis can bejustified and also a role in therapeutic guidance, especially for cancers. The use of genomicsin population screening and risk assessment, however, is not supported; excessive claims forgenomic technologies made by commercial providers should be resisted. The model ofgenome-based health care, in which newborns have their genomes sequenced and this thenremains available as a lifetime resource, is seriously flawed. In the area of rational, genebasedtherapies, some slow progress has been made but the field remains largelyexperimental.
The interpretation of genome sequence data requires a careful assessment of the patient'sphenotype as well as the critical interpretation of the sequence data. The simple reading fromgenotype to phenotype is not feasible.
Another constraint on the application of genomics to human health is the ethical dimension.
There are barriers to patients providing valid consent for genetic investigations, including theirlimited understanding of the potential results and their excessive expectations of thetechnology. These difficulties are more problematic because thereis no stable, professionalconsensusin in several areas, including how to manage (i) variants of uncertain significance,(ii) incidental findings, (iii) alterations in

the interpretation of results over time, (iv) thecompeting rights of family members, and (v) the appropriate way in which to challengepatients' thoughts as they make decisions.Another difficulty with the application of genomics to health is that it naturally emphasises thedifferences between people and distracts us from the collective interests we have in common.Some aspects of health, especially those relevant to disease prevention, are best tackledcollectively. The individualisation of genetic risks distracts us from how genomics impacts ondifferent social groups. Without a commitment to ensure that **all** benefit from genomics,especially those whose health is impacted most by their social status or by their geneticconstitution, genomics is likely to increase inequity in health care, following the 'inverse carelaw' of Tudor Hart. Justice and solidarity must be our guides if genomics is not to damage thehealth and welfare of many of our fellow citizens.

PL05 Application of Artificial intelligence in evaluation of rare genetic disorders

Nicole Fleischer
FDNA Inc., Boston, MA, USA

The role of objective facial analysis using automated facial recognition technology in making diagnoses following whole exome analysis (WES), has been shown in recent publications (Gripp et al. 2016; Mensah et al., 2107). Clinical phenotyping complementing next generation sequencing is reaching the level of next generation phenotyping (Hennekam, Biesecker, 2012) with the technology powering a free online tool called Face2Gene (FDNA, Boston, USA) . This technology is a form of artificial intelligence combining facial recognition algorithms with clinical feature annotation and anthropometric measurements, enabling detection of syndrome features from 2D facial photographs (Basel-Vanagaite et al.,2016; Valentine et al.,2017). Because facial dysmorphism is influenced by the ethnic background of the patient and of the evaluator, technology can be trained to learn to recognize the specific phenotype in different ethnicities. (Lumaka et al.,2016). The results of several studies conducted on syndromes as diverse as Angelman Syndrome, Cornelia de Langue and Fetal Alcohol Syndrome show that the technology's detection rate is comparable with dysmorphology experts, further suggesting that a clinical application utilizing such technology may be a useful tool for healthcare professionals in clinical settings as well in gene variants prioritization.

PL06 Cellular Senescence, Senolytics, and Age-Related Diseases

James L. Kirkland *and Tamara Tchkonia*
Robert and Arlene Kogod Center on Aging, Mayo Clinic, Rochester MN, USA

Interventions targeting fundamental mechanisms of aging hold promise for enhancing healthspan by delaying, preventing, or alleviating age-related diseases and conditions as a group, instead of one-at-a-time, the "geroscience hypothesis." Among these aging mechanisms is cellular senescence. Senescent cells, which are resistant to apoptosis, can secrete a range of pro-inflammatory cytokines and chemokines, proteases, factors that cause stem cell dysfunction, and other factors, the senescence-associated secretory phenotype (SASP). We developed senolytic agents – drugs that selectively eliminate senescent cells by inhibiting the pro-survival Senescent Cell Anti-apoptotic Pathways (SCAPs) that protect these cells from apoptosis caused from their own SASP. In animal experiments, intermittent senolyticdrug administration reduced frailty in progeroid mice, enhanced cardiac function in old mice, reduced age- and high fat diet-related vascular dysfunction, alleviated pulmonary fibrosis, restored hepatic function and reduced liver fat and fibrosis in diet-induced liver steatosis, restored bone mass and strength by reducing resorption without impeding bone formation in age-induced osteoporosis, and alleviated dysfunction caused by radiation. Thus, senolytic drugs are a new intervention that may delay, prevent, or treat multiple age- and chronic disease-related disorders. Early, proof-of-concept Phase 2 human clinical trials will be a critical step in translating these agents and others emerging from cell culture and animal preclinical studies into clinical practice.

PL07 Novel cell and gene therapy approaches for treatment of neurological diseases

Merab Kokaia
Lund University, Epilepsy Center, Lund, Sweden

Cell and gene therapy approaches are currently developing into alternative treatment strategiesfor various neurological disorders. In cease of cell therapy, not only the cells are transplanted toreplace lost neurons, but also can be genetically manipulated to encode and produce certainpeptides and proteins that can be then released into the brain after transplantation and therebyexert their designated therapeutic effect. An example of such approach is encapsulated cellbiodelivery (ECB) of glial cell-line derived neurotrophic factor (GDNF) in various models ofepilepsy, with a positive outcome in terms of significantly decreasing the frequency ofspontaneous recurrent seizures (SRS) in animal models of temporal lobe epilepsy.
On the other hand, direct genetic modification of neuronal cells in the target brain area based onviral vector delivery of therapeutic genes represents another, yet very powerful tool to alterneuronal excitability. We have used, in a chronic model of temporal lobe epilepsy, neuropeptide Y (NPY) and its receptor Y2 combinatorial gene therapy approach, which was effective indecreasing the frequency and duration of SRS. The NPY approach was validated in humanepileptic brain slices obtained from therapeutic respective surgery of the drug-resistant patients,whereby application of this neuropeptide significantly decreased epileptiform activity induced bychemical means. Taken together, these novel cell and gene therapy strategies are feasible approaches for developing alternative treatments for epilepsy, particularly drug-resistant group of patients that are not responding to currently available treatments. These patients constitute 30% of the epilepsy cases, which affects 1 % of the total population.

PL08 Genetic reprogramming of human somatic cells for cell therapy of neurodegenerative diseases

Zaal Kokaia
Lund University, Stem Cell Center, Lund, Sweden

Cells from different sources have been tested for their ability to reconstruct the forebrain and improve function after transplantation in animals subjected to stroke. We have recently shown improved functional recovery after transplantation of human reprogrammed induced pluripotent stem cells (iPSC)-derived cortical neuronal precursors in a rat model of cortical stroke. Grafted cells give rise to mature neurons that re-build the damaged tissue, receive functional afferent synaptic connectionsfrom host neurons, responded to sensory stimulationand send fibers to several brain structures.
Recent papers demonstrated rapid and efficient conversion of human somatic cell to mature neurons by overexpressing transcription factor combinations. We have attempted to generateprojection cortical neurons by direct reprograming of somatic cells. We demonstrated that a combination of three transcription factors convert human fibroblasts to functional excitatory cortical neurons. Single-cell analysisrevealed a complex gene expression profile, a subpopulation of neurons displaying a molecular signature similar to human fetal primary cortical neurons.
Our findings indicate that functional excitatory cortical neurons, generated by reprogramming of human somatic cells is feasible and could be further developed for cell therapy strategies.

PL09 Genetic Factors of Recurrent Pregnancy Loss (RPL)

J.Kristesashvili, *M. Rukhadze, N.Sigua*

I.Javakhishvili Tbilisi State University, Center for Reproductive Medicine "Universe", Association "Perinatology", Centre of Prenatal Diagnosis

Background: Balanced structural chromosomal anomalies revealed in 2-5% of couples with RPL. Unbalanced form of such anomalies, transmitted to embryos, can cause pregnancy loss.
Objective: Detection of frequency and types of chromosomal anomalies in couples with RPL.
Methods: 112 couples (aged 20-44yy.) with > 2 miscarriages (I trimester) were investigated in 2011-15. Besides of collecting comprehensive family and personal anamnesis, anatomic, hormonal, immunological, thrombophilic and genetic causes of RPL have been studied. Detection of karyotype was performed in peripheral blood lymphocyte culture (G-banding).
Results: Pregnancy and delivery with abnormal fetus, as well as karyotype of previous abortuses, were not detected in any cases. Chromosomal anomalies in one parent were revealed in 9 cases (8%). Balanced reciprocal translocation was detected in 4 men and 2 women, Robertsonian translocation – in 1 man. 2 from 5 men with translocations were subfertile. Total frequency of balanced translocations was 7 (6,25%). One woman had pericentric inversion of chromosome 9 and one woman – mosaic karyotype 46,XX/47,XXX. Mean number of previous miscarriages in common group of RPL was 3.15 and in the couples with chromosomal anomalies – 2.9.
Conclusion : In the couples with RPL and no history of delivery with abnormal fetus, when chromosomal status of previous miscarriages is unknown, significant frequency of balanced structural chromosomal anomalies indicates on reasonability of karyotyping of such couples, especially when male partner is subfertile. The reproductive risks (including miscarriage) are influenced by the size and the genetic content of the rearranged chromosomal segments and the sex of carriers.

PL10 Genome wide SNP-based array analysis in genome diagnostics: currently the best way to detect rare and recurrent chromosomal imbalances

Nicole de Leeuw
Department of Human Genetics; Radboud University Medical Center; Nijmegen Netherlands

Genome wide high resolution SNP-based array analysis has been used in our laboratory for the detection of copy number variations (CNVs) as a first tier diagnostic test since 2009 for patients with intellectual disability (ID) and/or congenital anomalies. Array is also performed prenatally in case of structural ultrasound anomalies or intra uterine foetal death and a normal QF-PCR test result as well as in patients with leukaemia. So far, more than 22,500 samples have been tested by SNP array in our diagnostic laboratory, including nearly 6,000 parental samples.
This diagnostic approach allowed us to reliably identify known and new, recurrent microdeletions and –duplications as well as rare, unique genomic imbalances with great accuracy. Moreover, the routine analysis of SNP genotypes revealed one or more significant stretches of homozygosity in 4 to 6 % of patients. Follow-up testing by either gene mutation analysis or patient-parent trio information analysis subsequently led to the respective identification of pathogenic mutations in recessive disease genes or uniparentaldisomies (UPD), thereby increasing the diagnostic yield with at least 1%. Using the SNP genotype information also improved the detection of mosaic copy number changes and enabled us to detect clinically relevant, mosaic, copy neutral changes of homozygosity.
Genome-wide high resolution SNP-based array analysis is a suitable and particularly effective technique in genome diagnostics to reliably detect various causes of rare and recurrent disorders including CNVs, UPDs and mosaic imbalances as well as pathogenic mutations in recessive disease genes. By using the right follow-up test procedures after initial SNP array analysis, a higher diagnostic yield and more knowledge of the mechanism underlying the genetic disorder are achieved, thereby enabling more adequate genetic counselling.

PL11 DNA damage as a driver of aging

Laura J. Niedernhofer
The Scripps Research Institute, Florida

Aging is believed to result from stochastic damage over time. However, it is not clear which type of cellular damage is the cause of aging, and which is the consequence. We are focused on discovering the role of spontaneous, endogenous DNA damage in the aging process. ERCC1-XPF is a DNA repair endonuclease required for the repair of multiple types of DNA damage that occurs in the nuclear genome. Mutations inh*XPF* that affect expression of ERCC1-XPF cause a progeroid syndrome or disease of accelerated aging. We recapitulated that disease in the mouse.
Ercc1$^{-/\Delta}$ mice express 5% of the normal level of ERCC1-XPF. The mice have a lifespan of 6-7 months and as adult animals spontaneously develop numerous age-related diseases including cardiovascular disease, cerebral atrophy with cognitive decline, loss of vision and hearing, peripheral neuropathy, pulmonary and hepatic fibrosis, chronic kidney disease and osteoporosis. *Ercc1*$^{-/\Delta}$ mice accumulate endogenous oxidative DNA damage more rapidly than wild-type (WT) mice. Importantly, the same DNA lesions accumulate in tissues of WT mice as they age. Tissue-specific deletion of *Ercc1* in mice yielded age-related diseases. Deletion in neurons of the forebrain caused cognitive decline. Deletion in renal podocytes caused chronic kidney disease. Deletion in myocytes caused congestive heart failure and deletion in β-cells caused Type II diabetes.Collectively, these data argue strongly that endogenous DNA damage plays a causal role in aging.

