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Translational Medicine in the Era of Omics**

Abstracts

May 11-12, 2019

Radisson Blu Iveria

Tbilisi, Georgia



საქართველოს მედიკალური გენეტიკისა და ეპიგენეტიკის საზოგადოება
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**Abstracts of
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Oral Presentations Plenary Lectures

Genomic Medicine Education Initiatives in Georgia: Key Messages and Core Ideas

Short Review

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Introduction

In the last decade, genomic medicine education initiatives have surfaced across the spectrum of medical training in order to help address a gap in genomic medicine preparedness among health professionals. The approaches are diverse and stem from the belief that 21st century physicians must be proficient in genomic medicine applications as they will be leaders in the precision medicine movement. Genetics will remain a foundational topic for all students aspiring for medical careers. In Georgia, Tbilisi State Medical University (TSMU) is one of the oldest higher education institutions in the whole Caucasus region. Here, in mid 90s, teaching of molecular and medical genetics gains priority and in past twenty years much work is done in order to develop and teach genomic medicine in Georgia. We reviewed the literature in genetics and genomic medicine education and training at the undergraduate, graduate, and continuing medical education levels within the TSMU, in order to identify current trends in genomic medicine education in Georgia.

Methods

We conducted a review of published literature and unpublished data (photo/video archive of the department of molecular and medical genetics at TSMU) in genomic medicine education and training for medical students, graduate medical education, and practicing physicians at TSMU between 1994 and 2019 to gain a picture of genomic medicine education in Georgia.

Results

The facts identified:

- *Medical Education at the Department of Molecular & Medical Genetics at TSMU: Rethinking, Reinterpreting and Restructuring.* 2006 – Department of Medical Biology, Genetics and Ecology was reorganized into the Department of Molecular & Medical Genetics. The initiator of the reorganization was professor Elene Abzianidze, which leads the department till now.
- *Departments's main priorities* – combining a strong foundation in basic sciences content with the critical thinking skills necessary to integrate new scientific discoveries into medical practice, competency-based education, problem-based learning and integrated study cases to develop critical thinking and applied empirical skills among undergraduate medical students.
- *What educational practices will best help future medical professionals to understand and embrace modern genetics and genomics?* Department's core curriculum embraces competency-based education by focusing on developing basic and applied empirical skills in genetics for the medical student as opposed to the memorization of a specific

constellation of symptoms associated with particular genetic diseases. Department implemented ASGH/APHMG MEDICAL SCHOOL CORE CURRICULUM IN GENETICS (1995, 2001, 2013) and states that it is necessary to integrate basic and clinical sciences, humanism, health population and ethic problems in the vertical axis, not only in the early years but also throughout the curriculum, presupposing the use of active teaching methods based on problems or cases in small groups.

➤ *Department's position on teaching genetics:*

– We think that genomic medicine should be valued, protected, expanded and changed over time. Curriculum designed by the Association of Professors of Human and Medical Genetics can provide guidance on which topics are best emphasized in a required medical school curriculum, both in preclinical and clinical phases of training, for the general graduate.

– Genetics should be integrated across all educational disciplines and healthcare

– It is important to state that molecular genetics is a basic science. An implementation of basic knowledge is crucial for genomic medicine.

– Continuing medical education should emphasize molecular and medical genetics

➤ *Continued Medical Education (CME):*

– From 2010 – Department of Molecular and Medical Genetics at TSMU is carrying out continued medical education (CME) program “Medical Genetics” for MDs.

– From 2017 – CME program “Genomic Medicine and Tools of Molecular Genetics” for MDs.

– From 2017 – CME program “Medical Genetics” for nurses.

➤ *Major breakthrough in Genomics education in Georgia:*

– “Thompson & Thompson Genetics in Medicine”, 7th edition, copyright translation, 2008. This is an instrumental book in the field. It has contributed to the modernization of medical education in Georgia. The new generation of doctors has acquired important knowledge in the genetics with the help of this textbook.

– In 2016 copyright translation of 8th edition of the book into Georgian language was done.

➤ *Intense translational and editing work at the department:*

– Karen Vipond’s “Genetics: A Guide for Students and Practitioners of Nursing and Health Care”, copyright translation, 2014

– Nessa Carey’s “The Epigenetics Revolution: How Modern Biology is Rewriting Our Understanding of Genetics, Disease and Inheritance”, copyright translation, 2014

– P. Kemp, K. Arms “Introduction in Biology” (adapted translation), 1997, 1998.

– E. Abzianidze, V. Berishvili, E. Imnadze, M. Tchipashvili, P. Pakhuridze, “Biology”, (in Georgian language) vol. I, II, III (1997, 1998, 2000)

➤ *Students' Activity at the Department – Vast amount of experience:*

– 1994-2014 Students' scientific debate club “FBG” Future Belongs to Genetics, more than 25 science conferences and debate activities

– From 2015 DNA Day Essay Contest for biomedical students. Different Georgian universities participate in this contest. Winners are awarded with various prizes including financial assistance, medical books and support for attending ESHG annual conferences.

- From 2016 Secondary school students under supervision of department participated in ESHG international DNA Day Essay Contest.
- ESHG “National” Fellowship for students’ interested and working in genetics.
- *Significant achievements of the Department: A story of hard work and success.*
 - 2014 – “Georgian Society of Medical Genetics and Epigenetics” was founded with the initiative of department’s personnel (www.geneticsgeorgia.org). Students’ scientific club “FBG” is now a part of society. It is a one of the most active society within Georgian scientific community. It became the member of European Federation of Human Genetics Societies (EFHGS) in 2016.
- *Focus on scientific research: From bench to bedside.*
 - 2014 – Experimental Laboratory of Molecular Genetics and Epigenetics was founded . The lab is equipped with modern techniques and several PhD programs are being carried out.
- *Major scientific directions– A fruitful work.* Department’s personnel and PhD students participate in local and international conferences, their works are published in peer-reviewed journals.
 - 2014-present – Epigenetic and Genetic Aspects of Inflammation.
 - 2000-present – Epigenetic and Neurobiological mechanisms of Pain.
 - Several PhD thesis were completed and several more are underway.
 - Department’s and PhD students research focuses on epigenetic changes in Cancer, Cystic Fibrosis, Endocrinological Conditions, Migraine, Neurogenetics, Inflammation and Pain , Nutrigenomic etc.
- *International multicenter epidemiological clinical study on biomarkers – successful collaboration.*
 - 2018 –Tbilisi State Medical University, Georgian Society of Medical Genetics and Epigenetics and CENTOGENE AG first time in Georgia announce international multicenter epidemiological clinical study on biomarkers. The aim of the study is to develop new MS-based biomarkers for the early and sensitive diagnosis of certain rare genetic conditions on patient’s dry-blood-spot sample.
- *Workshops:*
 - From 2015 – Workshop “Your Own DNA as a Gift” for high medical and secondary school students.
 - From 2018 – “Applications of artificial intelligence (Face2Gene) in medical diagnosis of rare diseases”.
- *Department is very active in organizing accredited conferences*
 - 2016-2017 – First and second accredited conferences “Genomics and Epigenomics in Precision Medicine- from Mechanisms to Treatment”, Tbilisi
 - *Human Genome and Health – First International Conference in Georgia, 2018* (<https://tsmu.edu/hgh2018>). Financed by Sh. Rustaveli Scientific Foundation (Grant No. MG_CG_10)
 - *Human Genome and Health – Second International Conference Translational Medicine in the Era of Omics, 2019* (<https://tsmu.edu/hgh2019>). Financed by Sh. Rustaveli Scientific

Foundation (Grant No. MG-ISE-18-574).

- *Public activities: We see ourselves not only in the field of science, but also in general public.*
 - 2008-2018 – Several charity events were organized by “FBG” Club and the Department’s personnel for children with disabilities and children in need. Activities included public exhibitions of students’ and children’s art works, performances of plays, etc.
 - Public lectures for general audience related to medical genetics and epigenetics.
 - 1997 – Unique Biological museum was rediscovered by the head of the department. Now it is a part of Georgian cultural life.
 - Media activity – Staff of the department is periodically invited in various Radio/TV programs to talk about trends in medical genetics, epigenetics, genetic disorders, etc.

- *The importance of collaboration in a connected world.*
 - Department has a tight collaboration with local and international institutions and organizations: Iv. Beritashvili Center of Experimental Biomedicine, TSMU Institute of Biotechnology, Genetics Institute of TSU, L. Samkharauli National Expertise Bureau, Arlene and Robert Kogod Center on Aging, Mayo Clinic, Rochester, KU Leuven University, Centre for Biomedical Ethics and Law, Leuven, Belgium.

- *Membership in International Scientific Societies.*
 - ESHG, IBRO, FENS, IASP, EPNS, ECA, SSIEM, FDNA etc.

Conclusion

We reviewed the literature in genomic medicine education and training at the undergraduate, graduate, and continuing medical education levels within the TSMU, between 1994 and 2019 in order to identify trends in genomic medicine education. We found evidence of innovative and creative genomic education initiatives and ideas to increase knowledge of and readiness for genomic medicine applications. In this way, the next generation of medical professionals will develop scientific critical thinking skills that will allow them to apply foundational genetic knowledge and ethical principles to patient encounters. They will be able to adapt to the rapidly changing practices that are accompanying the genomics revolution, understand the resources of molecular biology and bioinformatics that can serve them in their practice, and treat their patients with confidence, ethical soundness, and compassion.

Biobanking and genomic data sharing: harnessing the power of genomic data to improve patient care

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Biobanking and genomic research are considered essential to advancing the practice of medical genetics. Over the past three decades, there has been a proliferation of biobanks, that is, biospecimen repositories storing biological material for research use. Since the completion of the Human Genome Project in the early 2000s, biobanking has become increasingly intertwined with genomics and bioinformatics, progressively utilizing big data analytics to gain insights into human disease. This paradigm is epitomized by several large-scale genomic research initiatives, such as the 100 000 Genomes Project in the United Kingdom and the Precision Medicine Initiative in the United States. However, owing to rapidly diminishing costs of DNA sequencing, coupled with remarkable advances in bioinformatics, genomic research has become widely accessible to numerous organizations around the world. This has resulted in the fragmentation of genomic research, potentially hindering the progress in medical genetics.

In order to take full advantage of the ongoing genomic revolution, the importance of genomic data sharing among biobanks and genetic research centers has been emphasized. Recently, several data sharing initiatives have been established to facilitate federated access to genomic data across research organizations. However, the success of these initiatives is far from certain as various challenges to genomic data sharing remain to be resolved. In particular, issues relating to data ownership, privacy, anonymity, and the potential harm to individuals whose genomic data are analyzed pose material challenges to genomic data sharing.

Public Health and Genetics: priorities for 2025

Martina Cornelia Cornel

While genetic technology has developed fast in the last decades, medicine has not yet integrated many genomic applications. Especially if a high risk of disease can be detected before irreversible damage has occurred, and if interventions are available, public health priorities arise. Different settings in health care may integrate genomic applications: neonatal screening, testing healthy relatives of patients with oncogenetic or cardiogenetic conditions, preconception carrier testing, etc. Each country may choose its own policy at the interface of public health and genetics, depending on morbidity and mortality as well as on budgets that are available. Many countries however have started including more conditions in the neonatal screening programs. Providing screening for BRCA carriers and Lynch syndrome patients may prevent cancer morbidity. Recognizing familial hypercholesterolemia may help to prevent cardiovascular disease at a young age. Prevention becomes more urgent than treatment in populations where people live longer. For sustainable development, public health policy makers should prioritize affordable prevention programs taking into account where most people can benefit from public health genetic services.

Mammalian “retrobiome” (a variety of reverse transcription products in mammalian DNA) as a target for antiaging and anticancer therapies.

Andrei V. Gudkov

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Nearly half of mammalian genome is occupied by multiple families of virus-like repetitive elements expanded in evolution via the mechanism of reverse transcription and cumulatively forming a “retrobiome”. The majority of such retroelements use reverse transcriptase of LINE-1 family of repeats (RTL1) for their replication. Most of the LINE-1 repeats are genetically deficient due to inactivating mutations. A small subpopulation of technically intact LINE-1 elements is under a strict negative control by multiple mechanisms acting at transcriptional (epigenetic silencing), post-transcriptional (e.g., degradation of RNA and cDNA) and cellular levels (induction of interferon type I response). However, the expression of LINE-1 can occur rarely in somatic cells and frequently – in tumors. Desilenced retrobiome acts as a genomic instability driver contributing to aging (as a constitutive source of damaged cells) and cancer progression and adaptation to treatments. Cells with active retroelements can be considered as plausible targets for antiaging and anticancer therapies. The approaches to pharmacological targeting of retrobiome and their clinical applications will be discussed.