PL12 Stem Cells as Therapeutics for Extending Healthspan

Paul D. Robbins
Department of Molecular Medicine and Center on Aging The Scripps Research Institute, 130 Scripps Way, Jupiter, Florida USA

A universal characteristic of aging is loss of tissue regenerative potential, which leads to an impaired ability to respond to stress and, as a consequence, a dramatic increase in the risk of morbidity and mortality. This and the exponentially increased incidence of numerous degenerative diseases in the elderly has led to the hypothesis that aging is caused, in part, by the loss of functional stem cells necessary for maintaining tissue homeostasis. However, what drives age-associated stem cell loss and dysfunction, and the effects on aging have not been clearly defined. It also remains unclear if the defects in stem cell function arise due to defects in the stem cells (cell autonomous) or in the stem cell niche (non-autonomous). Thus we have using both naturally aged mice and mouse models of accelerated systemic and tissue specific aging to examine pathways that drive stem cell dysfunction with age as well as the ability of transplantation of functional stem cells to extend healthspan.

PL13 Role of MEFV gene mutations in development of autoinflammatory disorder

T.Sarkisian, H.Hayrapetyan, A.Yeghiazaryan, ***N.Kostandyan***
Center of Medical Genetics and Primary Health Care; Department of Medical Genetics, Yerevan State Medical University

Familial Mediterranean Fever (FMF) is a prototype of a group of inherited autoinflammatory disorders with diverse clinical manifestation. FMF is ethnically restricted disorder and caused by mutations in the MEFV gene. First information about recurrent inflammatory syndrome in Armenians was reported in ancient manuscripts in XII Century.
Carrier rate for MEFV mutations in Armenian population is about 1:3. Our data on more than 34000 individuals revealed that clinical significance of MEFV mutations is more than 98%. Molecular study revealed strong genotype-phenotype correlations for patients homozygous for M694V are at risk of developing early-onset and severe phenotype with complications. Carriers of M694V suffer from the

mild form of FMF. In some cases "mild" MEFV mutations, such as P369S, A744S and E148Q, are responsible for the determination of severity of inflammatory attacks in FMF, along with environmental or possible other genetic factors associated with inflammatory attacks.
The age of onset of FMF varies with about 60% and 90% of patients experiencing their 1st attack before the age of 10 and 20 year old, respectively with more severe disease phenotype in comparison with rare cohort of patients with 1st attack occurring at the age of more than 40.
Early diagnosis helps for immediate initiation of colchicine therapy of FMF patients and could prevent renal complications, reduce frequency of FMF attacks and facilitate patient management. No MEFV mutations were detected in 1.9% of patients with clinical features of FMF. Molecular study in these cases may be helpful to reveal other autoinflammatory syndromes.

PL14 THE PAIN MEMORY ENGRAM: AN EPIGENETIC SIGNATURE OF CHRONIC PAIN IN THE RODENT BRAIN

Tajerian M.
Stanford University

Background: Peripheral nerve injury can be accompanied by long-term pain-related manifestations, such as affective and cognitive disturbances, suggesting the involvement of supraspinal mechanisms. One particular region of interest is the prefrontal cortex (PFC), an area implicated in depression, anxiety and cognitive impairment, all of which are frequently associated with chronic pain. Clinically, pathological pain-related changes in the PFC in individuals with chronic low back pain can be reversed following effective pain management. However, the mechanisms behind pain-induced brain plasticity remain poorly understood.
Epigenetics is a term used to describe modifications to genomic DNA that alter gene expression. DNA methylation is an epigenetic mechanism that is involved in gene regulation mainly by silencing promoter activity. We propose that long-term alterations in DNA methylation could provide a molecular substrate for chronic pain-related changes in the CNS, forming a ''memory trace'' for pain in the brain.
Materials and methods: Spared nerve injury or sham surgery was performed in male mice and rats. Following the confirmation of mechanical hypersensitivity, brains and sera were collected and DNA and RNA were extracted. Global DNA methylation was measured by the luminometric methylation assay. Genome-wide promoter DNA methylation was analyzed by MeDIP. Promoter methylation of individual genes was assessed by sodium bisulfite sequencing and functionally validated using an *in vitro* promoter assay. Finally, mRNA levels of the target genes were measured by RT-PCR.
Results: Six to nine months following peripheral nerve injury, abnormal sensory thresholds and increased anxiety were accompanied by significant genomic DNA hypomethylation and transcriptional reprogramming. This was linked to the hypomethylation of individual genes, including (synaptotagmin 2) *syt2*, a known regulator of synaptic function. Furthermore, transcription of syt2 was regulated by differential methylation of its promoter in vitro and syt2 mRNA was upregulated in the PFC of injured animals. Finally, T-cell methylation landscape was shown to be similar to that of the PFC, thus suggesting the possible use of DNA methylation markers in T cells as noninvasive biomarkers of chronic pain susceptibility.
Conclusions: We show that peripheral injury produces long-term changes both in the PFC and T-cell methylomes,thereby potentially mediating the chronification of pain as well as the pain-related alterations in brain structure and cortical function.

Parallel Sessions (PS 01-15)

PS 01 Mutation spectrum of *PAH* gene in Georgian population affected by PKU

Agladze D.[2], *Gundorova P.*[1], *Margvelashvili L.*[3], *Kldiashvili E.*[4], *Polyakov A.*[1], *Kvlividze O.*[5]
[1] *Federal State Budgetary Institution «Research Centre for Medical Genetics», Moscow, Russia;* [2] *Research Institute of Clinical Medicine, Tbilisi, Georgia;* [3] *Children's New Clinic, Tbilisi, Georgia;* [4] *New Vision University, Tbilisi, Georgia;* [5] *Georgian Foundation for Genetic and Rare Diseases, Tbilisi, Georgia*

Introduction: Phenylketonuria is the most frequent inborn error of metabolism. PKU has 1:10 000 average incidence in Georgian population. PKU is a monogenic disease transmitted in an autosomal recessive pattern. Mutations found in the phenylalanine hydroxylase gene are the main determinants of PKU phenotype. Georgia screens patients for PKU since 2003 and there are 124 patients treated in the PKU national program.
Materials and methods: DNA samples of all 125 PKU patients from Georgia were analyzed for the presence of 25 common *PAH* gene mutations (S16* (c.47_48delCT), L48S, IVS2+5G>A, IVS2+5G>C, R111*, IVS4+5G>T, EX5del4154ins268, R158Q, D222* (c.664_665delGA), R243Q, R243*, R252W, R261Q, R261*, E280K, P281L A300S, I306V, S349P, IVS10-11G>A, E390G, A403V, R408W, Y414C, IVS12+1G>A) using allele-specific MLPA method.
Results: PAH gene mutations were detected on 85.1% of chromosomes. Severe mutations were detected on 68,9% of chromosomes, mild mutations – on 12,9%. Two severe *PAH* mutations were identified in 55.6% of examinees. In 21,8% of patients at least 1 mild mutation was identified.
Conclusions: Due to the *PAH* gene mutation spectrum, we can conclude, that Georgians are genetically far from nations living in contiguous territories. This feature is characteristic of most of the ethnic groups living in the Caucasus. DNA-diagnostics allow us to predict the treatment effect in PKU patients. Due to the high summary allele frequency of "severe" mutations among Georgians, more than the half of patients will not respond to the BH4 therapy. Nevertheless, there are about 22% of patients who may respond the therapy. The percentage is similar to the Eastern Europe. Such studies can help doctors start patient loading tests with sapropterin relying on the results of genotyping.

PS02 Contribution of NGS based applications for Hepatitis C elimination program in Georgia

G. Chanturia, *A. Kotorashvili, N. Kotaria, R. Sukhiashvili, P. Imnadze*
National Center for Disease Control and Public Health of Georgia, Lugar Center for Public Health Research

Approximately 71 million persons are living with hepatitis C virus worldwide. This number is expected to rise as the main route of transmission is through injection drug use. Georgia has a high burden of HCV infection with an estimated 5.4% of the adult population (150,000 people). The Government of Georgia, with strong support from US CDC and other international partners, declared intention to eliminate hepatitis C in Georgia. The national Hepatitis C elimination program became operational in April 2015.
To prevent new infections and reduce viral hepatitis-related morbidity and mortality efficient molecular surveillance for viral hepatitis is required. Global Hepatitis Outbreak and Surveillance Technology (GHOST), recently was developed by Division of Viral Hepatitis at Center for Disease Control and Prevention (Atlanta, Georgia). Establishing regional, independent and sustainable HCV GHOST-based

molecular surveillance centers are important to improve GHOST functionalities. One of the regional centers is set up at the National Center for Disease Control and Public Health (NCDC) / Lugar Center (Tbilisi, Georgia). The GHOST analysis pipeline is designed for sequencing of HVR1 (Hyper Variable Region 1) and NS5B (Non Structural region 5B) regions of Hepatitis C virus using Illumina Next Generation Sequencing system which is the main platform at NCDC/Lugar Center. NCDC/Lugar Center will serve as a regional lab for the CDC's working group focused on hepatitis C elimination program in the country and will identify, link and analyze hepatitis C genotypes and recombinant forms circulating in Georgia.

PS03 Prenatal screening and diagnostics of congenital anomalies in Georgia

Mariam Chipashvili, *Zaza Sinauridze, Ekaterine Bagrationi, Tamar Machitadze, Maka Jorbenadze*
"Perinatology", Tbilisi State University

Aim of the study: Prenatal screening and diagnostics are very important in antenatal care. Estimation of correlations between screen positive test results and diagnostic consequences is very important for elaboration of algorithms of antenatal care. The aim of the study was assessment of correlations between screening (age, biochemical, ultrasound) methods and prenatal cytogenetic test results.
Methods: We have analyzed the results of the prenatal screening and diagnostic results 2007-2017. In total 34233 biochemical tests, 9064 genetic consultancies, 8524 expert ultrasound examination and 1498 amniocentesis procedure were performed. Correlations between maternal age, biochemical test results and fetus ultrasound assessment and the consequences of amniotic fluid cytogenetic examinations was assessed.
Results: Cytogenetic abnormalities don't correlate with the typical biochemical changes. The very popular first trimester ultrasound marker - enlargement of nuchal translucency was noticed in 52.5% of fetuses with chromosome abnormalities. Second trimester ultrasound markers weren't identified in 35.9% of fetuses with chromosome aneuploidies. Also chromosome aneuploidies were less frequent among pregnant before 35 (37.84%), rather than over 35 years (62.16%).
Conclusion: Our study revealed, that typical changes in biochemical screening test or ultrasound markers aren't always presented. In the cases of suspicion on fetal aneuploidies it's better to perform prenatal cytogenetic diagnostics.

PS04 Influence of male reproductive ducts infections on oxidative stress and sperm DNA fragmentation

Didbaridze T*, Papava V, Shanidze T*
TSMU The First University Clinic, Georgian – Austrian Medical Centre

Sperm is particularly susceptible to reactive oxygen species (ROS) during critical phases of spermiogenesis. Male fertility depends on spermatogenesis process which produces the large numbers of cell by the testes known as spermatozoa. Mitochondria and sperm plasma membrane are two major sites of ROS generation in sperm cells. There is a long list of intrinsic and extrinsic factors which can induce oxidative stress to interact with lipids, proteins and DNA molecules. As a result, we have lipid peroxidation, DNA fragmentation, axonemal damage, denaturation of the enzymes, over generation of superoxide in the mitochondria, lower antioxidant activity and finally abnormal spermatogenesis. Oxidative stress is considered as one of the main causes of DNA damage in the germ cells. There is a long list of intrinsic and extrinsic factors which can induce oxidative stress, but the main generally

accepted etiologies are the followings: alcohol consumption, cigarette smoking**,**varicocele, obesity, diabetes, physical exercise, psychological factors and infections.
OBJECTIVE: We reviewed the influence of bacterial infection in the reproductive system of 12 males and determined the effects of oxidative stress on DNA fragmentation.
METHODS: All samples were collected according to the appropriate protocols of semen sampling. Bacteria in the semen were identified using API – system (BioMérieux). Susceptibility test was performed by Kirby-Bauer test (EUCAST 2017). To determine DNA fragmentation we applied in vitro diagnostic kit Halosperm (Halotech, Spain), as well as Oxisperm (Halotech, Spain) for measurement of oxidative stress.
RESULTS: Antimicrobial treatment was planned according to the local susceptibility test results and patients underwent three weeks course. After treatment, results of 9 patients (75%) to the reactive oxygen stress (ROS) were negative and DNA fragmentation dropped from 30% to 15%, which is normal reference range. In 3 patients (15%), oxidative stress was not observed but DNA fragmentation did not return from 50% to normal range (0-15%), but reduced to only 30% in total.
CONCLUSION: Activated leukocytes in response to different inducers like infection and inflammation can produce up to 100 fold higher levels of ROS compared with non-activated leukocytes. That is why bacteriological investigation of semen is very important to determine infection and select appropriate antibiotic according to the antibiogram. It shows significant decrease oxidative stress and DNA fragmentation before and after treatment.

PS05 Genome and Heath of Children living in Arsenic Polluted Area

A.Zedginidze,[1] ***M.Gagoshidze***[2] *N,Manjavidze*[2]
[1]*IvaneBeritashvili Center of Experimental Biomedicine, Tbilisi, Georgia.*
[2] *Tbilisi State Samedicino University.*

Environment impact on the human health was and will always be the major problem of earth science. Presently great attention is paid to studying of the impact of mutagen environment upon human health. Among chemical mutagens heavy metals are very important.In one of mountainous region of Georgia, namely Racha (Lukhuni Gorge) content of arsenic (As) in the environment considerably exceeds the admissible norms.As belongs to the group of high toxic and mutagenic substances. The mutagens first affect the cells, cause changes to genetic system, which can explain their effect on human healts. These disorders are expressed by changes on both molecular and cytogenetic level. Particularly sensitive to its effect are infants, children and adolescent, which is explained by the peculiarities of metabolism of the growing organism. The aim of our work was revealing the polluted by arsenic environment impact on genetic apparatus of childrenand estimating of correlation with morbidity By the help of cytogenetic methods, was determined the cumulative effect of arsenic on the children organism. Cytogenetic research was performed through several methods: in peripheral blood culture were studied the chromosomal aberrations and the level of micronuclei.The level of micronuclei was also studied in exfoliativebuccal cells. Chromosomal aberration(acentric fragments, chromosomal exchanges and singular dicentric chromosomes) and increased level of micronuclei in lymphocytes and buccal cells were stated and indicated on mutagen impact. By our own data the background level of MN in Georgia composed 0.014± 0.008 in lymphocytes and 0.0016±0.0012 in buccal cells (interval of statistical confidence was 95%) Index of MN in lymphocytes was higher then in exfoliative cells. The same correlation in investigated group was revealed. 143 children from Lukhuni Gorge, (I group) and 754 children living in a distance (>50 km) from the territory of arsenic mining, where arsenic pollution is significantly less (II group) have been carried out. At the arsenic polluted territory in school age

children health aberration is very high. Acute respiratory and other infections morbidity frequency (P>0,05) in I group was higher then in group II. Acute respiratory infection relative and atributiverisk was high. (p<0.05) Also the high risk of morbidity developmenti is found in other acute infections, atopic dermatits, allergic rhinitis, conjunctivitis, obstructive laryngitis and other deseases.. Chromosomal aberration and increased level of micronuclei in lymphocytes and buccal cells indicated on mutagen impact. Structural chromosomal aberrations (acentric fragments, chromosomal exchanges and singular dicentric chromosomes) only in children living in Lukhuni Gorge were revealed. Correlation between cytogenetic disorders and high morbidity of acute respiratory infections was detected (R = 0,789; p < 0,000001).Chromosomal aberration and increased micronuclei levels in lymphocytes and buccal cells were regarded as proof of mutagenic influence in studied region . To prove that mutagenic influence was caused by arsenic the level of it was estimated in blood .urine and hair. In children from Lukhumi level of arsenic was increased.Our researches give us an opportunity to identify the risk of morbidity in contaminated areas to conduct cytogenetic monitoring using a non-invasive, easily accessible method of micronuclei in buccal exfoliative cells

PS06 Regulation of Androgen Receptor Signaling by SIRTUIN2 Deacetylase in Prostate Cancer

*OzkanOzden and **Ilhami Gok***
Department of Bioengineering, Faculty of Engineering and Architecture, Kafkas University, Kars, Turkey

Aim: In this study, whether the androgen receptor whose activity is closely associated with prostate cancer is post-translationally regulated by a NAD^+ dependent and aging associated protein, SIRTUIN2.
Material and method: Immunoprecipitation and western blotting techniques were conducted to examine the association of the androgen receptor and SIRTUIN2 in cultured 293T and LNCaP human prostate cancer cell line. In addition, we performed *in vitro*deacetylation assays using purified SIRTUIN2 enzyme and androgen receptor.
Results: SIRTUIN2 gene removed mouse prostate had hyper-acetylated the androgen receptors. *In vitro* and *in vivo* protein-protein interaction assays revealed that SIRTUIN2 physically interacted with the androgen receptor in prostate cancer cell line, LNCaP. Finally, SIRTUIN2 deacetylated the androgen receptor *in vitro* conditions.
Conclusion: SIRTUIN2 interacted with the androgen receptor and deacetylated it. Identifying partners of the androgen receptor and molecular mechanisms of its regulation are curial for understanding the pathogenesis of prostate cancer. Using small molecules to activate SIRTUIN2 might be an important clinical approach to prevent, treat or delay the prostate cancer progression.

PS07 The Association between Genetic Markers and Arterial Thrombosis

***Sopio Garakanidze*[1], *Elisio Costa*[2], *Nona Kakauridze*[3], *Rusudan Khukhunaishvili*[1], *Marina Koridze*[1]**
[1]Department of Biology, Faculty of Natural Sciences & Health Care, Batumi ShotaRustaveli State University, Batumi, Georgia.[2] Faculty of Pharmacy, University of Porto, Porto, Portugal.
[3]Department of Internal Medicine, Faculty of Medicine, Tbilisi State Medical University, Tbilisi, Georgia.

Introduction and objective: Thrombophilia is considered as a condition predisposing to the development of thrombosis [1]. By the European cardiovascular disease statistics, cardiovascular diseases (CVD) remain the leading cause of mortality and a major cause of morbidity in Europe [2].

This is a very important problem in Georgia, due to the high death-rate associated with CVD (583.2 per 100 000 habitants) [3]. Our research aim was to study the association between genetic markers (Leiden V factorG1691A(FVL), Prothrombin (PT) G20210A and MTHFR gene mutations) and arterial thrombosis in Adjarian (Georgia) arterial thrombosis patients and control groups.
Materials and methods: This study involved 214 individuals, 101 arterial thrombosis patients (71.3% are males; 66.3 +/- 12.1 years old) and 113 healthy controls. The blood samples were collected at the Heart Disease Department and Medical Ward of Batumi Referral Hospital, Government of Autonomous Republic of Adjara, Georgia. The genetic research of samples was produced at the faculty of pharmacy, University of Porto.The genomic DNA was extracted from a dry blood spot on whatman filter paper according to the instructions (KAPA Biosystems, Wilmington, MA, USA). The polymerase chain reaction (PCR) was used to detect the genes as described previously [4] The amplification products were analyzed by electrophoresis in 2% agarose gel with ethidium bromide. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS, version 21.0) for Windows (SPSS Inc., Armonk, NY, USA).
Results: The results of the research carried out into the three genes in patients and control groups were the following: T/T genotype frequency was 8,9% in patients and 3,5 % in the control group (MTHFR). G/A genotype frequency was 4 % in patients and 1,8 % in controls (FVL). G/A genotype frequency was 3% in patients and and 4,4 % in controls (PT G20210A).However, A/A -homozygous forms were not detected in the research on FVL G1691A and PT G20210A genes in the patients and controls. **Conclusion:**According to the research results,FVL could be particularly associated with arterial thrombosis in the myocardial infarction patients. Our research has not found association between PT G20210A and arterial thrombosis. But MTHFR gene mutation might increase the risk of arterial thrombosis because the high allele and genotype frequencies were detected in Georgian arterial thrombosis patients.

PS08 Epigenetic Markers as Diagnostic and Prognostic Tools in Breast Cancer

K. Kankava, *T. Kvaratskhelia, M. Zarandia, G. Burkadze, E. Kvaratskhelia, E. Abzianidze*
Department of Molecular and Medical Genetics, Tbilisi State Medical University

Epigenetic changes are well known to be involved in breast cancer. Some of them are proposed as possible markers for tumor detection. Epigenetic change affects mainly gene promoters and repetitive sequences in genome - Long Interspersed Nuclear Element-1 (LINE-1) and Alu. Level of methyltransferases - the enzymes that are responsible for DNA methylation - is known to be representative of their activity and therefore global DNA methylation level. The aim of our study was to identify these epigenetic parameters in patients with benign and malignant breast tumors, also their correlation with morphologic and phenotypic characteristics of breast cancer.
Patients with biopsy-proved ductal invasive carcinoma of breast and various benign breast lesions were chosen for the study. Blood samples were collected preoperatively. Tumor and surrounding breast tissue sections left after histopathology investigation were used for methylation study. Methyltransferase activity, also LINE-1 methylation level was quantified using ELISA-based assay. For determination of Alu methylation status COBRA PCR technique was used.
In most tumors unmethylated LINE-1 predominated. LINE-1 methylation level was lower in normal breast tissue and lowest in blood samples. LINE-1 methylation in blood was not significantly different in patients with benign and malignant tumors. Lymphovascular invasion was the only aggressiveness-determining factor that was found to be at least weakly correlated with LINE-1 hypomethylation. The levels of different methyltransferases correlated with Estrogen receptor expression, tumor size and grade. We can conclude, that hypomethylation is a significant marker of tumor tissue, but no one of the investigated blood parameters can reliably be used alone for tumor identification or determination of prognosis.

PS09 Inherited Thrombophilia and Pregnancy Complications

K. Kartvelishvili*[1,2]*, *N. Pirtskhelani*[1,2]*, N. Kochiashvili*[2]*, L. Makhaldiani*[3]
[1]*Tbilisi State Medical University, Tbilisi, Georgia;*[2]*LevanSamkharauli National Forensics Bureau, Tbilisi, Georgia;* [3]*K. Eristavi National Center of Experimental and Clinical Surgery, Tbilisi, Georgia*

The hemostatic system plays a critical role in both the establishment and maintenance of pregnancy, and the dynamic balance between coagulation and fibrinolysis maintains a normal placental circulation. Hereditary thrombophilias are a group of genetic disorders of blood coagulation resulting in a hypercoagulable state, which in turn can result in abnormal placentation. Early in pregnancy this may manifest as spontaneous loss. In later pregnancy, thrombophilias have been associated with complications such as preeclampsia, intrauterine growth restriction, placental abruption and stillbirth.
Aim: The aim of this study was to determine to what extent inherited thrombophilia, V Leiden, Prothrombin G20210A and MTHFR C677T gene mutations, is associated with pregnancy complications:Miscarriage,Stillbirth,Preterm preeclampsia, intrauterine growth restriction (IUGR) and placental abruption.
Materials and Methods: 545 georgian women with different pregnancy complications and 100 control – women with three or more uncomplicated pregnancies, were genotyped by PCR analyses.
Results:relationships between pregnancy complications and FVL (6.3% in patients and 0% in control; $\chi^2(1, N=645)=6.586$, p=.003) and MTHFR (7.7% in patients and 1% in control; $\chi^2(1, N=645)=6.108$, p=.005) mutations were significant. Relationship between Pregnancy complications and Prothrombin mutation (4.2% in patients and 1% in control; $\chi^2(1, N=645)=2.446$, p=.091) was weak. Relationship between placental abruption and FVL mutation (20.5%; $\chi^2(1, N=138)=16.507$, p=.000), stillbirth and FVL mutation (8.8%; $\chi^2(1, N=202)=10.315$, p=.001), miscarriages and MTHFR mutation (7.6%; $\chi^2(1, N=645)=2.446$, p=.091), placental abruption and MTHFR mutation (13.6%; $\chi^2(1, N=144)=10.550$, p=.003) were significant.
Conclusions: This is the first study in our population and shows that women with inherited thrombophilia are at increased risk of developing both early and late complications in pregnancy. Taking into consideration received results, also the effectiveness of timely started adequate treatment, it's reasonable to investigate thrombophilia gene mutations in all Georgian women with pregnancy complications.
The study has been funded by the grant DO/166/7-140/14 of SRNSF of Georgia.

PS10 Epigenetic Approach to Gastrointestinal Cancers: Diagnosis, Prognosis and Treatment

Elmas Kasap*[1]*, *Seda Örenay Boyacıoğlu*[2]*, Mehmet Korkmaz*[3]
[1]*Department of Gastroenterology, Faculty of Medicine, Celal Bayar University, Manisa, Turkey*
[2]*Department of Medical Genetics, Faculty of Medicine, Adnan Menderes University, Aydin, Turkey.*
[3]*Department of Medical Biology, Faculty of Medicine, Celal Bayar University, Manisa, Turkey.*

Gastrointestinal (GI) cancers include malignancies in the esophagus, stomach, liver and bile ducts, gallbladder, pancreas, the small intestine, colon and rectum. There is 4.1 million new cases and 3 million deaths, annually worldwide due to Gastrointestinal cancer.
Epigenetic changes are common in all types of cancers, especially in GI cancers. Epigenetics' is defined as heritable changes in gene expression that do not cause permanent alteration of the underlying DNA sequences, and include e.g. DNA methylation, histone modifications and non-coding RNAs.
Recent studies of epigenetic changes have greatly extended our under- standing of the pathogenesis and pathophysiology of GI cancers and have provided novel epigenetic biomarkers for the diagnosis of tumors. There is a need for a preventive strategy to stratify patients into appropriate surveillance

programs. After than we believe that epigenetic findings will be of great benefit to treatment and as well as to understand which panel of biomarkers can be used to better define patient's prognosis and the best choice of available treatments.