Lecture “Non-coding structural variants in the “3D genome” and their relevance to congenital disorders”

Eva Klopocki

Institute of Human Genetics, University of Würzburg, Germany

Complex developmental processes require tightly controlled regulatory networks which ensure correct temporal and spatial gene expression during development. Gene expression programs are guided by *cis*-regulatory elements including promoters, enhancers, repressors and insulators. Together, promoters and enhancers constitute the regulatory landscapes of genes. By chromosome conformation capture technologies the 3D organization has been characterized and chromatin domains referred to as a topologically associating domain (TADs) were shown to be important to restrain and facilitate enhancer-promoter interactions. Alterations of the regulatory landscape can cause tissue- and stage-specific effects some of which have become recognized as a significant cause of developmental defects and human disorders. Different mechanisms underlie disruption of long-range gene regulation. These can give rise to phenotypes that differ from those associated with mutations in the coding regions of the affected genes.

Structural variants of the human genome contribute to phenotypic variation as well as pathogenic conditions. Copy-number variations (CNVs) constitute one group within these structural variants that arise from losses or gains, and as a consequence result in a copy-number change of the respective genomic region. CNVs may include entire genes, parts of transcripts, or only noncoding sequences. By now it is well accepted that CNVs affecting coding regions can have pathogenic effects i.e. due to changes in gene dosage. Noncoding variants which may encompass *cis*-regulatory elements and affect TAD structures, however, have only recently come into focus as disease-

associated variants. The consequences of CNVs in noncoding sequences are less obvious, although, the so far described phenotypes associated with alterations in noncoding elements with regulatory potential are striking and at the same time confined to a certain tissue/organ. Excellent clinical examples for this are duplications encompassing potential enhancer elements which cause limb malformations i.e. brachydactyly, polydactyly, and mirror-image duplications.

Besides CNVs in non-coding sequences structural variants such as inversions and translocations may disturb the regulatory landscape and chromatin architecture and have been associated with human disorders. One of the underlying mechanisms is known as “enhancer adoption” indicating a gene which is driven by an enhancer that is not its own potentially causing ectopic expression. Structural variants may also disrupt regulatory boundaries of topological chromatin domains (TADs) i.e. deletion of insulator elements resulting in aberrant gene regulation.

In addition to congenital anomalies non-coding regulatory variants have been identified in somatic disease conditions i.e. cancer.

In conclusion, genetic variants affecting regulatory elements and TAD structures are expected to be enriched among conditions which are due to disturbance of complex developmental processes. Integrating data from patients with the recently published data from the ENCODE project including HiC data will broaden our view of genes and their regulation and contribute to our understanding of pathomechanism underlying human disease and phenotypic traits in general.

ADHESION-LINKED PREDICTIVE MOLECULAR CLASSIFIERS IN PRIMARY CUTANEOUS ME-LANOMA

Alexander Meves, MD (Mayo Clinic, Rochester, MN)

ABSTRACT. In my presentation, I will address a problem that is common not just to melanoma, but also to other forms of cancer, such as prostate cancer, and that has been widely discussed recently. While we as dermatologists encourage patients to undergo skin cancer screening exams to detect melanoma early, it remains challenging to differentiate the truly aggressive from the indolent or nonclinical pigmented lesions. This is true even after a melanoma has been biopsied and examined under the microscope. Cancer Research UK, the world’s largest independent cancer research organization, named the ability to “distinguish between lethal cancers that need treating, and non-lethal cancers that do not” as one of the 2016 grand challenges that – if met – could revolutionize the way we prevent, diagnose and treat cancer. In our research we aim to address this challenge specifically in the context of cutaneous melanoma by applying our knowledge of a cancer-defining biological system, i.e. integrin adhesion, to distinguish between biologically indolent and aggressive melanoma. Staging of melanoma by AJCC 8th edition guidelines – the current classification system for describing the extent of disease and prognosis – is heavily dependent on the tumor invasion depth (aka Breslow depth) seen in the diagnostic biopsy sample and whether disease has metastasized regionally or beyond. However, this current staging system has a number of weaknesses. For example, many patients are currently falsely classified as lower risk, cannot be triaged to adjuvant therapy or be consistently enrolled into a clinical trial to assess whether adjuvant therapy alters their prognosis. There is a clear need for ancillary biomarkers that identify patients

who are likely to relapse but remain unidentified using conventional staging parameters. Our research in this area will be discussed.

New biomarkers in the clinical practice

Prof. Arndt Rolfs, MD¹

¹Centogene AG, Rostock, Germany

Due to the new developments in science and computing technology, today more than ever new promising approaches for the therapy of rare diseases are emerging such as ERT, Ab immunotherapy, proteins, gene therapy, stem cells, regenerative medicine, RNA-based therapies and small molecules. As a result, the diagnostic of rare disease patients becomes of the utmost importance. Due to the nature of the rare diseases, their insufficient description and known natural history as well as due to the lack of general knowledge of the practitioners and none in the least the necessity of rapid diagnostic, the burden of correct identification of the affected patients resides mostly on screening national programs and private targeted screening projects. Most of the typical screening projects will be 1-tier testing which is insufficient to correctly diagnose a rare disease patient. A more holistic approach is necessary including classical enzymatic testing, biomarkers (molecular fingerprint) quantification and confirmation by genetics. Large scale multiple tier screening include automatic sample preparation, multiple reaction monitoring mass spectrometry (MRM-MS), next generation sequencing and even CNV determinations. This approach is targeted for specific rare disease (e.g. Niemann-Pick, Gaucher) and all analyses are performed out of a minimal amount of sample. However, with the new field of untargeted metabolic profiling, combined with untargeted gene sequencing techniques such as genomic panels – the screening possibilities will be unprecedented, capable to compensate to the understanding of the disease and for the overlapping clinical pictures of specific diseases.

Epidemiological studies in rare hereditary diseases

Prof. Arndt Rolfs, **Dr. Volha Skrahina**

Centogene

All epidemiological studies in the environment of rare hereditary diseases are based on a particular population (the ‘risk population’) followed over a particular period of time (the ‘risk period’). Within this framework, the most fundamental distinction is between studies of disease ‘incidence’ and studies of disease ‘prevalence’. Once this distinction has been drawn, then the different epidemiological study designs differ primarily in the manner in which information is drawn from the source population and risk period. Incidence studies ideally measure exposures, confounders and outcome times of all population members. Incidence studies are usually the preferred approach to studying the causes of disease, because they use all of the available information on the source population over the risk period. However, they are often very expensive in terms of time and resources, and the equivalent results may be achieved more efficiently by using an incidence case–control study design.

Also, for some diseases (e.g. those rare diseases with a slow progress), incidence may be difficult to measure without very intensive follow-up. Thus, it is often more practical to study the ‘prevalence’ of disease at a particular point in time. This approach has one major potential shortcoming, since disease prevalence may differ between two groups because of differences in age-specific disease incidence, disease duration or other population parameters. For example, motor neurone disease and multiple sclerosis have similar incidence and mortality rates, but multiple sclerosis represents a greater burden of morbidity for the health services, because survival for motor neurone disease is so short.

Longitudinal studies (cohort studies) involve repeated observation of study participants over time. They represent the most comprehensive approach since they use all of the available information on the source population over the risk period. Longitudinal studies in rare diseases involve repeated assessment of categorical or continuous outcome measures over time (e.g. a series of linked cross-sectional studies in the same population), in our cases including the quantification of a biomarker.

In a cohort study the population under investigation consists of individuals who are at risk of developing a specific disease or health outcome. These individuals will then be observed for a period of time in order to measure the frequency of occurrence of the disease among those. This type of approach has been used to examine eg the frequency of Fabry disease in a pan-European young patient cohort (Rolfs et al, 2006). During the follow-up period, data are acquired on the symptoms experienced by the two cohorts (positively tested for the event (Fabry disease) vs negatively tested) using questionnaire interviews, objective investigations and biomarker. Studies like these are disclosing the deepest insight in the natural history of such rare hereditary diseases, including the change of time and the correlation of a biomarker with the clinical manifestations.

Parallel Sessions

GENOTYPE CORRELATED VITAMIN D SUPPLEMENTATION IN PKU PATIENTS

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Introduction: Due to restricted diet patients with PKU can develop deficiency of several vitamins. Aim of the study was to find correlation between the genotype and Vitamin D deficiency level in 148 Georgian PKU patients and to improve Vitamin D supplementation scheme according to patients Genotype.

Materials and methods: Study was made in Georgian population, the results of 148 PKU patients were analyzed. 25(OH) D Vitamin was measured in the blood plasma of patients. The only Vitamin D intake during last 3 month was exclusively from amino acid formula (8.7 mg in 100 g).

Results: In patients with clinically defined classic PKU the mean of Vitamin D was 12,4 ng/ml, in patients with moderate PKU the mean of Vitamin D was 18.7 ng/ml, in mild PKU cases mean was equaled 24.3 ng/ml and, finally, in patients with HPA Vitamin D mean made 35.1 ng/ml. (Normal range: 30.1-100 ng/ml). Genotype based analyze showed that the PKU patients with homozygous P281L mutation in PAH gene have the lowest Vitamin D level in blood.

Conclusions: There is a noticeable correlation between the genotype of PKU patients and the concentration of Vitamin D in blood. PKU patients with homozygous mutation P281L are more affected with low concentration of Vitamin D, then the patients with the same mutation on one allele, and the PKU patients with all the other mutations are less affected with Vitamin D deficiency. There is a need to supplement patients with the Vitamin D through taking in consideration their genotype, despite of patients supplementation with aminoacid formula containing Vitamin D, which is counted per patients weight.

Prognostic value of the first and second trimester ultrasound markers for the diagnostics of the fetal aneuploidies

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Laboratory of Cytogenetics "Perinatology"

Early diagnostics of congenital abnormalities as well as further management planning are the important part of maternal-fetal health. There are two main popular methods of prenatal screening in Georgia: First and second trimester biochemical screening and ultrasound screening. The third one-cell free DNA screening (non-invasive prenatal screening) is not widespread due to high cost.

The aim of the study was evaluation of correlations between first and second trimester ultrasound markers and fetal aneuploidies.

Methods: Prenatal genetic consultancy, expert ultrasound examination and prenatal cytogenetic diagnostics using second trimester amniocentesis were performed in 1665 pregnant women.

Results: The first trimester ultrasound marker- enlargement of the nuchal translucency was detected in 52.50% of fetuses with trisomy 21 and in 12.5 % of fetuses with trisomy 18. As for the second-trimester ultrasound markers they were presented in 64.10% of trisomy 21 and in all cases of trisomy 18.

Conclusion: It should be underlined that there is not just one important ultrasound sign which may considered characteristic for the particular aneuploidy. If there are any suspicion on fetus aneuploidies, prenatal cytogenetic diagnostics should be done and not to monitor only ultrasound markers which are not present always or make biochemical screening which is not the confirmation of diagnosis.

Molecular-genetic characteristics of the MEFV gene for the Familial Mediterranean Fever disease in patients from Azerbaijan Republic

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Familial Mediterranean Fever Gene (MEFV) is located in chromosome 16, being precise in 16.13.3. locus. Out of 177 MEFV gene mutations found, 154 are missense mutations.

We for the first time in Azerbaijan put up the goal: to study the molecular-genetic characteristics of the MEFV gene for the Familial Mediterranean Fever disease in patients from Azerbaijan Republic.

For this purpose, venous blood samples were taken from 18 patients. Positive Cycle Sequencing PCR samples, got by agarose gel electrophoresis, are purified by BIGDye XT dye remover. The purified gene samples were read by the Automatic DNA sequencing AB13130xI Analysis System. The obtained nucleotide sequences were read out with Seqscape V.2.7. programme, compared to normal MEFV nucleotide sequence by Blast Ce NCBI, and then polymorphisms and relative mutations were identified.

The molecular-genetic study of the MEFV gene has identified 7 mutations: R761H M694I, M694V, V726A, R202Q, M680I and E148Q. Three of 18 examined patients were heterozygotes, eight homozygotes, and seven double heterozygotes (compounds). Nine polymorphisms were found in three exons of the MEFV gene. Two R202Q and E148Q mutations were found in exon 2 (28.57%) of MEFV gene.

In order to prevent Familial Mediterranean Fever disease, parents of 18 patients have been consulted by geneticist for a healthy child prognosis for the next pregnancy and 25% of the risk of affected child.

Results of molecular genetic researches for the MEFV gene in patients with the diagnosis: periodic disease - are presented. Out of 7 mutations, two mutations E148Q and R202Q are located in exon 2, and the rest R761H, M694I, M694V, V726A, M680I five mutations in exon 10.

To carry out prophylaxes of periodic disease to families with genetic risk of affected child birth, medical genetic consultation is planned to be conducted with the following prenatal molecular genetic diagnostics of fetus in the first trimester of pregnancy.

Nutrigenetics: Folate Metabolism Gene Variants and Disease Prevention

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Introduction: The Nutritional genomic area includes two parts: first Nutrigenomics that is the study of interaction between dietary components and the genome, and the regulating changes in proteins and other metabolism; second Nutrigenetics that identify the response to dietary components with regard to genetic differences.