PS11 Genetic pattern of Cystic Fibrosis in Georgian Pediatric Population

Khurtsilava I.[1,2], *Parulava Ts.*[1], *Margvelashvili L.*[2,3], *Kvlividze O.*[3]
[1] *Cystic Fibrosis Center, Children's New Clinic;* [2] *Dept. of Genetics, Children's New Clinic;*
[3] *Georgian Foundation for Genetic and Rare Diseases; Tbilisi, Georgia*

Introduction and background: Cystic fibrosis (CF) is the most common life-threatening autosomal recessive disease, which occurs in 1:3000 between Caucasians and has increasing incidence in Asia. The median predicted survival is 37.3-41.4 years. Patients have abnormal transport of chloride and sodium across secretory epithelia, resulting in thickened viscous secretions in bronchi, biliary tract, pancreas, intestines and reproductive system. The usual presenting symptoms include persistent pulmonary infection, pancreatic insufficiency and elevated sweat chloride levels. CF is caused by mutation in a single gene on chromosome 7, hat encodes the CF transmembrane regulator protein. The most common mutation is F 508del, which is found in approximately 70% Caucasian patients. The diagnosis of CF requires clinical symptoms consistent with CF in at least one organ system and evidence of CFTR dysfunction (elevated sweat chloride, presence of two disease-causing mutations in CFTR). Epidemiology, clinical and genetic opportunities in Georgian population is unknown.
Aim and objectives: Retrospective analysis of patients under 18 year diagnosed and undergoing monitoring at CF center
Results: Total number of patients diagnosed during 2012-2017 years is 104. 2/3 of patients were diagnosed between age 1 month – 2 years. Lowest age at diagnosis was 2 weeks (newborns with intestinal obstruction)**,** medium age for patients underwent neonatal screening was 3 -4 weeks**,** for other patients – about 4,5 years. Sex distribution was same between male and female patients. Most patients were diagnosed after presenting with clinical symptoms: meconium ileus - 10%, respiratory infections – 26%, pancreatic insufficiency presented with chronic diarrhea, failure to thrive, malabsorbtion, electrolyte loss and anemia – 18%, mixed lung and pancreatic disease has 45%. Patients revealed due to neonatal screening is 16. The total number of patients who has undergone molecular diagnosis is 49. The most frequent mutation is 1667delTA
Conclusion: Study shows that in Georgian population low frequency of common mutations. Our ethnic group may be having genetic diversity and a wide range of unusual CF causing mutations.

PS12 Screening for DNA Mismatch Repair Genes Mutation in Turkish Patients With HNPCC\Lynch Syndrome by Next Generation Sequencing

Orenay-Boyacioglu Seda[1], *Caliskan Metin*[1], ***Korkmaz Mehmet***[2]**,** *Bozkurt Gokay*[1]
[1]*Adnan Menderes University, Faculty of Medicine, Department of Medical Genetics, Aydin, TURKEY;*[2] *Celal Bayar University, Faculty of Medicine, Department of Medical Biology, Manisa, TURKEY*

INTRODUCTION: Hereditary Non-Polyposis Colorectal Cancer (HNPCC) or Lynch syndrome (LS) is an autosomal dominant inheritance syndrome, with high penetrance that affects approximately 3% of the cases of colorectal cancer. Affected individualsgermline mutations in genes responsible for DNA Mismatch Repair (MMR): *MSH2, MLH1, MSH6, PSM1* and *PMS2*. This Retrospective study aimed to

investigate types and frequencies of mismatch repair (MMR) gene mutations in Turkish patients with HNPCC and to identify specific biomarkers for early diagnosis.
MATERIAL AND METHODS: Next-generation sequencing (NGS) was used to screen 5 genes involved in the DNA MMR pathway in constitutional DNA from 65 HNPCC and, plus 2 positive controls. Analysis of the crude data obtained by the NGS method was analyzed according to the reference genome in a web-based bioinformatics program (https://seq.genomize.com/).The ratio of the total number of readings obtained as a result of the analysis to the target regions was evaluated using the IGV (Integrative Genomics Viewer) program.All variants were confirmed by Sanger sequencing.
RESULTS: Ten variants were found in exons and flanking intron/exon regions for the 4 MMR (*MSH2, MLH1, MSH6, and PSM1)* genes. These variants were class 5 (pathogenic) or class 4 (likely pathogenic). Mutations in *MSH2, MLH1, MSH6, and PSM1* were contributed to13.8, 4.6, 3.07, and 1.5 % of HNPCC cases, respectively.
DISCUSSION: We suggest a possible role of *MLH1*, *MSH2*, *MSH6* and *PSM1* polymorphisms in the susceptibility to early onset of HNPCC. The present study adds new information about MMR gene mutation types in Turkish HNPCC patients.

PS13 Clinical Applications for Next Generation Sequencing

Adam Kotorashvili*[1]*, *N. Kotaria*[1]*, M. Murtskhvaladze*[1]
[1] *National Center for Disease Control and Public Health/Genome Center, Tbilisi, Georgia*

Overview: Genome Center (GC) at National Center for Disease Control and Public Health of Georgia became functional at the beginning of 2013. Whole Genome sequencing platform IlluminaMiseq located at GC makes Center unique not just for Georgia but for whole region including Azerbaijan, Armenia, eastern part of Turkey, Ukraine. GC is represented by the group of the scientists with high qualification. They have got trainings in leading universities of United States and European Union and are involved in many scientific project implementations using advanced techniques and technologies. Next Generation Sequencing (NGS) platform MiSeq(and all required equipment for library preparation and sequencing) was provided for National Center for Disease Control and Public Health by Department of Thread Reduction Agency (DTRA). On the IlluminaMiSeq platform we able to perform DNA and total RNA sequencing, metagenomics, gene expression profiling, methylation assay, micro RNA profiling and much more.Development of Next Generation Sequence based technologies enabled biomedical applied and basic science to reach new level in healthcare.
Methods: Illumina offers FDA (food and drug administration) approved platform MiSeqDx with wide range of clinical Applications. NGS can be applied in **Oncology**: Familial genetic testing, molecular Classifications of Diseases, Circulating Cell free tumor DNA and circulating tumore cells, treatment monitoring, biomarkers in clinical decision-making. In **Neurology and Psychiatry**: Epilepsy, Ataxia, Autism, movement disorders, neurodegenerative disorders with Dementia. In **Noninvasive Prenatal Tests**: NGS of fetal DNA, cell free fetal DNA, maternal – fetal DNA comparative analysis. In **Hematological Disorders**: acute myeloid Leukemia, CSF3R mutation in chronic Neutrophilic Leukemia, Non-Hodgkin Lymphomas, Thrombocytopenia Absent Radius Syndrome. In **Pharmacogenomics**: NGS in Transplantology, MultigenePharmacogenetics, And NGS in **Undiagnosed Diseases**: NGS testing for rare disease, pathogenetics.
Discussion: Utilization of genomic data in medicine has been recognized around the world. Georgia is particularly well placed to utilize genomic data. From a regional perspective, Georgia's strengths include a high standard of healthcare, uniform treatment practices, internationally recognized Genome Center with Next Generation Sequencing platform, and the willingness of the population to participate in scientific research.
Conclusion: Our Ultimate goals are: Widely use the genome data in Healthcare; provide opportunity for Individuals to use genomic data in their own lives; Make sure that Healthcare professionals have skills to use genomic data, closely integrate genomic research in healthcare, as a result Georgia will become attractive in the region for use of genomic data.

PS14 Enterococci Causing Vancomycin Resistant Infections

***Kurtulus M*[1]** *Kutluk I*[1] *Caglar K*[2]

[1] *Departmentof Pharmaceutical Microbiology, Faculty of Pharmacy, University of Gazi, Ankara, Turkey;* [2]*Departmentof Medical Microbiology, Faculty of Medicine, University of Gazi, Ankara, Turkey*

Introduction: Enterococci are members of the gastrointestinal systems of a wide variety of hosts—humans and other mammals, birds, reptiles and insects.Enterococci are an important cause of hospital-acquired infections. Some species of Enterococcus, mainly *Enterococcus faecalis*and *E. faecium*are leading causes of hospital-acquired infections.Rarespecieshas been isolated from humans, but clinical infections with these organisms are rare.

Materials and Methods: All enterococcal isolates from cultures of clinical samples were collected from January 2011 through December 2017. Isolates were assessed by disk diffusion and E test for antimicrobial susceptibility testing and by Vitek2 for species identification.DNA extraction was prepared with boiling method. Identification of vanA and vanBresistance genes in VRE was performed by PCR.

Results: Out of the 7551 Enterococci isolates,a total of 179 were identified as VRE between 2011-2017 in Gazi University Hospital. 95 of the VRE isolates had a *vanA*resistance gene and only one VRE isolate was found to have *vanB* resistance gene as detected by PCR testing.

van A genotype in the Enterococci species other than *E. faecalis*(5 isolates),*E. faecium*(51 isolates) and Enterococcus spp (33 isolates) was rare. Other *Enterococcus* species included *E. raffinosus*(one isolates), *E. hirae*(two isolates), and *E. avium*(three isolates).These species are infrequently isolated and/or difficult to be identified in clinical microbiology laboratories.

Conclusion: Clinical microbiology laboratories should be aware of the high probability that van A genes may be transferred from *Enterococcus faecium* or *Enterococcus faecalis* to other more rarely encountered Enterococcus species.

PS15 Epigenetics in Single Gene Disorders: Cystic Fibrosis

E. Kvaratskhelia*[1,2]*, *M. Gagua*[2]*, E. Maisuradze*[2]*, S. Surmava*[1]*, N. Kvaratskhelia*[1]*, E. Abzianidze*[1]

[1]*Tbilisi State Medical University, Department of Molecular and Medical Genetics;* [2]*V. Bakhutashvili Institute of Medical Biotechnology*

Introduction: Cystic Fibrosis is the most common lethal autosomal recessive disorder in the white population, caused by the CFTR gene mutation. The main clinical symptoms of CF are elevated sweat chloride concentrations, exocrine pancreatic insufficiency (in 85%–90% of patients), male infertility, and progressive obstructive lung disease. Lung disease is the major cause of morbidity and mortality in CF, arising from chronic bacterial infections with a persistent neutrophilic inflammatory response and leading to airway damage, bronchiectasis, emphysema, and finally to respiratory failure. Genotype-phenotype correlations in CF twins showed that environmental factors also contribute to pulmonary function variation in CF patients.

We hypothesize that the enhanced production of proinflammatory mediators observed in CF airways are in part controlled by an epigenetic regulatory program.

Material and Methods: In this study we profiled DNA methylation in healthy controls and homozygous and/or compaundheterozygous CF patients.

Results: $CD4^+$ T cells had a low DNA methylcytosine content in CF patients. In addition, both LINE-1 and Alu repetitive elements were hypomethylated. We observed that patients with CF showed a

significantly higher plasma level of IL-8 in relation to health individuals due to hypomethylation of IL-8 promoter.

Conclusions: Hypomethylation of mobile epements might be useful in developing a potential biomarker for the diagnosis and therapies for Cystic Fibrosis. Demethylation of inflammatory cytokine genes, such as interleukin IL-8, in $CD4^+$ T cells might participate in the progression of Cystic Fibrosis.

PS16 Host defense antimicrobial peptides (AMPs). AMP database – *DBAASP*

Pirtskhalava M*[1]*, *Vishnepolsky B*[1]*, Grigolava M*[1]*, Zaalishvili G*[2]*,Karapetian M*[2]

[1]*I.Beritashvili Center of Experimental Biomedicine;* [2]*Agricultural University of Georgia*

Human Genome is a repository of the genes which are responsible for the expression of the wide variety of the host defense peptides (AMP). AMPs are key components of the innate immune system shared by both invertebrates and vertebrates. Vertebrate AMPs can also have an impact on the adaptive immune system. So AMPs may show dual nature: rapid microbial killing and subsequent immune modulation.

About 100 different peptides protect humans from microbial infection. AMP have been identified in a variety of exposed tissues or surfaces such as skin, eyes, ears, mouth, airways, lung, intestines, the urinary tract, etc.Most of the AMPs are cationic, amphipatic and short with less than 50 amino acids.Modes of action of AMP relyon nonspecific peptide–membrane interactions. Consequently, a multistep mutations usually require to change microbial membrane composition and to evolve a resistance against AMP.

Although anti-infective resistance is one of the world's most pressing medical problem the antibiotic pipeline remains narrow. So novel approaches are urgently needed to combat the growing tide of antibiotic-resistant infections.A potential of AMPs as therapeutics, especially against organisms for which resistance to standard antibiotics is growing, allows to expect an increased interest in them.

Research interest and the promise of practical applications of AMPs resulted in the creation of several AMP web-databases.In the laboratory of Bioinformatics of theIvaneBeritashvili Center of Experimental Biomedicine manually curated database of AMPs (*DBAASP*) has been developed. *DBAASP*has presented as a cloud instance at http://dbaasp.org.