Folate (Vitamin B9, Folic acid) is essential for life-sustaining processes of DNA synthesis, replication, and repair which are naturally present in common foods such as peas, oranges, broccoli, and whole-wheat products. The Methylene tetrahydrofolate reductase (MTHFR) is the enzyme involved not only in the folate cycle, but also crucial to the 1-carbon cycle (1-CC), responsible for methylation. Methylation is essential for the regeneration and biochemical regulation of cells, imprinting genes and epigenetics. encodes enzyme which involve in folate metabolism. C677T polymorphism of *MTHFR* gene is a fairly common variant in human populations, with about 80 percent of individuals having a C allele, which codes for alanine, and the other 20 percent having a T allele, which codes for valine. The T allele protein is less stable and less active, and individuals carrying that variant have a lower folate status. In this study investigate frequencies of *MTHFR* C677T polymorphism in patients with Subclinical Hypothyroidism (SCH), In addition we studied methylation levels of Alu repetitive elements in patients with TT and CT genotypes.

Materials and Methods: 60 patients with SCH and 40 healthy individuals were enrolled in the study with mean age 45 ± 25.5 and 48 ± 25.1 , respectively.

Results: C allele is significantly prevalent in the control group compared to the SCH group ($p = 0.0012$), while T allele is prevalent in patients compared to the control group with a statically significant difference ($p = 0.0012$). In addition, Methylation levels of Alu transposons were significantly lower in patient group compare to control individuals ($p < 0.05$).

Conclusions: This study shows that *MTHFR* C677T polymorphism may play an important role in thyroid health. Furthermore, genome instability caused by abnormal methylation levels of DNA may trigger development of conditions such as Subclinical Hypothyroidism.

Diagnosics of intellectual disability using exome sequencing

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Introduction: Intellectual disability (ID) is widely spread in populations with an average frequency of 1% to 3%. Due to the huge genetic heterogeneity of ID only high throughput sequencing technologies (HTS) made it available to perform molecular diagnostics of ID. Optimal method of HTS is a whole (WES) or clinical (CES) exome sequencing.

Materials and Methods: There were 100 patients with various forms of ID included in the study. Traumatic and infectious brain damage were excluded in all patients. The patients were also tested for Martin-Bell, Rett syndromes and chromosomal pathology prior to HTS. WES was performed in 69 patients and CES (panel of 6500 HGMD genes, 2018) – in 31 patients. All (potentially) pathogenic genetic findings associated with ID were confirmed by Sanger sequencing in trios.

Results: Twenty two different forms of ID were diagnosed: (Kabuki, Cohen, Cowden, Nicolaides-baraitser, ZTTK, Smith-Magenis, Helsmoortel van der Aa, Cornelia de Lange-like, multiple congenital anomalies-hypotonia-seizures syndrome 1), metabolic diseases (MPS III type), early

epilepsy encephalopathy (type 4 and 7), mental retardation autosomal dominant (7, 8, 35, 36, 49 types, ID with a mutation in the *GRIAI*), mental retardation X-linked (98 and 102 types), mesomelic dysplasia with a mutation in the *AFF3*. Several new candidate genes were identified. As a result, 21 pathogenic, 3 probably pathogenic and 12 variants of unknown significance were found in 100 patients.

Conclusions: Exome sequencing allows to confirm molecular diagnosis of ID in 24% of patients (20% for WES; 29% for CES). High genetic and clinical heterogeneity of ID makes it difficult to establish precise diagnosis without high throughput sequencing. In these cases, exome sequencing is a method of first choice.

RBC aggregation index in hypertensive patients and in their hypertensive children

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Proved that there is a dependency aging rate of genotype features. This can be interpreted as evidence of the involvement of the genotype in the control of aging. But another point of view is valid. The reliability of the molecular, cellular, systemic mechanisms of the viability of the organism depends on the individual characteristics of the genotype. Our goal was to study the RBC aggregation in parents (>80 years old) and children (>60 years old). Both relatives had hypertension, inherited in a polygenic type. Although some forms of familial raising of cholesterol in the blood, which is a risk factor for coronary heart disease, are inherited monogenically. But this happens very rarely. We studied RBC aggregation in the group of parents and in the group of children. We used innovation Georgian method for count RBC aggregation index. Statistically processed pair values. It turned out that the aggregation of the parents was less than that of their children. But for parents and children, the aggregation was increased by a considerable amount compared with the norm. The presence of genotypic influences is supported by the fact that the process of RBC aggregation in blood of long-livers is slowed down. But in the children of these parents, who had hypertension, the aggregation capacity of the erythrocytes was much increased compared with the parents. This is evidenced by an increased likelihood of hypertony deasiasse in relatives (especially first degree relative) of people suffering from this disease. Our research can be interpreted as evidence of the involvement of the genotype in the control of aging.

Pharmacogenomics and personalized medicine - a modern approach for the diagnosis and treatment of Inherited Thrombophilia

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Introduction: Personalized medicine (PM) has the potential to tailor therapy with the best response and highest safety margin to ensure better patient care. By enabling each patient to receive earlier diagnoses, risk assessments, and optimal treatments, PM holds promise for improving health care while also lowering costs. Inherited thrombophilia is a genetic disorder of blood coagulation resulting in a hypercoagulable state, which has been suggested as a possible cause of recurrent thromboembolism. Family and twin studies have established a heritable component to venous and arterial thrombosis. For the vast majority of patients, thrombosis is a complex, multifactorial disease

caused by a combination of numerous, often unknown, environmental and genetic factors. The field of pharmacogenetics is rapidly expanding into many clinical disciplines, including hematology. Pharmacogenetics is based on the notion that genetic variations influence the clinical outcomes of drug therapies; ie, gene-drug interactions.

The aim of our study was to determine the role of inherited thrombophilia in thromboembolism and to estimate the frequency of genetic and allelic variants of CYP2C9 and VKORC1 genes in Georgian population patients with venous thromboembolism and their effects on warfarin dose requirement.

Materials and Methods: 1333 unrelated Georgians with thromboembolism and pregnancy complications were genotyped by PCR analyses for detection of inherited thrombophilia (Factor V Leiden (FVL), Prothrombin (PTH G20210A) and Methylenetetrahydrofolatereductase (MTHFR C677T) gene mutations) and also 50 Georgian patients with venous thromboembolism were genotyped by PCR analysis for the three single nucleotide polymorphisms (SNPs) of CYP2C9 and VKORC1 genes and were followed prospectively in determining the optimal starting dose of warfarin.

Results: As a result of our study it is possible to consider Leiden, Prothrombin and MTHFR mutations, especially its homozygous form and double heterozygous carriage as an independent high risk factors for development of thromboembolism, pregnancy loss and pregnancy complications in the Georgian population patients. In 40 (80%) patients were found *1/*1 wild-type variant of CYP2C9 gene in combination with GG or GA genotype of VKORC1 gene and starting daily dose of warfarin in this study group, considering physical constitution and age of patients, were more than 7 mg. Only in one patient was found rare malfunctioned genotype CYP2C9 *2/*3 and VKORC1A/A and daily dose of warfarin was 1mg. This patient had bleeding under observation of 2 mg. warfarin and only after this episode has performed pharmacogenetic testing. Alternative, less active alleles CYP2C9*2 and *3 were detected in 9 (18%) patients.

Conclusion: Distribution of studied polymorphisms in Georgian population and clinically effective dose of warfarin, considering patients' genetic profile, coincides with the recommendations of CPIC. Noteworthy the fact, that generally, doctors in Georgia prescribe genetic tests to patients when the average dose of warfarin is not effective. Although still in its infancy, the field of pharmacogenetics and pharmacogenomics already provides useful clinical information to enhance patient care and offers a growing potential to individualize drug therapy and improve clinical outcomes.

Structural abnormalities of chromosomes and reproductive failure

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Introduction: Cytogenetic studies in patients with reproductive failure (RF) is very important and is the one of the ways to determine the reason of this problem. In a number of patients balanced chromosomal anomalies are revealed. Unbalanced form of structural anomalies, transmitted to embryos, can cause pregnancy loss. Our goal was to estimate frequency and types of structural abnormalities in lymphocytes among patients with RF.

Material and methods: Blood samples of 25 patients with miscarriages and infertile persons were cultured for cytogenetic analysis and the metaphase chromosomes were subjected to banding treatment according to the slightly modified G-band method. All samples were cultured for 72 hr under the standard conditions. 30 cells for each examined individual were analyzed.

Results: According to their changes several variants of chromosomal abnormalities were distinguished. All of these patients were admitted to the clinic with similar histories, but we divided them in 2 groups: miscarriages and infertile individuals. 11 women and 14 men were studied. Different types of disorders have been identified: majority of them were translocations – 15 cases and 4 of them were Robertsonian translocations; it was also revealed deletions, inversions and other types of disorders.

Conclusions: We have been studying patients for last 5 years and among them (approximately 750) structural abnormalities of chromosomes were revealed in 25 patients, which is equal 3,3%. The structural aberrations of either sex or autosomal chromosomes were found in infertile men. If we add quantitative changes to this number, in our opinion, this gives us good reason to advise all the infertile couples to conduct cytogenetic research.

TRP Channels in pain and itch sensation

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Transient receptor potential (TRP) cation channels serve as cellular sensors for a wide spectrum of physical and chemical stimuli. Recent studies have established the role of temperature-sensitive TRP (thermo-TRP) channels as molecular thermometers in the peripheral and central nervous system. They also are involved in thermal and mechanical pain and itch (pruritus) sensations. Antagonists of these channels are likely promising targets for new analgesic drugs at the peripheral and central levels. Because some non-steroidal anti-inflammatory drugs (NSAIDs) are structural analogs of prostaglandins and NSAIDs attenuate heat nociception and mechanical allodynia in models of inflammatory and neuropathic pain, we investigated whether three widely used NSAIDs (diclofenac, ketorolac, and xefocam) affect thermal and mechanical hyperalgesia following the activation of TRPA1 and TRPV1 channels. We measured nociceptive thermal paw withdrawal latencies and mechanical thresholds bilaterally at various time points following intraplantar injection of the TRPA1 agonists, cinnamaldehyde (CA) and allyl isothiocyanate (AITC) or the TRPV1 agonist capsaicin, or vehicle. When pretreated with vehicle, intraplantar injection of CA, AITC and capsaicin each resulted in significant decreases in thermal withdrawal latency and mechanical threshold in the ipsilateral hindpaw that did not return to baseline for more than 2 hours. To test effects of NSAIDs either diclofenac, ketorolac or xefocam was pre-injected in the same hindpaw 35 min prior to CA, AITC or capsaicin. Pretreatment with each of the three NSAIDs produced strong anti-nociceptive and anti-hyperalgesic effects lasting approximately 60 min. Thus, we show for the first time that local administration of NSAIDs reduces thermal and mechanical hyperalgesia following TRPA1 or TRPV1 activation.

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Biomarker Clinical Study in Georgia: Results and Perspectives

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Introduction: There are more than 8000 rare diseases that affect >5% of the world's population. Often the awareness about these diseases is limited leading to delayed diagnosis and treatment. Georgia became a part of the international multicenter epidemiological protocol on the identification of new biomarkers for rare diseases. The goal of the study is to identify and validate new sensitive and specific biomarkers from the blood of the affected patients helping to benefit other patients by an early diagnosis and thereby with an earlier treatment.

Materials and Methods: Since November 2018 till May 2019 total of 107 patients were included in the study for various indications (Cystic Fibrosis - CF, Duchenne disease - DMD, Tuberosous Sclerosis Complex - TSC, Creatine Deficiency Syndromes - CDS, Hereditary Angioedema Type 1 – HAE1). Patients of both genders older than 2 months were included in the study, who have a confirmed diagnosis or high grade suspicion of a given diseases. In order to validate the biomarker the genetic analysis was performed for every patient for the corresponding disease.

Results: Genetic analysis of patients revealed interesting data. Total of 17 patients were involved in the CF study, 9/17 tested positive, 3/17 were carriers and 5/17 were negative. It turned out that the most common mutation in the *CFTR* gene in the study cohort is c.1545_1546del p.(Tyr515*), representing 60% of all the alleles in the Georgian cohort of patients. All patients involved in DMD study tested positive, in-frame deletions in the *DMD* gene were causative for Becker phenotype and out-of-frame deletions were causative for more severe Duchenne phenotype; one patients is a carrier of c.2665C>T p.(Arg889*) stop codon mutation for which treatment is available; another patient with large deletion encompassing exons 8-41 also presented with severe intellectual disability and autism spectrum disorder. For TSC study 5/6 patients had positive results and for HAE1 study diagnosis was confirmed in 10/12. For CDS study inclusion criteria was to have one or more of the following: developmental delay, intellectual disability, autism spectrum disorder, epilepsy and/or movement disorder; for this cohort of patients clinical exome was performed; it had 20% diagnostic yield, identifying various single-gene disorders, thus emphasizing the diagnostic yield of WES in intellectual disability/epilepsy/ASD patients.

Conclusion:

It is the first time that Georgia became a part of international multicenter epidemiological study on the discovery of new biomarkers, with the goal to recruit large amount of patients for various indications. Recruited patients benefit from molecular-genetic testing and opportunity for diagnosis to end the diagnostic odyssey. Personalized medicine approach will have a crucial impact on increasing the quality of medical care of rare disease patients, where customized and individualized treatment will be guided by the individual's specific biomarkers.