DBAASP hosts about 11 000 entries (peptides) and the rate of gaining of new entities equals about 100 peptides/month. It provides the information and analytical resources to the scientific community to perform structure/activity relationship studies and to establish the sequence-based antimicrobial potency predictive model. Establishment of the predictive model allows to develop tools which can help : a) to improve an annotation of known genes revealing their unknown antimicrobial features or to reveal new genes with antimicrobial potency b) to perform *de-novo* design of amino acid sequences being active against drug-resistant microbes.

de novo design of AMPmeans: 1. Prediction of the new amino acid sequences with antimicrobial potency; 2. Synthesis of the peptides using predicted sequences; 3.*In vitro* tests of the synthesized peptides on antimicrobial activities. Predictive model has been developed using data for*E.coli ATCC 25922*. Relying on this predictive model new amino acid sequences has been created that have to be active against *E.coli.,* Corresponding peptides has synthesized and tested by assessing the susceptibilities of *E.coliin vitro*. Results of *in vitro* tests show high efficiency of predictive model developed (accuracy> 0.95).Consequently DBAASP's prediction service is efficient to create a new anti-infective drugs. Predictive model can be used to annotate antimicrobial properties of genes.

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PS17 Inherited thrombophilia and Personalized Medicine

***N. Pirtskhelani*[1,2]**, *N. Kochiashvili*[2], *K. Kartvelishvili*[1,2], *L. Makhaldiani*[3], *N. Pargalava*[4]
[1]*Tbilisi State Medical University, Tbilisi, Georgia,*
[2]*LevanSamkharauli National Forensics Bureau, Tbilisi, Georgia,*
[3]*K. Eristavi National Center of Experimental and Clinical Surgery, Tbilisi, Georgia,*
[4]*Georgian Centre of Angiology and Vascular Surgery, Tbilisi, Georgia.*

Introduction: The increased interest in personalized medicine began with the sequencing the human genome in the early 2000s. Early analyses comparing genomes of different individuals confirmed the remarkable similarities of sequence, but soon gave way to expectations that the millions of nucleotide differences among different individuals would enable clinicians to not only recognize each individual's biologic uniqueness, but to translate this knowledge into more precise understanding of physiology, more refined diagnoses, better disease risk assessment, earlier detection and monitoring, and tailored treatments to the individual patient; i.e., personalized (or individualized or precision) medicine.

Aim: The aim of our study was to determine the impact of patient's genotype on the management and personalizing therapy of thromboembolism and pregnancy complications.

Materials and Methods: 1333 unrelated Georgians with thromboembolism and pregnancy complications were genotyped by PCR analyses for for detection of inherited thrombophilia (Factor V Leiden (FVL), Prothrombin (PTH G20210A) and Methylenetetrahydrofolatereductase (MTHFR C677T) gene mutations).

Resultsare presented in a table:

Table. Distribution of mutation in Patients

Genotype	Patients N=1333
FV Leiden Het	86 (6.5%)
FV Leiden Homo	6 (0.5%)
Prothrombin G20210A Het	58 (4.4%)
Prothrombin G20210AHomo	4 (0.3%)
MTHFR C677T Het	507(38.03%)
MTHFR C677T Homo	90 (6.8%)

Conclusion: Genetic and acquired factors play a role in the pathogenesis of VTE. The current management of VTE is primarily determined by the presence or absence of a significant provoking and modifiable factor. Currently, the data support 3 months of anticoagulation therapy for patients with provoked VTE. However, common practice may use up to 6-month (or longer) anticoagulation where the treating physician personalizes treatment based on clinical factors. The situation with unprovoked VTE is different, depends on kind of mutant genotype and typically involves long-term anticoagulation and in case of MTHFR gene mutation with hyperhomocysteinemia,is necessary combined folic acid and B-vitamin therapy, which substantially reduces homocysteine level. When there is a question regarding the feasibility of long-term anticoagulation, patient's genotype, characteristics (sex, age, and body mass index), nature of the initial VTE (distal deep vein thrombosis vs proximal deep vein thrombosis vs pulmonary embolus) and assays of global hemostasis are used for recurrence risk assessment and personalizing therapy.

PS18 The structure and risk of chronic morbidity in some villages of the Upper Imereti region of Georgia and their molecular and cytogenetic markers

T. Sanikidze*[1]**, *A. Zedginidze*[2], *E. Tikaradze*[4], *M. Buleishvili*[1, 3], ***R. Kverenchkhiladze[1], *G. Kvarackhelia*[5], *I.Chkhikvishvili*[1], *N. Gogia*[1], *G. Ormotsadze*[2]

[1]Tbilisi State Medical University,[2]Beritashvili Center of Experimental Biomedicine, Laboratory of Radiation Safety Problems,[3]N. Kipshidze Central University Clinic,[4]Sachkhere District Hospital,[5]Davit Aghmashenebeli University of Georgia,Georgia

The paper presents a preliminary analysis of the comprehensive study results of the structure and development risk of major and concomitant chronic diseases, as well as levels of redox balance and cytogenetic status in the population of the villages Khreiti, Perevisa and Rgani of Chiatura district of Georgia. The above-mentioned villages differ both in their remoteness from the sources of environmental pollution (manganese mining quarries), and scale of its extraction, which allows to rank they according to the degree of ecological tension (Khreiti - low, Perevisa- average, Rgani - high.
It was proposed, by analyzing of the possible association between the structure and risk of primary and concomitant chronic diseases and the mechanisms supporting the redox balance and the stability of the genetic apparatus in the body, to identify the role of the disruption of these mechanisms in the pathogenesis of chronic disease, potential predictors of the risk of the diseases and the dose markers of the adverse effects of environmental factors on the population.
Material and methods. The inhabitants of the Chiatura region, living in villages characterized by the different degrees of environmental ecological burden (Khreiti, Perevisa and Rgani) were examined (total 400 persons). Clinical, laboratory and instrumental studies were performed. Determination of blood redox balance of patients was carried out by modified DPPH (2.2-diphenyl-1-picrylhydrazyl) test. For investigate the genetic status the level of micronuclei in exfoliative cells of oral cavity was detected; light microscopy was used for the analysis.
Results and Discussion. It was revealed that the distribution of chances of chronic bronchitis and COPD development between the Khreiti/Perevisa, Perevisa/Rgani, and Khreiti/Rgani villages (1.9; 3.6; 7, respectively) and also their total value were statistically significant, which with a high degree of reliability indicates for the presence of a causal relationship between the level of morbidity in the surveyed populations and the degree of environmental stress in the places of their settlement. Analysis of prevalence of accompanying pathologies for chronic obstructive pulmonary disease COPD has shown a close correlation between the primary COPD and the accompanying hypertension. This fact supports the hypothesis on the role of COPD as a risk factor for circulatory diseases, but similar pattern does not appeared in primary hypertension cases - in Perevisa and Rgani if the prevalence is significantly higher as in Khreiti and between Rgani and Perevisa no difference was fixed. From the other hand, a hypothesis about the important role of systemic inflammatory process in pathogenesis of COPD, could be the link between this disease and different comorbidities. High level of inflammatory factpors (TNF-α, IL-6, CRP, fibrinogen), oxidative stress and genom's destabilization, the possible mechanism for which is p53 dysfunction, indicatesis on the integral role of the inflammation process.
Analysis of the study results of patients' blood redox balance revealed that the blood antioxidant activity in Perevisa statistically significantly exceeds that in Khreiti and Rgani, while the average level of the blood antioxidant potential of the residents Khreiti significantly exceeds its value in Rgani, which indicates depletion of the antioxidant protection system's resource. A similar pattern has been observed in level of the micronucle.
Conclusion. The obtained results with a high degree of reliability indicate the leading role of inflammatory processes, associated oxidative stress stress and level of micronuclei in the development of COPD and circulatory diseases. In this case, there is a competitive relationship between the probabilities of COPD and hypertension, with a clear dependence of the probabilities from the level of oxidative stress - with moderate stress (physiological and mildly stress conditions), the likelihood of development is almost the same, whereas under severe stress, the severity of COPD increases dramatically.

PS19 Syndromic autism

[1]Tkemaladze T., *[1]Ekaladze E., [1]Delibashvili D.,[1]Partskhaladze B., [1]Tabatadze N.*
[1]Tbilisi State Medical University, Department of Molecular and Medical Genetics

Autism and autism spectrum disorders (ASDs) are a collective of conditions that have in common impaired socialization and communication in association with often stereotypic behaviors. Recently there has been increase in the number of referrals to clinical geneticists for the evaluation of persons with ASD. The major role of geneticist in this process is to define the etiology when possible, in order to provide genetic councelling and to aid in case management. To date the etiology of autism is largely unknown. About 20% of ASD is thought to be of genetic in origin. Single most common inherited cause of ASD is Fragile X syndrome (FXS) that contributes to up to 6% of all ASDs. Rett syndrome deserved a special attention in girls with autism and developmental regression. Certain microdeletion/microduplication syndromes and single-gene diseases, including metabolic and mitochondrial conditions comprise additional 15% of all ASDs. Thus, it is important to recognize expanded phenotypes of well-described syndromic and metabolic conditions that overlap with ASD and plan appropriate diagnostic tests to search for underlying reasons of ASDs, among them testing for Fragile X syndrome, Rett syndrome, chromosomal microarray (CMA) and first and second-line metabolic tests. Autism itself is a clinical diagnosis, however having genetic diagnosis of underlying cause will aid clinicians in better management of the patients and will end diagnostic odyssey for the families. A clinical approach to assess and treat metabolic dysfunction in ASD will be further reviewed.

PS20 Microbiome, Parasites and Epigenetics

***Tskhomelidze D.**, Kokaia N., Chiladze N., Iashvili N.*
Tbilisi State Medical University, Department of Medical Biology and Parasitology
Georgia Research Institute of Medical Parasitology and Tropical Medicine

According to Rodney Dietert “the microbiome is a major player in establishing our developmental programming, in part though control of the gene switches”. Parasites are able to make changes inside microbiome and via this they may have some influence on human epigenome, using horizontal gene transfer (HGT) and molecular mimicry. It is known that the microbiome composition depends on the mode of delivery of newborn. Our aim was to find out which parasites affect more frequently the children born by cesarean section in Georgia. The provided investigations showed us that C-section born children were infected with L.donovani more frequently to compare with Giardia lamblia and Enterobiusvermicularis. 20 individuals have been diagnosed for L. donovani in this year at the Georgia research institute of medical parasitology and tropical medicine, the majority of Visceral leishamniasis (VL) cases were diagnosed in children under 8 years of age and all patients were residents of Georgia. Confirmation of VL diagnosis was based on microscopically observing parasites on a Giemsa-stained smears of bone marrow, obtained by iliac crest aspiration. Among 20 patients with VL 8 children were born by cesarean section. In addition to laboratory investigation, the special questionnaire was developed, which helped us to find out the new cases of VL in cesarean delivered children. Findings of prevalence about VL underline that cesarean section is associated with increased sensitivity for immune and metabolic disorders. We suppose it exists some risk factors for treatment and developing relapses of VL in cesarean-delivered children.

Poster Presentations
P 01-19

P01 The Relationship Between Paclitaxel Resistance and *PTEN* Status in Prostate Cancer

Boyacioglu Olcay[1],Orenay-Boyacioglu Seda[2], Korkmaz Mehmet[3]
[1]Adnan Menderes University, Faculty of Engineering, Department of Food Engineering, Aydin, TURKEY; [2]Adnan Menderes University, Faculty of Medicine, Department of Medical Genetics, Aydin, TURKEY; [3]Celal Bayar University, Faculty of Medicine, Department of Medical Biology, Manisa, TURKEY

Introduction: Prostate cancer (PCa) cells with *PTEN* gene mutation are susceptible to the treatment methods such as ionizing radiation, UV, mitomycinC, paclitaxel, and docetaxel. In this study, the effect of *PTEN* mutation on paclitaxel resistance (PtxR) in PCA cells was investigated by establishing PtxR in three PCA cell lines.
Materials And Method: PCa cell lines with different *PTEN* statuses, DU-145 (*PTEN*+/-), 22Rv1 (*PTEN*+/+), and PC3 (*PTEN*-/-), were used in the study. Cells were incubated with increasing doses of paclitaxel (0.1-400 nM) to gain resistance against paclitaxel. The correlation between the *PTEN* status and level of PtxR observed in cell lines was determined by applying 400 nM paclitaxel to all resistant and parental cell lines, and results were measured by Cell Titer-Glo Luminescent Assay. Test results were compared with analysis of variance (ANOVA). $P<0.05$ value was regarded as the level of significance.
Result: The paclitaxel-resistant PC3 cell line was found to be the least resistant cell line than other cell lines. There was no difference in the level of PtxR between 22Rv1 and DU-145 in spite of the *PTEN*heterozygosity of DU-145 ($P > 0.05$).
Conclusion: There are several studies in the literature that show the effect of *PTEN* status on cancer cell lines, but there is no study to question the effect of *PTEN* status on the development of PtxR. By indicating the importance of *PTEN* status, this study suggests that mutated *PTEN* status in PCa patients may be of potential benefit in treatment by hindering the development of PtxR