About Some Peculiarities of the Inheritance of Twins

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It is well-known that monozygotic twins have essentially the same DNA but even though there are many dissimilarities in their phenotype. This is because of their different epigenetic patterns allowing for difference in their gene expression. Moreover, scientists recognize that human microbiome so called human second genome is a source of genetic diversity, especially a modifier of diseases and an essential component of immunity (e.g. it is estimated that twins produce different amount of antibodies after flu vaccination). Researchers also suggest that the colonization of the microbiome founding microbes occurs largely at birth. Our aim of studying was to establish about susceptibility of twins to measles, chickenpox and influenza. For revealing the cases of twins first we visited ten schools of Tbilisi, among their pupils 5 % appeared as twins. Using the special questionnaire 30 pairs of monozygotic and 20 pairs of dizygotic twins and their parents were interviewed. The provided investigations showed us that there is 60% more of chance for the second born to be effected by infectious diseases, e.g. after catching the common influenza virus always second born individuals were frequently infected, usually with pneumonia like symptoms and needed more time to recover, generally the second born had less developed immunity than the first born. This data allows us to propose that at natural birth of monozygotic and dizygotic twins the first born is exposed to more microorganisms than the second born and the unequal distribution of microorganisms during the birth results in different resistance to different diseases. As Caesarian birth prevents the vital bacteria from being passed on newborn, in case of twins, second born always will much more sensitive to infectious diseases. If it is not contraindicated better to choose the natural mode of delivery for twins.

The experience of enzyme replacement therapy in children with mucopolysaccharidosis in Kazakhstan Republic

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Introduction: Mucopolysaccharidosis (MPS) - group of hereditary lysosomal storage diseases caused by impaired glycosaminoglycan metabolism due to lack of lysosomal enzymes participating in their degradation. According to the nowadays classification, there are 7 different types of MPS. Clinical manifestations of MPS are variable and characterized by multiple organs dysfunction. The point of this message is to share with experience of enzyme replacement therapy (ERT) in children with MPS in Kazakhstan Republic.

Material and methods: 23 patients: MPS I - 7, MPS II - 11, MPS VI - 5. The average age on time of examination in years: MPS I - 7, MPS II - 8, MPS VI - 8. All patients underwent following methods of examination: anthropometry, echocardiogram (ECHO), abdominal ultrasound investigation (USI), spirometry, 6-minute walk test (6MTW).

Results: The average age on ERT start in years: MPS I - 7, MPS II - 8, MPS VI - 8. The average duration of ERT in years: MPS I - 2, MPS II - 2,6; MPS VI - 3,4. All examined patients have multisystemic impairment. In 100% patients showed significant improvement in growth on treatment. According to abdominal USI in 100% ERT had positive impact in decreasing of hepatosplenomegaly. The distance of 6MWT as a clinical indicator of the functional capacity of

cardiorespiratory function increased in average in all MPS types in 22.5%. ECHO revealed absence of myocardium hypertrophy progression in 100%. According to spirometry, all MPS patients had respiratory impairment as obstructive, restrictive or mixed dysfunction. ERT had positive effect on respiratory function only in MPS II patients, all parameters increased in average in 16,8%.

Conclusions:

1. In 100% patients with MPS have multisystem impairment.
2. ERT in children with MPS I, II and VI types: positive impact in physical development; increased tolerance to physical exercise; hepatosplenomegaly decreased; the pathological process in the myocardium stabilized.

Targeted high-throughput sequencing for somatic mutations identification in thyroid cancer diagnostics

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Introduction: There are problems of low sensitivity of preoperative differential diagnosis of thyroid neoplasms and lack of methods for predicting progression and choosing the optimal treatment strategy. Identification of driver and secondary mutations with targeted high-throughput sequencing is a promising approach in diagnostics and management of thyroid cancer.

Methods: Point mutations, fusions and CNVs associated with sporadic thyroid cancer were selected from COSMIC and TCGA project data. Additionally, exons of *RET* gene harboring germline mutations were included. Design of primers for targeted amplification of regions with pathogenic point mutations was done with AmpliSeq Designer v. 5.4.1, the length of amplicons was set in the range of 125-375 b.p. The technical validation was performed on cell lines SW620, MCF7. Samples of thyroid carcinomas (15 samples), benign nodules (3 samples) and normal thyroid tissue (11 samples) were analyzed. Preliminary assessment of analytical sensitivity was done via introduction of DNA with known mutations into DNA of normal tissue, which did not harbor pathogenic mutations.

Results. The design included about 450 point mutations in 25 genes, 3 regions of CNVs and 25 types of fusions. Mutations expected in cell lines (according COSMIC CLP and Cancer Cell Line Encyclopedia (CCLE)) were detected: *APC* p.Q1338* and *KRAS* p.G12V – in SW620; *PIK3CA* p.E545K – in MCF7. Analytic sensitivity was 3% of alleles in the sample.

In carcinomas following mutations were detected: *BRAF* p.V600E (5 samples); *KRAS* p.G12V, *IDH1* p.V178I; fusions *ETV6-NTRK3*, *PAX8-PPARG*, *NCOA4-RET*; CNVs 22q-del и 9q-del. No mutations were detected in benign nodules and normal tissue.

Conclusion. Tests limited to *BRAF*, *K/N/HRAS*, *RET/PTC1,3*, *PAX-PPARG* mutations have low diagnostic value. The developed panel allows analyzing an expanded range of mutations, including recently described rearrangements and CNV.

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Poster Presentations

Combination of two genetic disturbances: G6PD enzyme deficiency and Duchenne muscular dystrophy in one patient

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The Duchenne muscular dystrophy (Becker's muscular dystrophy) was first described by English anatomy-surgeon Charles Bella in 1830. The disease occurs in approximately one of 4,000 newborn boys. Dystrophin gene is the largest of the known genes and consists of at least 4 promoters consisting of 2.6 million nucleotides, 79 exons, and 78 introns. The length of the dystrophin protein, which is a cytoskeletal protein, is 147 kDa.

Enumerous world population studies on glucose-6 phosphate dehydrogenase enzyme (G6PD) show its high frequencies. Up to now more than 400 abnormal variants of G6PD enzyme are identified, and around 100 of them differ as endemics.

Both diseases inheritance types belong to X-linked chromosome recessive.

Patient A.N. and his family members' venous blood was sampled. All of them live in Balakan administrative area of Azerbaijan.

Activity of G6PD was identified with fluorescence method. Diagnostics of Duchenne muscular dystrophy was based on creatine kinase enzyme activity analysis.

A.N. patient with G6PD enzyme hemizygote inheritance type has got enzyme activity in blood as 12.3% of norm, his mother with heterozygous carriage and his sister have got activities values as 52.1% and 48.9% of norm.

Creatine kinase activities were in A.N. proband >2000 U/L (N38-137 U/L), his mother – 879 U/L (26-140 U/L), his sister - 896 U/L (26-140 U/L).

Out of 28 family members as a result of clinic-genealogy analysis: 2 persons revealed Duchenne muscular dystrophy, 6 women were heterozygous carriers of Duchenne muscular dystrophy and 5 women manifested G6PD enzyme deficiency (1 hemizygote and 4 heterozygotes).

Taking into account reproductive age of patient's parents, the ways of prophylaxis as prenatal diagnostics of Duchenne muscular dystrophy disease for the certain family are being discussed.

Erythrocyte antigens expression among patients with Blood Tumor and Anemia

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Introduction. Several studies have reported a correlation between the distribution of ABO blood group antigens and a risk of developing specific types of cancer, although no consensus has been reached. Several studies have reported a correlation between the distribution of ABO blood group antigens and a risk of developing specific types of cancer, although no consensus has been reached.

One plausible hypothesis encompasses a dysregulation of the enzymatic activity of the ABO glycosyltransferases, which are specifically involved in the processes of intercellular adhesion and cellular membrane signaling as well as in the immune response to the host. The alteration of these surface molecules may promote the process of malignancy.

Aim of research. Study of Immunogenetic features of patient with Blood tumor and Anemia; Detecting of correlation between Blood Tumor and blood group antigens; ABO Genetic and Epigenetic Features.

Materials and Methods. The cohort study consisted of 115 blood samples Among them: Blood Tumor - 42 Patients (39 before and after chemotherapy), Anemia - 30 Patients before and after transfusion. The patients were diagnosed at Republican Clinical Hospital, Hematology Department and Hospital 'MEDCENTER' - Diagnostic Laboratory between 2016-2018. The Research took place in Batumi Shota Rustaveli State University (BSU) and Bau International University Batumi (BAU). The research methods

were immunoserological withThe research methods were taken immunoserological methods with anti- A, -B -AB, -D, -C, -c, -E, -e, -K, -k, -M, -N monoclonal antibodies as forward as reverse methods.

Results. The study has resulted in revealing of the two cases of change of ABO system antigens (a patient diseased with chronic myeloid leukemia and a patient with anemia). We think that the reason of the mentioned changes is a change of enzymatic activity of glycosyltransferase of ABO system in malignant circumstances or suppression of the respective gene expression under the influence of chemotherapy. Based our studies, blood cancers correlate with phenotypic groups 0 (I), MN, K+.

Conclusion. ABO antigens expression decrease after chemotherapy.

MOLECULAR CHARACTERISTICS OF GBA GENE IN PATIENT WITH GOCHER DISEASE TYPE 2 OF AZERBAIJAN REPUBLIC

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Gocher disease is the most often encountered form of inherited enzyme pathologies which are compiled into lysosome storage diseases group. The base of the disease consists of inherited activity deficiency of acidic β -glucosidase (glucocerebrosidase – GCB) – lysosomal enzyme participating in degradation of cell metabolism products.

Gocher disease has frequency of 1:40 000 to 1:60 000 in all ethnic groups; in Ashkenazi Jews it reaches 1:450 – 1:1000. Disease is inherited as autosome recessive. Gene, that encodes GCB, is located in q21 locus of chromosome 1. Presence of 2 mutant alleles of GCB gene (homozygous inheritance) is associated with reduction (or absence) of GCB catalytic activity leading to storage of non-utilized lipids macrophages and specific cells – macrophages overloaded with lipids – formation.

Patient N.F., 9- month-old, after analytic examination and during treatment manifested hepato- and splenomegaly, anemia and thrombocytopenia. Biopsy taken from the bone marrow identified specific to Gocher disease cells.

Blood of patient was taken on DBS cards. Diagnostics for Gocher disease has been done in ARCHIMED Life GmbH laboratory, Austria.

Analytical method was tandem mass spectrophotometrics. Result of analysis has shown that β -glucocerebrosidase enzyme activity was 1.1 $\mu\text{mol/l/h}$ lower than norm (N >2.5 $\mu\text{mol/l/h}$). And acidic sphingomyeliase was normal - 25.5 $\mu\text{mol/l/h}$ olmuşdur (N >0.9 $\mu\text{mol/l/h}$).

Molecular genetic diagnostics of GBA gene identified substitution of Thymin nucleotide with Cytosine nucleotide in 1448 position. Mutation was found as homozygote (1448 T-C/1448 T-C). Leucine was substituted with Proline aminoacid in the position 483 as a result of mutation. Parents of the patient were children of two sisters – second consanguinal marriage. As to prophylaxis of the disease in the family where parents are of reproductive age, prenatal diagnostics of fetus in prospective pregnancy is planned.

Genomic Literacy, Education, and Engagement Perspectives at the Department of Molecular & Medical Genetics at Tbilisi State Medical University

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Introduction

Genomics is revolutionising medicine. The ability to sequence the genetic code of a large sample of the population is revealing how small variations in our DNA can change our vulnerability to different diseases – and thus how medicine can be personalised for better prevention, diagnosis and treatment. Genetics is an essential subject to be mastered by health professional students of all types. A medical genetics education focused on developing scientific critical thinking skills in health care professionals has the potential not only to increase the number of specialists available to the medical community for genetics-related fields but also to increase the overall proficiency level of all medical professionals in utilizing the vast informational resources available in today's rapidly evolving medical landscape. The rapid pace of discoveries in genomics requires that genomic medicine education initiatives are designed to incorporate continuously updated, novel information. We conducted a review of literature in genomic medicine education and training at the Department of Molecular & Medical Genetics at Tbilisi State Medical University – one of the oldest higher education institutions in the whole Caucasus region, in order to gain a picture in genomic medicine education.

Methods

We extensively reviewed the published literature and unpublished data in genomic medicine education and training at the Department of Molecular & Medical Genetics at Tbilisi State Medical University between January 1994 and May 2019 in order to identify trends in genomic medicine education.

Results

We found evidence of progress in the development of new and innovative educational programs and other resources aimed at increasing physician knowledge and readiness at Tbilisi State Medical University. The main achievements identified: Department is the only Molecular & Medical Genetics department in Georgia and in Post-Soviet states generally, implementation of ASGH/APHMG Medical School Core Curriculum (1995, 2001 and 2013), intense translational and editing work, including translation of *Thompson & Thompson Genetics in Medicine* (2008 and 2016), which transformed georgian biomedical field and significantly improved the knowledge of genetics among the new generation of georgian health professional students and practising physicians, founding of Students' Scientific Society "Genetics in Medicine" (1994-2000) and Scientific Debate Club "FBG – Future Belongs to Genetics" (2001 –), organizing more than 25 students' scientific conferences and open debates, holding National DNA Day Essay

Contest for Biomedical Students from 2015, providing national fellowships for students interested in genetics to attend ESHG conferences, founding of Georgian Society of Medical Genetics and Epigenetics in 2014, which is a member of European Federation of Human Genetics Societies (EFHGS) from 2016, organizing accredited conferences – 1st and 2nd accredited conference “Genomics and Epigenomics in Personalized Medicine” in 2016 and 2017, organizing two international conferences in Georgia – Human Genome and Health, in 2018 and 2019, Continued Medical Education (“Medical Genetics” for MDs, “Genomic Medicine and Tools of Molecular Genetics” for MDs, “Medical Genetics” for nurses). Department’s position on medical education shows good pedagogical standards, namely implementation of problem-based learning, support for developing integrated medical education curriculum and use of integrated study cases to develop critical thinking and applied empirical skills among undergraduate medical students.

Conclusion

we examine the general trends of genetics education at both the preclinical and clinical levels and the ways in which medical and pedagogical research have guided reforms to current and emerging teaching practices in genetics at the Department of Molecular & Medical Genetics at Tbilisi State Medical University. These trends will produce future health professionals with the skills and confidence necessary to embrace the new tools of medical practice that have emerged from scientific advances in genetics, genomics, and bioinformatics.

Study of variability of P53, PRB genes and ribosomal cistrons activity during colon cancer

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The colon cancer (CC) is belongs to genetically predicated multifactor diseases. Mutation of the p53 and pRB genes are one of the commonest genetic changes in the development of human colorectal cancer. Established connection between pRb and p53 genes.

It is shown that the system of the ribosomal cistrons involved in the neoplastic vulnerability of tumors. This facts shows the importance of studying the variability of the genes pRb and p53 products in carcinogenesis and the effectiveness of ribosomal in the cells of patients with CC.

The study material was the cells of stimulated peripheral blood lymphocytes from colon cancer patients and healthy donors. P53 and PRB gene products have been evaluated by the ELISA method. The activity of ribosomal genes was studied on chromosome prepare derived from peripheral blood lymphocyte cultures. The method of silver impregnation to reveal active nuclear organizers. It was found that the contents of p53 and pRB gene products in patients with CC vary in blood serum of according to individuals, and therefore the role of mutations of these genes can be varied in case of specific tumors.

As a result of the analysis show: the patients with CC is characterized general instability of genome. In addition, as the chromosomal fragility test indicates, changed the distribution of damaged chromosomes by groups, what should be a specific feature for a tumor of this type. Against the background of general high instability, the genome of patients with CC is characterized by the presence of specific areas of the greatest vulnerability (damageability). The cells of patients CC are characterized by a high level of intensity of synthetic processes, which is provided by additional activation of ribosomal cistrons.

Phenotypic manifestation of the coinheritance of α and β globin gene mutations

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Introduction. Human α (*HBA1*, *HBA2*) and β (*HBB*) globin genes encode for globin proteins which are key parts of the hemoglobin molecule. Mutations in these genes can cause a decrease in the production of the corresponding globin protein leading to thalassemia, or to the synthesis of an anomalous chain leading to the production of variant hemoglobins. Patients with these disorders have severe clinical presentation and they depend on lifetime blood transfusions. 4% of the population are carriers of β -thalassemia and 10% are of α -thalassemia in Azerbaijan. This indicates the high possibility of the coinheritance of the mutations of these two genes. Therefore, our aim was to study the phenotypic manifestations of the cases with a coinheritance of *HBB* and *HBA* genes.

Materials and Methods. We included the patients among those referred to the Hemoglobinopathy Laboratory of the Institute of Hematology and Blood Transfusion. Hematological indices and hemoglobin fractions were evaluated by Sysmex2000i analyzer, and BioRad Variant II analyzer, respectively. *HBB*, *HBA1* and *HBA2* gene mutations were determined by polymerase chain reaction followed by reverse dot-blot hybridization method (β -Globin Strip Assay AZE1 & AZE2; α -Globin Strip Assay. ViennaLab GMBH).

Results. Coinheritance of α -globin gene mutations increased the total hemoglobin level in a β -thalassemia patient and decreased the HbS level in carriers of sickle cell disease, increasing microcytosis in all patients.

Patients with the coinheritance of *HBB* and *HBA* gene mutations (* - control patients were given for comparison; ND - not detected, mutations were found in heterozygous form, unless otherwise noted).

№	HBB gene mutation	HBA1 and HBA2 gene mutation	Hematological indices				Hb fractions		
			Hb	RB C	MC V	MCH	HbA ₂	HbF	HbS
1	Codon 8 [AA] hom	[- 20.5 kb DEL	8.7	4.31	58.0	20.2	4.2	72.4	---
2	Codon 8 [AA] hom*	[- ND	5.8	2.74	65.3	21.2	1.0	62.3	---
3	Codon [A>T]	6 20.5 kb DEL	10.1	6.51	50.2	15.5	3.2	0.27	18.4
4	Codon [A>T]	6 20.5 kb het.	10.9	5.19	67.1	21.0	3.4	1.9	25.0
5	Codon [A>T]	6 MED DEL	10.6	6.40	52.2	16.6	3.0	1.7	21.3
6	Codon [A>T]	6 α 2 Poly A-1 [AATAAA>AATA AG]	10.3	5.94	54.5	17.3	3.4	0.32	19.2
7	Codon [A>T]*	6 ND	12.0	4.97	74.7 4	24.21	2.89	0.84	32.5 4

Conclusion. We observed an alleviating effect of α -thalassemic mutations, on the phenotypic manifestation of β -thalassemia and sickle cell disease.

Public Knowledge of Genetics in Georgian Population

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In March-April 2019 we conducted a cross-sectional study with the aim of evaluating the knowledge of medical genetics in the lay Georgian population. Every person was eligible to participate. Topics included gene biology, genetic diseases, and genetic testing.

Data was collected via scripted and confidential in person interviews. Interview questions were closed ended or multiple choice and were divided into five topics: 1) general knowledge about genes and disease, 2) disease related concepts, 3) facts related to cancer development, 4) perceived genetic knowledge of participants, and 5) DNA testing.

Interviews were conducted with 415 participants, diverse in age, religious affiliation, education level, and profession. Most participants were 18-45 yrs. (71,6%), followed by 46-65 yrs. (22,1%), and >65 yrs. (6,3%). Most participants held an academic degree from a university (75,9%), while those remaining had only obtained a professional bachelor's diploma (12%) or only completed secondary education (12%). The majority identified as Orthodox Christian (86,8%), while small minorities identified as non-religious (9%), Muslim (2,4%), or other (1,5%). Of these, most considered their religious involvement somewhat active (45%) or passive (44,6%), with only a minority being active (9,9%).

Topic 1- General knowledge about genes and diseases: There were 12 questions on this topic. The question which the most participants answered correctly (84,2) was, “is gene a disease?” The question which the most participants answered incorrectly (45,4%) was, “the genotype is not susceptible to human intervention.” In addition, most participants (66.7%) did not know that a person is estimated to have 22000 genes.

Topic 2 – Disease-related concepts: There were 5 questions on this topic. The question which the most participants (91.3%) answered correctly was, “the onset of certain diseases is due to genes, environment and lifestyle”. 18.9% did not know whether the following statement was valid or not: the child of a disease gene carrier is always also a carrier of the same disease gene.

Topic 3 - Facts related to cancer development: There were 6 questions on this topic. The majority (54.1%) of participants correctly knew that this statement is not true: genetic testing is only used to determinate whether you have cancer right now.

34.5% of participants were incorrectly informed that only women can have an altered breast cancer gene. 42% didn't know that a father can give an altered breast cancer gene to his daughters.

Topic 4- Perceived Genetic Knowledge of Participants: There were 5 questions on this topic. 34% of participants were well informed about the possibility of early detection of certain disorders using DNA-testing. 39.5% of participants had the least information about the possibility to use genetic knowledge to prevent or treat a disorder.

48.4% of participants didn't know the possibilities and risks of gene therapy.

Topic 5 – Genetic Testing: There were 6 questions on this topic. 48.3% of participants thought they had adequate knowledge of their rights to refuse DNA testing (and this was highest result of „well know”) 29.7% of participants were less informed about the consequences of DNA testing for their daily life.

The majority of participants (57.7%) indicated that they didn't have any information about the consequences of DNA testing for their work.

The results of this research serve to improve genetic knowledge depending on performing educational activities. This therefore will improve healthcare level.

Glutathione S-transferase M1 and T1 genes polymorphism association with antituberculosis drug-induced hepatotoxicity in Georgian population

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The glutathione S-transferase (GST) enzyme system constitutes a family of multifunctional enzymes which play an important role in biotransformation and detoxification of many different xenobiotic. Human cytosolic GSTs are polymorphic, and have ethnic-dependent polymorphism frequencies, and have been associated with several types of diseases. In the double-null variant of GSTM1 and GSTT1(GSTT1(-) and GSTM1(-)) are respectively associated with a higher risk of different forms of liver injury, cancer, cardiovascular diseases etc.

Comparisons between GST null genotype frequencies in the worldwide populations show different patterns in Asian, African, and European populations.

Some detailed studies of GST variants in various geographic regions can increase the knowledge about relationship between the ethnicity and the prevalence of certain diseases. Thus, it becomes necessary to consider the genotypic differences for reducing the risk of anti-tuberculosis drug-induced Liver injury.

The aim of our research was to determine the polymorphism of GSTM1 and GSTT1 genes in Georgian population – in healthy individuals and in patients with lung tuberculosis.

In the studies was used the peripheral blood samples obtained from individuals randomly selected groups and from patients with lung tuberculosis. The GSTM1 and GSTT1 genotypes was investigated using an Ese-Quant tube scanner-by SmartAmp Method.

The study of GST genes polymorphism in Georgian population revealed that only 1% of healthy individuals have double null genotype by GST genes (GSTT1(-) and GSTM1(-)).

As for patients with lung tuberculosis, there was revealed that GSTT1(-) GSTM1(-) genotype was in 14% of investigated patients. All patient with double null genotype has violation of liver functional indicators.

The results will play the most important role in personalized medicine, in the appointment and management of drugs and for prevention of adverse drug reactions in patients of Georgian population.

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Distribution of Inflammatory Cytokine Gene Polymorphisms with Inflammatory Bowel Disease in East Black Sea Region

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Key words: Inflammatory Bowel Disease, Cytokines, Genetic Polymorphisms

Aim: To investigate IL-1 α , IL-1 β , IL-1R, IL-4RA, TGF- β , TNF- α and IFN- γ gene polymorphisms in Turkish patients having Inflammatory Bowel Disease (IBD).

Methods: An analysis was carried out at Karadeniz Technical University, Medicine Faculty, Gastroenterology clinics between March 2004 and May 2010 on 69 patients with IBD (cases) and 100 healthy individuals (controls). PCR-SSP and cytokine gene panel (Helderberg kits) based techniques for analysis of gene polymorphisms were used.

Results: Changes in allelic frequencies of each of the investigated polymorphisms in eight cytokine genes in patient with IBD were found. Among the allelic genes analysed, the highest statistically significant change was observed in some positions. The following increases were observed in IL4RA + 1902A and TNF- α -308 G. In other analysed genes, allelic changes were found to be decreasing for IL- 1 α -889T , IL- 1 β 3962T , IL4RA + 1902G , IFN- γ UTR5644A, TGF- β KO10 C/T and TNF- α -308 A/T. There is no change in the frequencies of other alleles ($p < 0.005$). In addition, there were no changes in genotypic frequencies investigated one polymorphic site and only one of cytokine gene IL-1R pst1 1970 T/C was not changed.

Conclusion: Gene polymorphism is not itself the only determining factor for clinical diagnoses. However, it can be used in the clinical diagnosis of IBD in order to determine the low level or high level variations in cytokine gene polymorphisms.