P02 Targeted Treatment For Cervical Cancer: A Src-tyrosin Kinase Inhibitor 'Bosutinib'

Esat Calik[1], Pinar Solmaz Hasdemir[1], Burcu Erbaykent[2],Tevfik Guvenal[1],Mehmet Korkmaz[3]
[1]Celal Bayar University Medical School, Department of Obstetrics and Gynecology, Manisa, Turkey
[2]Department of Molecular Biology and Genetic, Faculty of Science, Uludag University, Bursa, Turkey
[3]Celal Bayar University Medical School, Department of Medical Biology, Manisa, Turkey

INTRODUCTION: The aim of this investigate the anti-proliferative effect of a tyrosinekinase inhibitor 'bosutinib' in cervical cancer cell line.
MATERİALS AND METHODS: A human cervical cancer cell line named as CCL-62 HeLa contaminant was propagated in a humidified incubator at 37 °C and 5 % CO2. A src-kinase inhibitor 'bosutinib' (SKI-606) was administered in different concentration values to this cell line. 50 % lethal doses (IC50 values) for cytotoxic or anti-proliferative effects of bosutinib were determined by MTT assay. The amount of DNA damage occurred with bosutinib were determined by immunofluorescence through phosphorylation of H2AX (Ser139) andpATM(Ser1981) as well as the rate of apoptosiss was evaluated by imaging the annexin-5 andcaspase 3 activity. Furthermore, colorimetric caspase-3

analysis was used for activation of apoptosis via bosutinib in cervical cancer cell line. Lastly, the levels of γH2AX(Ser139), pATM(Ser1981), pp53(Ser15) were evaluated as DNA damage markers and cleaved PARP, caspase 3 and 9 were evaluated as apoptosis markers by western-blot. For γH2AX(Ser139) foci counts and pATM(Ser1981) measurements as well as for densitometric analysis Image J software was used.

RESULTS: We suggest that bosutinib leads to DNA damage by causing DNA double strand breaks and had anti-proliferative effect by activating caspase 3.

CONCLUSIONS: Bosutinib, a thyrosinekinase inhibitor, causes damage in DNA with antiproliferative effect and induces apopitosis in cervical cancer cell lines. Thus, it might be a new treatment option for cervical cancer. This finding needs to be confirmed with further animal and human studies.

P03 Phase 1 Study of Autologous Bone Marrow Stem Cell Transplantation in Patients with Spinal Cord Injury

Konstantine Chutkerashvili, Gocha Chutkerashvili, Irakli Chelishvili,
Tbilisi State Medical University, Innova Medical Center, Tbilisi, Georgia

Introduction. A total of 18 patients, with complete motor deficits and paraplegia caused by thoracic and lumbar spine trauma without muscle atrophy or psychiatric problems, were included into this study. All patients signed a written informed consent. The study protocol was confirmed according to ethical guidelines of the 1975 Declaration of Helsinki and was approved by InnovaMedicalCenter, Tbilisi, Georgia.

Materials and Methods. The bone marrow was aspirated from the anterior iliac crest under local anesthesia and the mononuclear fraction was isolated by density gradient method. At least 750 million mononuclear-enriched cells, suspended in 2 mL of saline, were infused intrathecally.

Results and Discussion. The study reports demonstrated improvement of motor and sensory functions of various degrees observed in 9 of the 18 (50%) cases after bone marrow stem cell transplantation. Measured by the American Spinal Injury Association (ASIA) scale, 7 (78%) out of the 9 patients observed an improvement by one grade, while two cases (22%) saw an improvement by two grades. However, there were no cases in which the condition was improved by three grades.

Conclusions. Analysis of subsequent treatment results indicated that the transplantation of mononuclear-enriched autologous BMSCs is a feasible and safe technique. However, successful application of the BMSCs in the clinical practice is associated with the necessity of executing more detailed examinations to evaluate the effect of BMSCs on the patients with spinal cord injury.

P04 Polymorphism of the Homologous Recombination Repair Pathway Genes *RAD51* and *XRCC2* in Colorectal Cancer in a Turkish Population

Ilhami Gok[1], OzkanOzden[1]
1Department of Bioengineering, Faculty of Engineering & Architecture, Kafkas University Kars, Turkey

Aim: RAD51 and XRCC2 genes are crucial parts of the homologous recombination DNA repair pathway and genetic variability in these genes has been associated with increased cancer risk. In the present study, the association between polymorphisms in these two DNA repair genes and the risk of colorectal cancer formation was evaluated.

Material and method: Polymorphisms selected in this study were *RAD51*-135G/C and *XRCC2*-A/G. Each polymorphism was genotyped using Polymerase Chain Reaction-restriction fragment length polymorphism (PCR-RFLP) in study cohort of 71 colorectal cancer patients and 86 age matched controls.
Results: Arg188His polymorphism of *XRCC2* genes was observed in 42.2% (30 of the 71 cancer patients) while this frequency was 24.2% (21 out of 86 controls). Secondly, while 21 of the 71 patients (29.5%) carried the RAD51135G/C, the same polymorphism was observed in 11 of the 86 controls (12.7 %; $p < 0.05$).
Conclusion: Our results suggest that Arg188His polymorphism of XRCC2, and 135G/C polymorphism of RAD51 may be associated with increased colorectal cancer risk in Turkey.

P05 Association between Polymorphisms of the Interleukin-6 and Interleukin-1Beta Patients and Obstructive Sleep Apnea Syndrome in a Turkish Population

Ilhami Gok[1], Ozkan Ozden[1], Dogan Ilhan[2]
[1]Department of Bioengineering, Faculty of Engineering and Architecture, Kafkas University, Kars, Turkey; [2]Departments of Molecular Biology and Genetics, Faculty of Science and Literature; Kafkas University, Kars, Turkey

Aim: To investigate the relationship of IL-1β and IL-6 cytokine gene polymorphisms with obstructive sleep apnea syndrome (OSAS) in 61 patients admitted to the neurology clinic in Kafkas University Hospital with insomnia problem who were diagnosed with OSAS in sleeping labs, and 80 healthy subjects not associated with the syndrome.
Methods: Blood samples were taken to isolate DNA from patients diagnosed with OSAS based on polysomnography results and healthy controls. DNA amplification of the genes was performed with PCR. Amplification products were cut with the restriction enzymes in order to determine IL-1 gene (TaqI) and IL-6 gene (Lwel) polymorphisms. The cut DNA fragments were carried out in agarose gel electrophoresis, and RFLP analysis was performed by utilizing the images with gel imaging system. PCR products were sequenced with an Applied Biosystems Automated Sequencer.
Results: Polymorphic changes were observed for IL-1β gene in 26 of 62 patients (41.9%), and 16 of the 80 (25.8%) in the control group. The incidence of polymorphic changes in IL-6 gene was in seen in seven (of the 62 patients) (11.3%), and in the 16 (20%) controls.
Conclusion: The findings on the genomic level in OSAS may provide an important contribution to diagnosis of obstructive sleep apnea syndrome in clinical practice, as well as it helps to obtain the results easily about environmental and genetic interaction of OSAS patients.
Keywords: OSAS, cytokine genes, RFLP, interleukin

P06 Human Infections with the novel Orthopoxvirus in Georgia – case study

Khmaladze E[1], P.Imnadze[1], Reynolds M[2]
[1]National Center for Disease Control and Public Health (NCDC), Tbilisi, Georgia
[2]Centers for Disease Control and Prevention (CDC), Atlanta, GA, USA

Orthopoxviruses (OPXV) are large DNA viruses within the family Poxviridae. OPXV pose a threat to public health based on their ability to cause zoonotic outbreaks; OPXV members of this genus that cause infection in humans are variola virus (agent of smallpox), vaccinia virus (smallpox vaccine), cowpox virus, and monkeypox virus.
In 2013, some cows in a herd of cattle in a rural part of the country of Georgia started developing lesions on their teats; simultaneously first time in Georgia, human infection with orthopoxviruses has been identified when two herders presented with severe febrile rash illnessand skin lesions at infectious diseases hospital.

Virus was initially found to have DNA-level signatures consistent with an OPXV using genus specific TaqMan based real-time PCR method; species level tests validated for identification of known zoonotic OPXV revealed negative. Amplification and sequencing of a DNA target generally conserved among OPXV suggested the virus was a new OPXV species. Subsequently, the virus was grown in cell culture and virus DNA was submitted for genome sequencing. The genomic data confirmed that the isolate represents a new, early divergent Eurasian OPXV species (Akhmeta virus) which is clearly distinct from all currently described OPXV. Human anthrax cases have also been identified in most of the regions and it is possible that the similarity of the disease presentation caused by this OPXV, could be confused with cutaneous anthrax. To investigate this possibility, limited set of archival DNA samples from suspected anthrax cases where anthrax had been ruled out were tested using real-time PCR. As a result of this retrospective study a second (DNA from 2010), similar to the Akhmeta virus was detected. The patient was presumably infected near Vani, more than 200km from the Akhmeta case.
In August 2016, swab sample was collected from a patient with severe skin lesions, DNA was extracted and real-time PCR assay for generic detection of OPXVwas applied; Sample tested positive on Orthopoxvirus infection; for species differentiation sanger sequencing using Panpox Low-GC PCR assay was performed; Raw sequence data was analyzed and de novo assembly was performed in Sequencher 5.0 software; phylogenetic trees were generated in MEGA 7.0. Sanger sequencing confirmed that infection was caused by cowpox virus. It has to be mentioned that until this case, no detection of human infection with CPXV has been reported in the country.
This study confirms that orthopoxviruses are spread across the country and cause human infection for at least several years. In addition to this, a rodent survey performed in 1986 (Tsanava et al 1986) resulted in the isolation of an OPXV (then identified as cowpox virus) from the southeastern border of Georgia, however here we demonstrate first confirmed OPXV cases in human in Georgia.

P07 ABO, Rh and MN Blood Group Systems in Gastric Cancer

Marina Koridze, Marina Nagervadze, NatoZosidze, Irina Nakashidze
Department of Biology, Natural Science and Health Care, Batumi ShotaRustaveli State University, Batumi, Georgia

Background: Gastric cancer is the second most common cause of death worldwide. The ABO system group antigens are known to stimulate the development of some cancers. However, the precise molecular mechanism underlying the relationship between blood groups and gastric cancer is unclear. **Material and Methods:** We studied the distribution frequencies of blood group systems (ABO, Rh-Hr, MN) antigens and phenotypes in control group and gastric cancer patient. In the control group, we examined 100 healthy donor patients and 39 patients in diseased. Internationally recognized immunoserology methods were used to reveal the erythrocyte group antigens. The clinical stages of the disease were estimated by exploring the cytological, morphological, ecosophical and computer methods. **Results:** Our present investigations have revealed high-frequency B(II) and AB(IV) phenotypic groups among gastric cancer compared to healthy control. From Rh-Hr system D, E, e and e antigens, the frequencies of D and E antigens were increased in cancer group. It's notably, that, the carriers of CCDee, ccDEE, ccDEe and ccDee phenotypes have the high risk for development of gastric cancer. From MN system antigen, the frequency of M antigen was high, but N antigen frequency was decreased in patients with gastric cancer. In our study, the frequency of MN phenotype was high among patients with gastric cancer compared to control group. **Conclusion:** In comparing the gastric cancer group with the healthy controls, it notably that the risk of gastric cancer in carriers of B(III)) and AB(IV) phenotypes, were significantly high. From Rh-Hr system antigens, carriers of D and E antigens have the tendency to develop gastric cancer.

P08 Alpha7 subunit of the nicotinergic acetylcholine receptor gene (CHRNA7) and perception of coherent motion in aging

Nato Kotaria[1], Karin Pilz[2], Adam Kotorashvili[1], Michael Herzog[3]& Marina Kunchulia[4]
[1]Genome Center, National Centre for Disease Control and Public Health, Tbilisi, Georgia
[2]School of Psychology, University of Aberdeen, Scotland, UK
[3]Laboratory of Psychophysics, Brain Mind Institute, ÉcolePolytechniqueFédérale de Lausanne (EPFL), Switzerland; [4]Institute of Cognitive Neurosciences, Free University of Tbilisi, Tbilisi, Georgia

Genetic variations of the alpha7 subunit of the nicotinergic acetylcholine receptor gene (CHRNA7) are linked to cognitive deficits in aging and schizophrenia. However, little is known about associations of the CHRNA7 gene with aged-related decline in visual perception. In the present study, we tested whether variations in the alpha7 subunit of the nicotinergic acetylcholine receptor gene (CHRNA7) interact with the perception of coherent motion in healthy aging.
We assessed motion coherence for 125 adults, including 63 younger adults ranging from 18 to 29 years (M=22.5, SD=2.99) and 62 older adults ranging from 60 to75 years (M=64.5, SD=3.56). Prior to the experiment, visual acuity for each participant was determined with the Freiburg visual acuity test (FrACT, Bach, 1996). A single nucleotide polymorphism (SNP) [rs2337980] of the CHRNA7 was genotyped. There were allele frequencies 0.59 for C allele and for 0.41 T allele. There were 43 C allele homozygotes, 62 heterozygotes, and 20 T allele homozygotes.
Overall, older adults had higher motion coherence thresholds than younger adults. We did not find any age-related associations of motion direction discrimination with the CHRNA7. However, regardless of age group, participants carrying the T/C genotype performed the task significantly better than C/C carriers. Our results therefore, indicate a strong relationship between the nicotinic system and motion perception.