Investigation of genotypic distribution of ABO blood groups and Rh system in South Kazakhstan and Eastern Anatolia populations

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Keywords: Blood Groups (ABO), Rh Factor, Turks and Kazakhs Populations

In this study, we aimed to compare the Kazakh citizens living in South Kazakhstan with the Turkish citizens living in Eastern Anatolia in terms of ABO blood groups and Rh system. Information was obtained from individuals by using questionnaire and determining of blood group. Accordingly, phenotypic/genotypic distributions and allelic frequencies of blood groups were compared in terms of ABO blood system and Rh system in individuals living in Kazakhstan and in Anatolia. We aimed to emphasize the importance of blood transfusions and the blood disorders due to Rh incompatibility in these two populations. Our primary results which are important with respect to genetically and anthropologically as follows: a total of 232 samples were collected from those living in Eastern Anatolia. A blood group 42.4%, B blood group 28%, AB blood group 5.4%, O blood group 24.2% and Rh (+) ratio 85.6% and Rh (-) ratio was 14.4%. A total of 324 samples were collected from those living in Kazakhstan. Genotype distributions are as following: A blood group 38.5%, B blood group 22.2%, AB blood group 5.4%, O blood group 33.9%, and while Rh (+) was 93%, Rh (-) rate was 7%. To conclude, the prevalence of ABO blood groups were A > B > O > AB,

and Rh (+) distribution was higher than Rh (-) distribution in Eastern Anatolia Region. In the South Kazakhstan Region, the prevalence of ABO blood groups is A> O> B> AB, and again the Rh (+) distribution was higher than Rh (-)

The role of MTHFR gene mutation in epileptic pregnant women with RPL

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Context: Epilepsy is one of the most common neurological conditions in pregnancy. The most women with epilepsy are at increased risk for a range of perinatal complications and their offspring might have major congenital malformations and adverse neurodevelopmental outcomes. Antiepileptic drugs (AEDs) can cause neural tube defects and interfere with folate metabolism by inhibiting glutamate formyltransferase (McAuley JW et.al, 2002). Mounting evidence suggests that long-term administration of AEDs may affect the metabolism of serum folic acid and vitamin B12, further increasing the serum level of homocysteine and leading to cerebrovascular events. The abovementioned risk increases even more if pregnant women at the same time has MTHFR GM (C677T; A1298C), especially homozygous form. During this genetic thrombophilia, enzyme MTHFR deficiency occurs and the first reaction of folic acid metabolism – folic acid recovery to L-5-methyl-tetrahydrofolate (L-5-methyl-THF) – fails. Considering that to take recommended 4mlg folic acid might be ineffective for pregnant women with epilepsy, especially for those with MTHFR GM.

We reckon to use the active form of L-methyl folate instead of folic acid. L-5-methyl-THF is effective in decreasing **plasma homocysteine**, as well as increasing folate in plasma and erythrocytes, in pregnant and breastfeeding women or those who wish to conceive.

Objective: Detection MTHFR gene mutations (GM) in women with unexplained RPL and epilepsy.

Methods: In all women with RPL, thrombophilia gene mutations (GM) detected by PCR method.

Patients: In 2017, we have studied 191 women who had had 2 or more discontinued pregnancies. 144 out of them were with MTHFR GM, and 11 patients were diagnosed with epilepsy and had been treated with AEDs.

Results: Administrated with Metafolin in all 11 patients, we received successful outcome.

Conclusions: Women who take AEDs and plan pregnancy have to conduct of thrombophilia gene mutation tests (MTHFR GM -C677T; A1298C). It should be noted that usage of L- methyl-folate (Metafolin) has less risk because it is a supplement and not drug. The advantage of L-methyl-folate vs. to folic acid, in epileptic pregnant women with MTHFR-GM needs further research with neurologists because there is not enough evidence-based information regarding this issue.

Association between Different Pregnancy Complications, VTE during Pregnancy and Thrombophilia Gene Mutations

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Introduction: Normal pregnancy is associated with extensive changes in hemostasis, involving pro-coagulants (intrinsic and extrinsic pathways), the natural anticoagulation (antithrombin III, protein C, protein S) and fibrinolysis (tPA, PAI-1;PAI-2; TAFI). Prothrombotic state protects against severe hemorrhage during delivery, but in addition with inherited thrombophilia it may also predispose both the mother and the fetus to complications during the pregnancy. Thrombotic complications in pregnancy are the major cause of morbidity and mortality. Numerous studies have confirmed a connection between inherited thrombophilia and adverse pregnancy outcomes, such as recurrent miscarriages, stillbirth, intrauterine growth restriction, placental abruption and pre-eclampsia. Predominant inherited thrombophilias include FV Leiden, prothrombin G20210A, Methylenetetrahydrofolate reductase (MTHFR) C677T mutations, protein C, protein S, antithrombin III (AT) deficiencies.

Aim: The aim of the study was to evaluate the association between inherited thrombophilia (Factor V Leiden, Prothrombin G20210A and MTHFR C677T gene mutations), different pregnancy complications (miscarriages, stillbirth, preterm preeclampsia, intrauterine growth restriction (IUGR), placental abruption) and venous thromboembolism in women during pregnancy and postpartum period (Deep and superficial venous thrombosis/pulmonary embolism).

Materials and Methods: 545 Georgian women with different pregnancy complications, 40 Georgian pregnant women with VTE and 100 controls (women with three or more uncomplicated pregnancies) were investigated for detection of FV Leiden, prothrombin G20210A, Methylenetetrahydrofolate reductase (MTHFR) C677T mutations. In all cases common causes of pregnancy complications (anatomic, endocrine, chromosomal alterations, APS, etc.) have been excluded. We investigated the distribution of mutations as in general during pregnancy complications, as well as separately for individual complication.

Results: Relationships between both pregnancy complications, VTE (during pregnancy and postpartum) and FVL and MTHFR mutations were significant, but between Prothrombin mutation was weak. P values for FVL were 0.003 (6.3%) and 0.005 (22.5%) respectively, for MTHFR homozygote mutation – 0.005 (7.7%) and 0.0173 (12.5%). Statistically significant relationships were seen between placental abruption, stillbirth and FVL mutation (20.5%, $p=0.000$; 8.8%, $p=0.001$) and placental abruption, miscarriages and MTHFR homozygote mutations (13.6%, $p=0.003$; 7.6%, $p=0.091$).

Conclusion: Our study shows that women with inherited thrombophilia are at increased risk of developing both pregnancy complications and VTE during pregnancy and postpartum period. Anticoagulants are considered a possible therapy for women with pregnancy complications, VTE and inherited thrombophilia. Prescription of anticoagulants to pregnant women produces specific risk at the time of delivery, where bleeding and clotting risks interface. Altered metabolism rates of anticoagulants in pregnant women often requires closer monitoring than is required outside of pregnancy in order to ensure efficacy and safety. Low-molecular-weight heparins (LMWHs) and unfractionated heparin are the mainstay of treatment of venous thromboembolism (VTE) in pregnancy, as they don't cross placenta. Maternal and fetal concerns must be considered at all times, with a careful assessment of the risks and benefits of anticoagulant therapy in each patient.

NEWBORN CYSTIC FIBROSIS SCREENING: DIAGNOSIS IN ABSENCE OF SYMPTOMS

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Introduction: Cystic fibrosis (CF) is a life-shortening recessive genetic disease which can be diagnosed by newborn screening (NBS) CF is caused by mutations in a single gene on chromosome 7 that incodes the CFTR protein. Clinical disease requires disease causing mutations in both copies of CFTR gene.

NBS in Georgia have been in existence from 2013 year. There have been no prospective studies assessing infants with positive results. Consequently, little is known about phenotype, genotype and clinical outcomes of these infants.

Aim and objectives: Retrospective observational study of children revealed by NBS for CF.

Materials and methods: All NBS-positive infants underwent sweat chloride testing and subjects with positive or intermediate results - repeated sweat chloride testing and molecular analysis. Infants were defined as CF if there was sweat chloride ≥ 60 mmol/l and/or CF-causing mutations on both alleles. Infants were defined as CFTR-related metabolic syndrome if there was: a) CF-causing mutation on 1 allele and intermediate sweat chloride (30-59mmol/l), b) CFTR mutation on both alleles but only 1 is known to be CF-causing and c) no detect mutations, but a very high IRT concentration and an intermediate sweat test. Subjects were monitored every month during the first year of life and every 3 months thereafter (anthropometry, specific respiratory and gastrointestinal symptoms, bacterial culture, serum Na, K, Ca, fecal testing).

Results: 23 infants were enrolled. Positive sweat chloride test has 17 and intermediate 6 infants. CFTR mutations on both alleles was revealed in 22 cases, no mutations in 1 case. 3 has CFTR-related metabolic syndrome (1 case with negative DNA results, but very high IRT concentration and 2 cases with intermediate sweat test and heterozygotes for 1 disease-causing mutation and 1 currently unknown consequence. The majority of these children are well. The weight and height of 5 subjects with CF were significantly lower by the time of first assessment, but normalized after prolonged enzyme replacement therapy Only 2 patient has history of recurrent respiratory infections, 4 patients are underweight

Conclusion. NBS can improve early detection of CF and access to specialized medical care. Monitoring of NMS-positive infants lead to maintain a growth pattern, earlier intervention and improved outcomes. Monitoring of children with CF-related metabolic syndrome can give additional diagnostic information and help clarify the diagnosis over time.

Frequency of Methylenetetrahydrofolate reductase (*MTHFR*) gene C677T polymorphism in Georgian female population with Subclinical Hypothyroidism

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Introduction: *MTHFR* gene C677T polymorphism, which is related with elevated levels of plasma homocysteine, is known as a predisposition factor for many diseases. Subclinical hypothyroidism (SCH) is defined as a state of increased thyroid stimulating hormone levels. Since there are consistent reports demonstrating that homocysteine is high in hypothyroid patients, we hypothesized that *MTHFR* C677T polymorphism may contribute to the development of SCH.

Materials and methods: 62 Georgian female patients with SCH (mean age 45 ± 25.5) and 42 Georgian healthy female volunteers (mean age 48 ± 25.1) participated in this study. Genomic DNA was extracted using quick-DNA universal kit. *MTHFR* gene was genotyped by PCR-RFLP method using *HinfI* enzyme.

Results: Genotype frequencies for the patients and controls, respectively, were: 27 (43.55%) and 29 (69.05%) for CC genotype, 21 (33.87%) and 11 (26.19%) for CT, 14 (22.58%) and 2 (4.76%) for TT. There is an increase in CC genotype distribution in the control group when compared to the SCH patient group OR (CI) 0.35 (0.15 to 0.79) $P = 0.0116$, the CT was not increased significantly OR (CI) 2.05 (0.84 to 5.03) $P = 0.1172$ and there was increase in TT genotype distribution in SCH group versus control group OR (CI) 7.52 (1.56 to 36.2) $P = 0.0119$. However the C allele is significantly prevalent in the control group compared to the SCH group OR(CI) 0.33 (0.17 to 0.65) $P = 0.0012$, while T allele is prevalent in patients compared to the control group with a statically significant difference OR(CI) 3.01 (1.55 to 5.84) $P = 0.0012$.

Conclusions: This study indicates that the *MTHFR* 677 TT genotype could correlate with development of Subclinical Hypothyroidism. We suggest that molecular studies of *MTHFR* gene could be valuable for refining the clinical diagnosis of SCH leading to more precise management of this condition.

PREVALENCE OF HIGH ONCOGENIC RISK HPV GENOTYPES 16, 31, AND 33 IN GEORGIAN WOMEN WITH CERVICAL INTRAEPITHELIAL NEOPLASIA GRADE 1

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Although the immune system clears most of the human papillomavirus (HPV) infections, in a few cases these infections persist. About 1 in every 200 women will develop cervical cancer during their lifetime, and almost all of them are caused by infections with high oncogenic risk HPV. There are at least 14 high-risk HPV genotypes and diagnosing their infections may provide increasingly significant health benefits. Unfortunately, very sparse data are available on the prevalence and distribution of high-risk HPV genotypes among Georgian women.

The present study aimed to detect and genotype high-risk HPV viruses in cervical swab specimens of Georgian women. In total, 95 women (ages 24 to 67) were tested and genotyped. All patients had cervical intraepithelial neoplasia grade 1 (CIN1) by Pap cytology or inconclusive colposcopy. DNA was extracted from the specimens and genotyping were carried out by RealLine™ HPV kit that detects 12 high-risk HPV genotypes 16, 18, 21, 33, 35, 39, 45, 51, 52, 56, 58, and 59.

In this cohort of patients, we detected all tested genotypes, except HPV52. We found that 23% (18/95) of women tested positive for high-risk HPV with 33% (6/18) of all positives presented as multiple infections. Most prevalent genotype was HPV16 that was identified in 39% of positives (7/18) and 86% (6/7) of multiple infections. Other frequent infections were with HPV31 at 21%,

HPV33 at 13% and HPV56 at 8% of all positive samples. Hence, we conclude that genotypes 16, 31 and 33 are the most frequent high-risk HPV in Georgian women.

DNA methylation of proximal CpG motif of interleukin-10 promoter in Cystic Fibrosis

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Introduction: Cystic Fibrosis (CF) is an autosomal recessive disorder characterised by a chronic infection and neutrophil-mediated airway inflammation. Epigenetic mechanisms such as DNA methylation may play a role in the development of chronic inflammation in CF. Interleukin-10 (IL10), a cytokine crucial for suppressing inflammation and regulating immune responses. In the present work we analyzed DNA methylation status in the promoter region of the IL-10 gene.

Materials and Methods: Genomic DNA from activated CD4+ T-cells of 8 CF patients and 7 controls were purified and subjected to bisulfite modification using EpiTect Fast Bisulfite Kit (Qiagen, USA). Methylation status of the IL-10 gene was determined by methylation-specific polymerase chain reaction (MSP) analysis. PCR products were electrophoresed on 3% agarose gel and visualized.

Results: We observed that in CF individuals the level of complete methylation was 62%, the level of unmethylation was 18%. In the control group the level of complete methylation was 22%, the level of unmethylation was 58%.

Conclusions: This study suggests that DNA methylation plays an important role in the pathogenesis of CF and promoter status of IL-10 gene may help clinicians initiate the correct treatment strategy in Cystic Fibrosis.

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Problems of Prevention of Genetic Stomach Cancer

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Genetic gastric cancer develops when the genome is damaged. Cancer-associated oncogenes, regulatory genes, and proteins are involved in the process to maintain uncontrolled cell growth. Consequences of these changes this damage and abnormal expression of mucins, enzymes and hormones by tumor cells. Most cases of genetic stomach cancer develop in stages. In our study, the question of cytogenetic research was not. And thus identifying the sequence of chromosomal abnormalities was not possible. It has been suggested that many changes are nonspecific or secondary to malignant changes. Stomach cancer occurs sporadically with the participation of external risk factors such as Helicobacter pylori infection, dietary habits and lifestyle. However,

approximately 10% of patients have a burdened oncological family history. Often, genetic predisposition leads to the development of ring-cellular cancer and poorly differentiated adenocarcinoma. We, as doctors of the department of research and treatment of gastric cancer, consider endoscopic control. The most effective method for the prevention of genetic stomach cancer. Preventive gastrectomy is only an opportunity to prevent the development and / or spread of hereditary gastric cancer. However, not every mutation carrier is willing to undergo this kind of prophylactic disabling operation. This applies mainly to young patients who do not understand the problems and are worried about the effect of such treatment on physical and psychological health. Endoscopic observation is especially important in case of refusal from prophylactic gastrectomy. However, endoscopic control may be imperfect, as the tumor may progress under normal gastric mucosa. Even late stages of cancer with the involvement of the tissues surrounding the stomach may not cause visible changes in the surface epithelium. For oncotherapists, gastroenterologists, interaction with geneticists is very important. We are happy to start our collaboration within Human Genome and Health - 2nd International Conference.

BRAF AND RAS MUTATIONAL STATUS IN THYROID NODULES WITH INDETERMINATE CYTOLOGY AND PAPILLARY THYROID CANCER IN GEORGIA

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Malignancy risk evaluation and precise determination of the nature of thyroid lesions requires a multidisciplinary approach that includes molecular testing. BRAF and NRAS mutations are frequent genetic alterations found in thyroid nodules. These molecular markers establish a differential diagnosis and facilitate clinical decision-making. Prevalence of thyroid nodule associated mutations has not been studied in Georgia. Hence, we evaluated BRAF, NRAS and HRAS mutations in Georgian patients with indeterminate cytology or diagnosed with papillary thyroid cancer (PTC).

BRAF (V600E), NRAS (G12C, G12D, Q61R, Q61K) and HRAS (G12C, G13R, Q61R) were determined in the DNA extracted from fine needle aspirate specimens. In total, 116 patient samples were analyzed using Competitive Allele-specific TaqMan PCR (CastPCRTM). In these samples, 36 were diagnosed as papillary thyroid carcinoma, and 80 were indeterminate by Bethesda System for Reporting Thyroid Cytopathology (BSRTC III-V).

BRAF (V600E) mutation was the most frequent genetic alteration found in 31% of all analyzed samples. Specifically, this mutation was present in 61% of PTC cases and 18% of cases classified as indeterminate (BSRTC III-V). NRAS mutations were present in 16% of PTC and 30% of indeterminate cytology samples. NRAS G12D and Q61R were most prevalent at 22% and 25% of all NRAS mutations. BSRTC IV category of indeterminate cytology had the highest frequency of NRAS mutations at 43%. From analyzed samples, HRAS (Q61R) mutation was present in one PTC case. Finally, 29 out of 36 PTC cases were positive for the mutations indicating 81% of diagnostic sensitivity of the test.

Pathogenic Variants in Mitochondrial dna Mutations: MELAS (A Clinical Case Study)

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INTRODUCTION: MELAS (*mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes*) is a rare multisystem mitochondrial disorder that presents with variable clinical features including stroke like episodes, altered consciousness, vision and hearing loss, loss of motor skills and intellectual disability. Symptoms and physical findings associated with MELAS syndrome vary greatly between affected individuals as a result of several mutations in the mtDNA gene. The aim of this study is to show the impact of several pathogenic variants in mitochondrial DNA mutations with regards to their penetrance and phenotypic presentations.

MATERIAL & METHODS: Using the case report of a documented clinical case of MELAS in a 15 year old boy to investigate the mutations in mitochondrial DNA causing the known clinical features of MELAS, This study showed that mutations in MT-TQ, MT-TH, MT-TK, MT-TS1, MT-ND1, MT-ND5, MT-ND6, and MT-TS2 are all associated with MELAS syndrome but about 80% of MELAS are caused by mutations in m.3243A>G pathogenic variant *MT-TL1*, other important genes of interest are pathogenic variants m.3271T>C and m.3252A>G in *MT-TL1* and m.13513G>A in *MT-ND5*. A genetic analysis was done which identified a heteroplasmy mutation in MT-ND1 gene, m.3764 GP (Thr153).

RESULT: the genetic sequencing and analysis showed mutation in the MT-ND1 gene detecting a change in position 153 Thr (m.3764 GP) which is one of the pathogenic variants causing a wide spectrum of symptoms like bilateral progressive hearing loss, hypotrophy of muscles, weight loss, loss of appetite, neurologic presentations like ataxic gait, nystagmus, hypotony, eye manifestations of cataracts, bilateral eyelid clonic movements. Lab test showed increased level of lactic acid and EEG exams showed left sided periodic lateralized epileptic form of discharges. According to this findings, it is clinically suspected as MELAS.

CONCLUSION: there are several pathogenic variants that have been implicated in mutations of mtDNA, it could also be a result of spontaneous mutation or mutations in a nuclear gene and the findings in the genetic analysis and sequencing like the MT-ND1 gene could provide an important and valuable contribution to the diagnosis of mitochondrial genetic disorders like MELAS.

Atrial Fibrillation and KCNQ1 (new overview information)

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Medical genetics is a section of human genetics devoted to the study of the role of hereditary factors in human pathology. The main section of medical genetics is clinical genetics, which studies the etiology and pathogenesis of hereditary diseases. Atrial fibrillation is an heart rhythm characterized abnormal pathological rhythm. Some case with abornrmal phaze have no symptoms, another case occasionally there may be heart palpitations, fainting, lightheadedness, shortness of breath, or chest pain. High blood pressure and valvular heart disease are the most common alterable risk factors for

this disease. The disease is associated with an increased risk of heart failure, dementia, and stroke. It is a type of supraventricular tachycardia. Franco-Chinese international research has shown that there is a gene that causes a heart rhythm disorder. It was found that the cause of atrial fibrillation is a gene located on chromosome 11. That he may be responsible for the condition that is observed in patients with atrial fibrillation.

In order to identify the atrial fibrillation gene, researchers had to observe four generations in a family whose representatives suffered from this disease. It turned out that the gene is involved in the movement of potassium ions in the heart muscle. This is a very large and important study. Apparently, the KCNQ1 gene is not the only cause of cardiac arrhythmias, since some patients with atrial fibrillation do not have this mutation. Studies were conducted on a small number of people, but received positive feedback from the British Heart Association. We are going to connect to this study, which is ongoing now in real time.

The diverse genetic basis of neurodevelopmental disorders: experience from the SYNaPS Study

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Introduction: Neurodevelopmental disorders (NDDs) are defined as highly prevalent and debilitating paediatric neurological conditions caused by impairments in brain growth and development. Although causative mutations in more than one thousand genes have been associated with various NDDs, many patients remain without a defined genetic diagnosis. The SYNaPS Study, which is an IRB/ethics approved and aimed at analysing unexplained ultra-rare neurological conditions, aim to identify underlying genetic causes in patients with NDDs by high-throughput genetic investigations. **Objective:** To identify genetic causes of NDD in a large multi-ethnic cohort of patients by genetic investigations. **Methods:** As part of SYNaPS study a large cohort of well-phenotyped families affected by NDD were recruited from multiple paediatric neurology clinics around the world with a diverse ethnic background. Exome sequencing was performed for probands of around 1000 families with any forms of NDDs. Some of the unsolved individuals were subjected to a combination of SNP-Array genotyping/homozygosity mapping, whole genome sequencing and coupled with deep-phenotyping. **Results:** Overall, in this study we manage to resolve around 50% of the patients with NDDs. We also uncovered novel disease-causing genes in various families. Re-annotating/re-analysing the exome data along with more extensive data sharing and also employing homozygosity mapping in some of the families increased the rate of diagnosis. **Conclusion:** We made a molecular diagnosis for around half of the families and characterised multiple new genes and ultra-rare NDDs. Additionally, an accurate genetic diagnosis improved the management for some affected individuals with a genetic diagnosis, underlining the significance of early and specific diagnosis.

Down syndrome and epilepsy

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Introduction: Down syndrome the most common chromosomal disease (6) is associated with malformations, cognitive impairments and psychosocial difficulties (4). Mosaic or non-mosaic forms influences on Down syndrome development (5, 8) as well as its phenotypic variability's are under the control of genomic variability (1, 9). The Frequency of epilepsy in case of Down syndrome is 1-17% (2, 3) amongst them most frequent is West syndrome (10). West Syndrome in its own has complex genetic backgrounds (7). Our goal is to observe the peculiarities of epileptic seizures and Down syndrome comorbidity.

Material and Methods: Four histories with mosaic and non-mosaic Down syndrome with epilepsy were retrospectively investigated. Patient's age 3 month-20 years.

Results: Patients diagnosed with Down syndrome were classified according to the cytogenetic testing. One case was mosaic and others were non-mosaic forms. The seizure onset varied from 5 to 17 years old. In case of late onset seizures neurodevelopmental delays were milder in compare to West syndrome. The West syndrome was found in both mosaic and non-mosaic forms.

Conclusion: The Down syndrome association with epilepsy is complex. The age of seizure onset could be different. The severity and outcome of West syndrome is similar with mosaic and non-mosaic forms.

Management of Recurrent Thromboembolism in Patient with Inherited Thrombophilia: a case report

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Introduction: In the past decade, knowledge of thrombophilia has greatly increased with successful identifications of risk factors from everyday life, as well as genetic correlations, all leading to hypercoagulability states. Factor V Leiden and prothrombin gene mutation, as well as homozygous mutation in methylenetetrahydrofolate reductase (MTHFR) gene are known to be predisposing factors of thromboembolism. Inherited thrombophilia is one of the main risk factors of deep venous thrombosis (DVT), as well as other hypercoagulability complications, such as pulmonary thromboembolism, which can be developed after cessation of blood thinners. The aim of this case report presentation is to share our experience to the other healthcare professionals with similar potential challenge of personalizing care for patient with mutations in thrombophilia genes.

Materials Methods: A 63-year-old female with the previous medical history of venous thromboembolism (DVT) who was admitted to the emergency department with chief complaint of excruciating pain in both shoulders and chest. Chest CT scan was used for the detection of blood clots in pulmonary arteries. Genetic testing was also performed to detect potential mutations in Factor V Leiden, prothrombin, and MTHFR genes. Other laboratory tests include Anticardiolipin antibodies G and M, Anti-beta2-glycoprotein 1 G and M.

Results: Patient N.G. 63-year-old with family history of thrombophilia and previous medical history of deep venous thrombosis (DVT) in the left lower leg, followed by surgery. CT scan results: Pulmonary embolism; Patient's genotype: heterozygous variant of Factor V Leiden and MTHFR gene mutations; Other laboratory test results was negative.

Conclusion: Patients with this kind of genetic exposure who show the signs of early thromboembolism must be tested for the thrombophilia gene mutations. Early detection of inherited thrombophilia would prevent recurrent thrombosis and pulmonary embolism. Continuous monitoring and lifelong management of disease with Rivaroxaban can show positive results on patients' health and well-being.

Study of Genome characteristics in the case of lung cancer

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Cancer is one of the leading causes of morbidity and mortality in the world. According to World Health Organization data, the current level of death caused by cancer, which is about 8.2 million in the world, will increase by more than 13 million before 2030 (Cancer Statistics Center 2016).

The purpose of our work was: to determine the variation of the genomic parameters in individuals with lung cancer; to study the activity of ribosomal cistrones; to determine the level of mutations of the chromosomes (aberrations, aneuploidy, polyploidy, early chromatid segregation (ECS), fragile sites); to measure the frequency of acrocentric chromosomes and ribosomal cistrones.

The study material was the cells of stimulated peripheral blood lymphocytes from lung cancer patients and healthy donors.

The results show that in cells of lung cancer patients statistically significant was increased the structural (aberrations, fragile sites) and quantitative (ECS, aneuploidy, polyploidy) disorders of chromosomes compared to the control group. In the 58% of male patients the aneuploidy was induced by the lost of Y chromosomes. In patients with lung cancer, the frequency of centromeric fringe sites have increased in comparison to the control group, while the frequency of medial fragile sites decreased.

In patients with lung cancer, the frequency of acrocentric chromosomes is relatively higher than in control group that is indicating the increase of transcriptional activity of ribosomal genes.

The intensity of 15 chromosome entering in associations in all patients with lung cancer was increased compared to the control group. The frequency of Ag+ chromatids in patients with lung cancer is significantly increased and the frequency of 2-point Ag+ chromatids is reduced compared to the control data.