P09 Two siblings with *MTHFR* gene C677T variant related epigenetic changes and subclinical hypothyroidism,a family case report

Kvaratskhelia T., Kvaratskhelia E., Kankava K., Abzianidze E.
Tbilisi State Medical University, Department of Molecular and Medical Genetics; Tbilsi, Georgia

Introduction: Subclinical Hypothyroidism (SCH) is defined as a state of increased TSH levels, with circulating thyroxine (T_4) and tri-iodothyronine (T_3) concentrations within the population reference range. Since there are consistent reports demonstrating that plasma homocysteine(tHcy) is high in hypothyroid patients, we hypothesized that *MTHFR* C677T polymorphism and its influence on levels of DNMT1 and DNMT3a may contribute to the development of SCH.
Case report: 51 and 52 years old sisters were diagnosed with SCHabout 16 years ago (elevated TSH and normal FT4 levels). They were enrolled to a research study related to the link between MTHFR C677T variant, levels of DNMTs and SCH.
Methods: MTHFR gene was investigated by the PCR-RELF method using HinfI (NEB Inc) enzyme. Levels of DNMT1-3a were measured in nuclear extracts of PBMC (Abcam). Total serum homocysteine concentrations were also measured.
Results: Both siblings revealed *MTHFR* gene 677 TT genotype and two other siblings - CC genotype with no history of SCH. Their mother has CT genotype and had an episode of thyroid failure. Family members with TT and CT genotype had elevated levels ofDNMT3a compared with CC genotype. There was no significant difference in DNMT1 levels.tHcy levels were significantly elevated (16.7 and 15.8umol/l) in study subjects with TT genotypes and slightly elevated in the individual with CT genotype.

Conclusions: We suggest that molecular studies of MTHFR gene and its related epigenetic changes could be valuable for refining the clinical diagnosis of SCH leading to more precise management of this condition.

P10 Medical genetics. Difficulties in teaching and diagnosing hereditary pathology

Mkheidze Medea
St.Petersburg Academy of Postgraduate Pedagogical Education, Russia

Medical genetics is an important part of the healthcare system of any civilized world, which is concerned with the prevention of public health. The development of medical genetics was blocked and difficult in the former USSR and the Post-Soviet space.
The analysis of 45 years of activity in this field of medicine allows us to characterize the main problems in teaching and in the diagnosis of hereditary pathology.
Main challenges in this regard are as a next:
1. The training of medical geneticists should be long (4-6 years of training in medical genetics after six years of study at a medical university) against the vicious practice of a one-year internship or two-year residency in this specialty).
2. Continuous training of work with a patient suffering from hereditary pathology, and his family under control of the supervisor.
3. Access to up-to-date information and international discussion of clinical cases.
4. Providing high-tech methods for diagnosis of hereditary diseases
5. State financing of diagnostic procedures in full (taking into account low solvency of the population)
6. Creation of an independent medical genetic service with clear tasks and ways to resolve them
Without the implementation of these provisions, it is impossible to expect real improvement in the health of the population, a significant part of which is related to genetic factors.

P11 Assessment of Immunogenetic features of donors' blood

M. Nagervadze, L. Akhvlediani, I. Tsintsadze, D. Surmanidze, T. Gorgoshadze
Batumi ShotaRustaveli State University, Laboratory of Immunogenetics

Introduction: Erythrocytes, leucocytes, thrombocytes are the carriers of blood group antigens. For clinical medicine erythrocyte group antigens are very important in as much as they are they precondition blood compatibility and are the main reasons of post-transfusion complications. These antigens represent a genetically firmly-determined peculiarity. Erythrocyte group systems are sharply distinct features of immunogenetic polymorphism. The goal of the research is to study regional immunogenic features of official donors. We will study the frequency of spreading of A, B, C, c, D, E, e, K, k, M, N antigens in donor populations.
Material and methods: The blood of 656 donors has been investigated on erythrocyte blood group antigens. The sample has been provided from diagnostic laboratory of Medina Ltd Health Centre of Batumi. Lab analysis of the sample has been carried out on the basis of immunogenetics laboratory of Batumi Shota Rustaveli State University. In order to reveal the specific antigens of erythrocyte group plasma as well as the erythrocyte mass have been applied. While carrying out the research the following internationally acclaimed immuno-serological methods was used: direct and cross-sectional reaction of ABO system determination; while determining rhesus system antigens: a) express-method using universal reagents; b) express method with the complete shape antibodies on the plane-table; In order to reveal MN, Kell system antigens the express method with universal mono-clone antibodies was used. The following specific test-systems was used during the research: anti –AB, -B, -A, -D, -C, -c, -E, -e, -K, -k, -M, -N, standard O(I), (II), (III) group erythrocytes and standard O(I), A(II), B(III), AB(IV) serums.
Results: The blood of 656 donors has been investigated on erythrocyte blood group antigens. The results of the investigation of the frequency of allele of ABO system in donors revealed that r is the

high frequency of allele spread and it equals to 0,70. Frequency of q allele appeared to be 0,23 whereas p allele was recorded as the allele with lowest frequency equaling to 0, 07. The results of investigation of phenotypes of rhesus system in donor populations displayed the following characteristics: 16,3±1,43% of investigated donors bear Rh(-) phenotypes; relevantly Rh(+) phenotype is found in 83,7±1,43% of donors. CcDee phenotype with its frequency that equals to 29,9±1,78% is frequently spread phenotype among phenotypic groups of rhesus system of the investigated donors. It is followed by CCD-ee - 17,2±1,47%, ccddee - 14,9±1,38% and CcD-Ee - 13,9±1,34%. ccD-Ee phenotype is the least spread phenotype with 11,1±1,22%; ccD-ee - 5,5±0,88%; same phenotype indicators -2,1±0,55 were observed for CcD-EE and ccD-EE; CCD-Ee phenotype frequency equals to 1,4±0,45%, CCD-EE phenotype frequency is 0,4±0,26% and frequency of Ccddee phenotype amounts 1,1±0,40%, ccddEe and CCddee phenotypes were recorded with the frequency of 0,2±0,17%. Investigation of the frequency of Kell system allele revealed p allele low frequency equaling 0,05, whereas the frequency of q allele was observed to be 0,95.

Conclusion: The study of the obtained data is of great importance for the rational preparation of blood components for the purpose of their use in transfusion. The obtained results can be used by medical institutions, especially hematological and transfusion centers.

P12 CALR and JAK2 mutation status in Turkish patients with BCR-ABL1-negative myeloproliferative neoplasms

Orenay-Boyacioglu Seda[1], Caliskan Metin[1], Korkmaz Mehmet[2], Bozkurt Gokay[1]

[1]Adnan Menderes University, Faculty of Medicine, Department of Medical Genetics, Aydin, TURKEY

[2] Celal Bayar University, Faculty of Medicine, Department of MedicalBiology, Manisa, TURKEY

INTRODUCTION: The myeloproliferativeneoplasms(MPNs)are chronic myeloid cancers characterized by the overproduction of mature and immature blood cells. Essential trombocythemia (ET), polycythemiaVera(PV)and primary myelofibrosis (PMF) are three of the *BCR-ABL* negative chronic myeloproliferativeneoplasms.In this study, it was aimed to determine the frequency of JAK2 and CALR gene mutations and to compare them clinically and hematologically in patients with BCR-ABL1-negative MPN.

MATERIALS AND METHOD: The mutations of *JAK2* V617F and *CALR* gene at exon 9 in 561 Ph negative MPNs patients were detected by Sanger sequencing and Real-Time PCR.

RESULTS: The *JAK2*V617F mutation was found in 100 (32.3%) of 310 PV patients, 45 (24.3%) of 185 ET patients and 6 (9%) of 66 PMF patientsrespectively, with the total mutation rate as 26.9% (561\151).The *CALR* mutation was found in 1 (6.66%) of 15ET patients and 1 (20%) of 5 PMF patients respectively, with the total mutation rate as 10% (2/20). There was no significant difference in median onset age between *JAK2*V617F [(24-80) years] and those without mutations [(21-78) years] and *CALR* [(21-81) years] mutations.Patients with *JAK2*V617F had higher white blood cell count and hemoglobin level when compared with patients with CALR mutation and without mutations. The platelet count of patients with *CALR* mutation was significantly higher than of with *JAK2*V617F mutation.

CONCLUSIONS: New retrospective studies with larger cohort will help us to understand the effect of *JAK2* V617F and *CALR* mutations in the development and prognosis of myeloproliferative neoplasms.

P13 Mutations of maturity-onset diabetes of the young (MODY) genes in early-onset type 2 diabetes mellitus in Turkish patients

Orenay-Boyacioglu Seda[1], Caliskan Metin[1], Korkmaz Mehmet[2], Bozkurt Gokay[1]

[1]Adnan Menderes University, Faculty of Medicine, Department of Medical Genetics, Aydin, TURKEY; [2] Celal Bayar University, Faculty of Medicine, Department of Medical Biology, Manisa, TURKEY

INTRODUCTION: Maturity-onset diabetes of the young (MODY) is a form of diabetes that is characterized by an early onset diabetes.The aim of this retrospective study is to investigate both novel and proven mutations of 11 MODYgenes in Turkish patients with MODY and early-onset type 2 diabetes.
MATERİALS AND METHODS: Unrelated 197 patients with early-onset type 2 diabetes and MODY were analysed for nucleotide variations in promoters, exons, and exon-intron boundaries of 11 known *MODY* genes, including *HNF-4alpha, GCK, HNF-1alpha, PDX1, HNF-1beta,NeuroD1, KLF11, CEL, PAX4, INS,* and *BLK*byNext-generation sequencing (NGS). Missense mutations or mutations located in regulatory region were classified as potentially pathogenic mutations.
RESULTS: We found that mutations of the four known MODY genes (*HNF-4alpha, GCK, HNF-1alpha, HNF-1beta)* account for a small proportion of classic MODY (10%) and early-onset type 2 diabetes (13.5%) in Turkish patients. One of these mutations are novel including *GCK.* Mutations of *PDX1, IPF-1,NeuroD1, KLF11, CEL, PAX4, INS,* and *BLK*were not identified in the studied patients.
CONCLUSIONS: *GCK* and *HNF-1alpha*are the most frequent type of MODY in our study population. Mutations of the 11known *MODY* genes may not be a major cause of MODY and early-onset type 2 diabetes in Turkey. Therefore, unidentified genes await discovery in a majority of Turkey and patients with MODY and early-onset type 2 diabetes.

P14 Dyslexia and attentional functions

Parkosadze K., Kunchulia M., Kezeli A.
Laboratoryof Vision physiology, IvaneBeritashviliCenter of Experimental Biomedicine, Tbilisi Georgia

Dyslexia is a specific learning disability affecting about 8-12% of population. It is characterized by difficulties with accurate or fluent reading and/or spelling abilities. Dyslexia is neurological in origin but underlying mechanisms are not fully understood and are debated for a long time. Among the possible risk factors inherited factors are estimated to account for up to 80%. Comparing identical and nonidentical twins has shown that genes account for about half of reading skills and thus the heritability of dyslexia is about 50%. Visual attention is one of the most relevant neuro-cognitive functions that is involved in reading process and deficits in visual attention could contribute to reading difficulties in dyslexia. Indeed, it is evidenced that attention problems and dyslexia co-occur frequently. Here we investigated states of attention using visual and auditory attentional tasks in Georgian children with dyslexia and their age and IQ match typically developed children.
Twenty-four dyslexic (age between 8-12 years old) and twenty-three typically developed children (age between 7-12 years old) participated in our experiments. All children with dyslexia were evaluated by Multi-disciplinary Group of Ministry of Education and Science of Georgia and were free from all kinds of vision, hearing and other neurological disorders.Here we mention that among the participants of our experiments were two dyslexic brothers, and also two siblings of dyslexic children who were not evaluated as having dyslexia. Participants performed visual search task, visual working memory task (n-back), and auditory statistical learning task. In Search Task participants were searching for a target - green horizontal line among the distractors - red and green vertical lines. In n-back task a sequence of visual stimuli (pictures of different objects) were presented and participants were answering whether the current stimulus matched to the previous stimulus (1-back), or the current stimulus matched to the stimulus presented two stimuli before (2-back). During auditory task the stimuli were the trisyllabic "words" that have no meaning and sound like a foreign unknown language (pagote, bagote, bupada and so on). Participants were listening to 3 min duration "text" and after they performed tests phase – they

heard pairs of trisyllabic “words” and answering which “words” in each pair sounded more like the sounds they heard before.
We found that accuracy of performances of search task was similar for both groups, but reaction times were significantly increased in dyslexic children indicating delayed bottom-up attentional mechanisms. Performances of 1-back visual task were significantly different between two groups, dyslexic children performed worst then their age match typically developed children, whereas when the task became more difficult, e.g. in 2-back task there were no differences in performance. Regarding auditory statistical learning task dyslexic children performed worst then typical children, indicating deficiency in auditory rather than visual attention. We found no correlation between IQ scores and data performances in both groups.
Our results showed that even when children with dyslexia show some deficits in attentional processes for both visual and auditory attention,attentional deficits alone cannot be accounted for specific deficits ofcomplex neurobehavioral disorders in dyslexia and can be the result of ongoing developmental processes in children in general.
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P15 Interphase Nuclei Damage asPreliminary Index of Mutagenic and Toxic effect of Environmental Factors

Pirtskhelani A., Mamaladze N., Pirtskhelani N.
Iv. Javakhishvili Tbilisi State University
Tbilisi State Medical University

Introduction: Environmental pollution which is basically caused by human industrial and agricultural activities comes back to them as a harmful factor to genetic apparatus of organisms which are connected not only to hereditary diseases, also to inherited abnormalities, malignant tumors,cardiovascular, digestive, nervous system diseases and others. Environmental pollution factors (pesticides, heavy metals,fertilizers, carcinogens, viruses and others) are characterized by mutagenic and toxic activities. It is important Identification and application of effective antimutagens which can minimize the frequency of spontaneous and induced mutations.
Materials and Methods: Mutagenic and toxic effects of pesticides (trichlorfon, cuprozan, Bordeaux mixture, Zinc sulfate), and cancerogen (Benzo[a]pyrene) have been studied on grown laboratory mice (without line). Administration of pesticides and Benzo[a]pyrenehave done orally to mice, dose ½ LD50 and 1/5 LD50.
Chromosome preparations from bone marrow cells of animals were made, in accordance with the methods of Ford and Woollam. All digital data have been processed with various statistical method, t-criteria was determined with Student’s t-distribution.
Results: Above mentioned factors are characterized by mutagenic and toxic effects. Per oral administration of these substances in animals (doze -1/5ld 50, 1/2ld 50) causes statistically significant increasing of the frequency of interphase nuclei damage(hollowed nuclei), chromosome abnormalities(single and pair fragments,triploidy, tetraploidy) and pathologic mitosis ($P<0.001$). Damages of Interphase nuclei, detected by us, are interested from the point of view, that they are preliminary indexof mutagenic and toxic effects of environment factors.
Conclusion: Numerous comparative cytogenetic tests showed, that disorders of Interphase nuclei are in positive correlation with chromosome abnormalities and pathologic mitosis. Detection of Interphase nuclei disorders are easierthan testing chromosome abnormalities and pathologic mitosisand due to

thisfact, for the purpose of preliminary evaluation of mutagenic and toxic effects of different environment factors, it is allowed and more economical to use Interphase nuclei test-analysis.

P16 The Interesting Cases of X-chromosomewith quantitative andstructural changes

Rukhadze M., Kvaracxelia E., Jangulashvili N., Sigua N.
Association "Perinatology", Centre of Prenatal Diagnosis;David Metreveli Medical Centre

Introduction: Cytogenetic study of chromosomes in patients with different disorders is very important and isthe only way for establishment of an etiologyof any chromosome disease. Patient's individual genetic heterogeneity defines the clinical polymorphism of the disease. So, the study of chromosomes in the Ullrich-Turner Syndrome (UTS) cases is reasonable. Our goal was to estimate variability of chromosome changes in lymphocytes from patients with UTS.

Materials and methods: Blood samples of three patients with disorders were cultured for cytogenetic analysis and the metaphase chromosomes weresubjected to banding treatment according to the slightly modified G-band method. All samples were cultured for 72 hr under the standart conditions. 30 cells for each examined individual were analyzed.

Results: According to their changes were distributed several variants of chromosomal abnomalites. All of these patients were admitted to the clinic with similar histories but at different ages: the mean age of admmittion to the endocrinologist was 11 years (9y, 11y and 14 y respectevely). Short stature developed postanally during childhood, frequent t episodes of Otitis Media, typicalphenotypical features: short neck, with inverse hair growth, low set ears, high arch palate, micro and retrognaty, wide chest and hypertelorism, cubitus valgus, shortened 4^{th} metacarpals were seen in all of them. Difficulties with Math, Autoimmune thyroditis but still euthyroid state and hypergonadotropichypogonadism with stucturalabnormnalitries of the ovaries and uterus was confirmed in all patients, cardiac abnormalities and celiac disease were not found in any of these patients.

Cytogenetic examination indicated 46,X,del(Xp),45,X/46,X,i(Xq) (81%:19%) - Mozaic form of Turner's Syndrome and 45,X/47,XXX (66%:34%) - Turner's Syndrome with Tryplo-X.

Conclusion: We present 3 different cases of UTS with rare abnormal female karyotipes based on analysis of G-banded chromosomes in cultured lymphocytes.All of these patients wereadmitted to the endocrinologist at different ages but with similar anamnesis, common phenotypic and laboratory findings were found. Significant changes in the abberant cells were found in these patients with all studied forms of deseases. Very interesting and rare is the patient with quantitative changes and two lines of cells. To conclude, with the development of HTS and aCGH technology, and its comprehensive application combined with G banding analysis and FISH, it is promising to conduct a more sophisticated study in derived chromosome, which will allow for a detailed elucidation on the association between the genotype and phenotype.

P17 Thrombocytopenia absent radius (TAR) syndrome: a case report of Georgian patient

[1]Tkemaladze T., [1]Abashishvili L, [1]Gogolashvili A., [1]Bagrationi G., [1]Tskhakaia I.
[1]Tbilisi State Medical University, Department of Molecular and Medical Genetics

Thrombocytopenia-absent radius syndrome (TAR) is a rare genetic disorder characterized by low platelet count and bilateral radial aplasia with both thumbspresent. Affected individuals may also express intolerance to cow's milk and cardiac and renal malformations. TAR syndrome is caused by a 200 kb minimally deleted region on chromosome 1q21.1 and the presence of low frequency noncoding *RBM8A hypomorphic allele.* Pattern of inheritance is autosomal recessive. Here we present a case of 18 months old girl, who presented with thrombocytopenia, bilateral radial aplasia with present thumbs, frequent diarrhea, ventricular septal defect and asymmetry of lower limbs, which is not a common feature of TAR syndrome. To investigate the TAR syndrome associated microdeletion 1q21.1 and one of two predisposing SNPs in *RBM8A* in patient, a quantitative PCR (qPCR) and sequencing on genomic DNA was performed. The patient revealed a reduced copy number for all investigated amplicons (NK, A13, A15, C) on chromosome 1q21.1. Sequencing detected the rare allele (c.1-21G>A) of SNP rs139428292 in the 5'UTR of *RBM8A*. The SNP rs201779890 in intron 1 of *RBM8A* showed the wild type allele. Thus, presence of compound heterozygous state of 1q21.1 microdeletion and a *RBM8A hypomorphic allele confirm the diagnosis of TAR syndrome in our patient. This case report illustrates, that children with* TAR syndrome should be examined for other associated malformations of various systems and followed up regularly. Additionally, the presence of the thumbs in the absence of the radius is an important finding differentiating this syndrome from related conditions.

P18 De novo mutation of *RPS6KA3* gene in a double consanguineous family: a case of Coffin-Lowry syndrome

[1]Tkemaladze T.,[1]Pati S.,[1]Peesapati S., [1]Pradeep R., [1]Shahare T., [1]Galal A.
[1]Tbilisi State Medical University, Department of Molecular and Medical Genetics

Introduction: Coffin Lowry syndrome is very rare X linked disorder characterized by severe to profound intellectual disability, growth retardation, typical dysmorphic facial features, skeletal anomalies and sometimes stimulus-induced drop attacks (SIDAs) . Condition mostly affects males whereas females may be either asymptomic or mildly affected due to the pattern of X inactivation.
Materials and methods: Here we describe a 5 year old boy who presented with typical features of Coffin-Lowry syndrome:severe cognitive and motor developmental delay, characteristic features with low set protruding ears, hypertelorism, epicanthus, downslanting palpebral fissures, broad eyebrows, anverted nares, long phyltrum, thick and averted lips, high palate, unilateral single palmar crease, tapered fingers, pectusexcavatum, bilateral cryptorchidism and scoliosis.
Results: Gene sequencing of the patient's DNA revealed abnormalities in *RPS6KA3* gene. A hemizygous G>A change was detected at nucleotide 632 (c.632-1 G>A). *RPS6KA3* is located on X chromosome at Xp22.2 and encodes the serine threonine protein kinase RSK2. RSK2 is animportant growth factor that is involved in the ras-mitogen-activated protein kinase signaling pathway. Mother's gene sequencing of *RPS6KA3*was also performed to check for the presence of c.632-1 G>A identified in the boy and she tested negative.
Conclusion: Although this specific mutation (c.632-1 G>A) in *RPS6KA3* gene has never been reported in the Human Gene Mutations Database, this particular change interrupts a consensus splice site that would result in abnormal splicing. Thus we can classify the novel variant of the gene as pathogenic. Despite the fact that the patient comes from the double-consanguineous family, the disease-causing mutation is a de novo event. Hence, it is important to take into consideration that de novo mutations are not rare in consanguineous families and they can become the causative factors of the disease.

P19 The importance of genetic biomarkers for detecting the effect of irradiation and prognosis of complications during radiotherapy

Alla Zedginidze[1], Ema Namchevadze[2], George Ormocadze[1], , Tamara Nikuradze[1] ,
[1]IvaneBeritashvili Center of Experimental Biomedicine, Tbilisi, Georgia
[2]Tbilisi state University, E. Andronikashvili Institute of Physics, Tbilisi, Georgia

One of the intensively developing part of medical genetics is radiation genetics. Ionizing radiationbeing a strong mutagen primarily act on human genetic apparatus, causes its disorders and consequently affects human health. A major part of the mutagens has cancerogenic features as the key moment in the development of tumor cells is a mutation. Today the usage of ionizing radiation is increasing day by day. Irradiation of persons is possible not only at nuclear disaster, or environmental contamination, but also during routine diagnostic procedures and radiotherapyGenetic investigation is the best method for detecting the impact of irradiation on live organisms In the context of increasing use of ionizing radiation in medicine, especially in oncology, great importance is u given to determination of individual sensitivity of patients, which the effectiveness of treatment depends on . The goal of this study was to detect the significance of cytogenetic, molecular and certain physiological disorders for revealing the individual reaction to the irradiation of cancer patients undergoing radiotherapy forprognosis of complicationsThe effect of the local fractionated gamma-irradiation with doses of 40-70 Gy was studied in dynamic of 12 cancer patients with localized head and neck tumors in dynamic (before, during and after the radiation exposure) Detection of chromosomal disorders, yield of micronuclei in buccal cells, assessment of DNA strand break damage by comet assay and functional state of red blood system were performed. There were two groups of cancer patients : 1.- with the first stage of disease and II – with II-IV stages and local spread of disease. In the first group investigation before irradiation did not show any differences compared with our control data according to all parameters..In the second group changes in all estimated parameters were observed even before the irradiation. However, following the first course of irradiation (as part of radiotherapy),in patients of 1 group all end points studied showed a significant increase with differential sensitivity among patients (Mnb level 4,33.±0.99;, amount of comets – 26-30% , chromosomal aberrations (acentric single and paired fragments) 0.02-0.05 per cell, number of dicentrics per cell -0.02-0.03). After the last irradiation, most of the patients did not show increase in the tested parameters. In II group increase of all parameters during radiotherapy was also observed in all casesWe observed more evident individual differences among estimated specific radiation biomarkers: dicentrics and other chromosomal damage, micronuclei in exfoliate buccal cells and amount of DNA-comets. Despite one and the same tumor localization and identical received dose of radiation, changes in the studied parameters were not homogeneous. The study of chromosomal abnormalities, the DNA damages by the comet assay and the micronuclei detection of the buccal cells revealed a statistically significant correlation between the initial cytogenetic indices in cancer patients and their dynamic changes during and after the radiation exposure. In addition, the correlation has been detected between the initial cytogenetic parameters and the functional stage of red blood system. After four months of radiotherapy clinical data was obtained in the patients' conditions. The correlation between clinical state and the level of biomarker changes was demonstrated.We believe that **the** biomarkers we have chosen are more appropriate for the determination of geno- and cytotoxic effects of ionizing radiation during radiotherapy.The application of the above-mentioned geneticbiomarkers should help in the individualizing medical management of patients undergoing radiotherapy.