SCREENING OF 22Q11 COPY NUMBER VARIATIONS IN CONGENITAL HEART DISEASES

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Introduction: Congenital heart defect (CHD) is the most common form of birth defects and approximately 1% of newborns are affected. Copy number variants (CNVs) are important genetic contributors to CHDs, usually in the presence of additional birth defects and developmental delay. Chromosome 22q11 microdeletion syndrome is often associated with congenital heart defects. It is the most common human microdeletion syndrome and occurs in approximately one in 4000 live births in the general population. 22q11 CNVs are caused by non-allelic meiotic recombination events in the flanking low copy repeat regions (LCRs), labeled A-H. Our aim was to evaluate the prevalence of 22q11 CNVs in Hungarian CHD patients.

Materials and Methods: 175 blood samples were obtained from children and adults with CHDs (tetralogy of Fallot, septal defects, stenosis or coarctation of the aorta, transposition of the great arteries and complex defects). Samples were analyzed using multiplex ligation-dependent probe amplification (MLPA) to detect CNVs in the given chromosomal region.

Results: CNVs were detected in overall ten cases (5,7%): typical microdeletions (between LCRs A and D) - also known as DiGeorge syndrome - in six cases (four tetralogy of Fallot, one ventricular septal defect and one bicuspid aortic valve), microduplications in two cases (one ventricular septal defect and one congenital aortic stenosis) and combined microdeletions and duplications in two cases (one tetralogy of Fallot and one coarctation of the aorta). Positive MLPA results were confirmed with conventional cytogenetic methods. Genotype-phenotype comparison and family screening was also performed. In three families the CNVs were also detected in other family members.

Conclusions: The diagnosis of 22q11 microdeletion/microduplication syndrome may be challenging due to its variable clinical manifestation. Thus, systematic genetic testing of CHD patients may be beneficial in setting up the diagnosis, in the prevention or early treatment of comorbidities and positive family planning.

The Importance of genetic biomarkers for the diagnosis of radiotherapy in Oncopatients.

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Mutagen is an agent influencing genetic apparatus and changes the cell DNA. As a strong mutagen the ionizing radiation first of all causes changes in genetic apparatus of living organism. That's why cytogenetic indices are excellent biomarkers for detection of the effects of ionizing radiation.

The goal of the research was: Determining the regularity of complex genetic disorders during the radiotherapy of oncologic patients with one tumor localization (larynx) to define the predictors of radio sensitivity and predict the side effects of radiotherapy.

To evaluate the side effects of local radiation we examined the patients with larynx tumors. The studies were carried out in dynamics. The stage of the disease, age and gender of the patient were recorded in all cases. The number of leukocytes was observed and made cytogenetic analyzes. Except for all the rest, all planned analysis were carried out Comet assay for DNA, count of the micronuclei level in erythrocytes, examination of red blood functional stage and redox-systems

examination. The irradiation was carried out on a linear accelerator with the following level: 2Gy/fraction, total dose (44-70 Gy), 70 Gy was given during with radical program.

The scientific value of the research was in the fact, that in the process of the radiotherapy of cancer patients, to do the complex genetic studies of the local radiation, allows to register and assess the biologic effects on the human body.

If we summarize the above mentioned our research gives as the opportunity to develop the radio-sensitivity predictors (comets and buccal micronuclei) and to assess the radiation gravity (buccal micronuclei and dicentrics).

The application of above-mentioned genetic biomarkers should help in the individualizing medical management of patients undergoing radiotherapy.

The Role of Sirtuin2 in Regulating the Inflammatory Process and Tumor Development

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Keywords: Sirtuin, inflammation, STAT3, cervical cancer, deacetylation

Interleukin 6 (IL-6) is a pro-inflammatory cytokine important in immunoregulation. Aberrant IL-6/JAK/STAT pathway has been implicated in various diseases including chronic inflammation-associated cancer. Anti-IL-6 agents have been sought as therapeutics for several cancer types. SIRT2 (sirtuin2) is a cytoplasmic and nuclear member of sirtuins and plays significant roles in the regulation of stress response and cancer. It plays dual roles as a tumor suppressor and oncogene in various tumors. SIRT2 gene removed mouse develops tumors in many organs, including the liver and breast tissues. SIRT2 has been shown to deacetylate numerous substrates and the molecular mechanisms how SIRT2 decreases inflammation and inflammation associated disorders has been poorly understood up-to-date. Materials and Methods: the roles of SIRT2 on IL-6 induced inflammatory response was investigated in genetically modified mouse embryonic fibroblasts (MEFs) with SIRT2 gene removed and HeLa cervical cancer cell lines that overexpress SIRT2 gene. Results: SIRT2 enzyme interacts with JAK1, which is one of the downstream proteins of IL-6. Overexpression of SIRT2 in HeLa cervical cancer cells decreases IL-6 induced inflammatory response by decreasing the activity of JAK1. Conclusions: SIRT2 interacts and decreases the activity of JAK1 kinase; and ultimately, it decreases the activity of IL-6 induced JAK/STAT3 signaling cascade, suggesting that SIRT2 have an immune response regulatory function through IL-6 in cancer cells.

Effects of Expression of a BARD1 Splice Variant in Homologous Recombination DNA repair in Colorectal Cancer

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Keywords: BARD1, colorectal cancer, DNA repair, splice variant

Introduction: Colorectal cancer (CRC) is the third most common cancer worldwide, and there are more than 50,000 deaths from CRC every year in the U.S. The tumor suppressor functions of

BRCA1 are largely attributed to stabilization of BRCA1 by its binding partner BRCA1-associated RING domain protein (BARD1). Presence of a BARD1 splice variant (SV) in various cancers may render BRCA1 dysfunctional and allow cells to become sensitive to homologous recombination targeting therapies. Materials and Methods: We exogenously expressed BARD1 β in different human colon cancer cell lines and evaluating homologous recombination (HR) DNA repair and differential sensitivity to PARP inhibition.

Results: Overexpression of BARD1 β stimulated a decrease in BRCA1 functional capabilities with decreased HR DNA repair. BARD1 β sensitizes colon cancer cells to poly ADP ribose polymerase 1 (PARP-1) inhibition. Our findings indicate therapeutic targeting of deficient HR with PARP-1 inhibition therapy may be beneficial to the colon cancer patients who express this SV.

Conclusion: Expression of BARD1 β may serve as a future biomarker to assess suitability of colon cancers for HR targeting with PARP-1 inhibitors in treatment of advanced colon cancer.

Polymorphism of serotonin, dopamine and δ opioid receptors genes and drug dependence in Georgian Population

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Introduction: Drug-dependence disorders (cocaine, opioid, and nicotine dependence) are genetically influenced. Risk genes have been located based primarily on genetic linkage studies, and identified primarily based on genetic association studies. The probability of initial use and the probability of progression toward a pathologic pattern of use are influenced by intrinsic factors (eg, genotype, sex, age, age at first use, pre-existing addictive disorder, or other mental illness), extrinsic factors (eg, drug availability, peer influences social support, childhood adversity, parenting style, socioeconomic status), and the nature of the addictive agent (eg, psychoactive properties, pharmacokinetics, mode of use or administration). The use and abuse of legal and illegal psychoactive substances is a worldwide public health priority with repercussions on the individuals, their families, and society.

Materials and Methods: 49 unrelated Georgians with drug dependence and 40 healthy controls were genotyped by PCR analyses for detection of association between polymorphisms of dopamine receptor DRD2, δ opioid receptor OPRD1 and serotonin receptor 5HT2A genes and drug dependence.

Results: The frequency of alleles and genotypes, the Taq1 polymorphism dopamine receptor DRD2 gene, T921C polymorphism of δ opioid receptor OPRD1 gene and 1438G/A polymorphism of serotonin receptor 5HT2A gene, were not statistically different in drug dependent patients, compare to the healthy controls from Georgian population, but the frequency of malfunctioned allele A1 of the DRD2 gene was higher in patients than in control group (23% and 15%). The increase frequency of A/G genotype of the 5HT2A gene was also marked in the group of drug dependent more than in control group (51% and 45%) and the C/C genotype of DRD2 gene was increased as well (28.6 % and 22.5 %).

Conclusion: Molecular-genetic analyses of three above mentioned genes polymorphisms don't revealed significant association between malfunctioned alleles and drug dependence in Georgian population, which can be result of the high frequency of malfunctioned alleles in Georgian population compare to other populations. Significant differences were found in the frequency of genotypes and alleles among Georgian population group than in Swedish, French and German populations.

Key words: Genetic polymorphism, DRD2, OPRD1 and 5-HT2A genes, drug dependence.

THROMBOCYTOSIS IN JAK2 V617F POSITIVE MYELOPROLIFERATIVE NEOPLASMS

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Mutations in JAK2, MPL, and CALR genes are defined by the World Health Organization as diagnostic criteria for major subcategories of myeloproliferative neoplasms (MPNs) which are polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). Although JAK2 V617F mutation is most prevalent and can be found in all subcategories, MPL and CALR mutations are not present in PV. All these mutations are mutually exclusive and provide significant prognostic and therapeutic value.

The purpose of our study was to evaluate the prevalence of JAK2, MPL and CALR mutations in a cohort of 32 patients, pre-diagnosed with myeloproliferative neoplasms. To detect JAK2 V617F and Exon 12ins/del, MPL W515K/L and CALR L367fs*46del/K385fs*47ins mutations in patient blood or bone marrow samples, we used Multiplex ligation-dependent probe amplification (MLPA) methodology. JAK2 V617F and MPL W515K/L mutation status were always confirmed by Competitive Allele-specific TaqMan PCR (CastPCR™).

JAK2 V617F mutation was identified in 50% (16/32) and CALR L367fs*46del in 6.3% (2/32) of the patients. No patients were identified with MPL W515K/L or JAK2 Exon 12ins/del mutations. We also observed elevated complete blood count values in JAK2 V617F positive patients. Elevation was significant only in the case of platelet counts. Specifically, platelet counts in JAK2 V617F negative patients were $379 \pm 37 \times 10^6$ cells/ml and in JAK2 V617F positive patients $788 \pm 42 \times 10^6$ cells/ml. The difference was highly significant (One-way ANOVA, $p=6.6 \times 10^{-8}$) indicating an association of the mutation with thrombocytosis.

Population-based Cancer Screening Programs in Georgia

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According to population-based cancer registry in Georgia, 2015, the incidence rate of breast cancer per 100 000 women in Georgia is smaller than Europe but higher than the average rate of Commonwealth of Independent States (CIS). The incidence rate of colorectal cancer per 100,000 women decreased by one-third, and the incidence rate per 100 000 men decreased after a slight increase in 2016. The incidence rate of cervical cancer per 100 000 women in Georgia is higher than the average rates of both the Europe and CIS. According to cancer localization, prostate cancer is on fourth place, while in the previous years was on second place.

Population-based cancer screening programs were implemented in Georgia in 2011. Target population for breast cancer screening is defined as women 40-70 years, using the methods of clinical breast examination, mammography and ultrasound in every two years. Cervical cancer screening is done in women with the age range of 25-60 years, using the Pap Test, and Colposcopy with Biopsy in every three years. The screening of colorectal cancer is recommended in both gender annually, with the age range of 50-70 years using the method of gFOBT, and Colonoscopy with

biopsy. Prostate cancer management of targeted population implies the identification of specific antigens in blood, annually.

The coverage rate of breast cancer was 18% in Tbilisi, and 9.2% in regions with nearly the same rates were observed with cervical cancer screening, 18% in Tbilisi, and 11.5% in regions, respectively. The lowest coverage rates were reported for colorectal cancer, 3% in Tbilisi, and 1.6% in regions. The coverage rate of prostate cancer management was 5% in Tbilisi, and 3.3% in regions (Georgia, 2016).

As the coverage rates of population-based cancer screening programs are very low in Georgia, we need to increase the role of primary health providers in raising the population awareness about screening programs and eliminate asymmetries between regions with regard to cancer screenings at a national level.

Relationship Between Population Genetic, Biomedical and Ethnological Data in North-West Georgia

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The populations of Georgia are unique for their cultural and ethnic diversity and its geographical distribution. From this point of view, they may be the best model for ascertaining the principle structuring of ethnicity in the Near East and particularly Anatolia. When making comparative studies of the genetic diversity of these populations, scientists often refer to the genetic background of the Georgian population, based on limited and non-representative data. For this reason, it is crucial to properly interpret genetic data in the context of historical, archaeological and ethnological evidence in order to develop a fully picture of the history of the Caucasus.

In this analysis, we will review the results of the study of Upper (Zemo) Svaneti conducted in 2012 (103 male, 97 female, total 200) and Samegrelo region (372 male, 112 female, total 484) in 2016 together with the American scholars (University of Pennsylvania and Bryn Athyn College) and research carried out in East Georgia in 2017 by our research team.

While population genetic research in Georgia (the Caucasus) naturally attracts significant international scientific interest, in this study, we will focus on patterns of intra-population diversity. In particular, we will focus on family surnames, which are considered a pseudo-genetic marker, especially by Georgian scholars. We expect to obtain highly informative results about tribal, clan and other types of social status from our participants.

During our population genetic analysis with study participants, we obtained the following types of demographic and life history data, with informed consent:

1. Reproductive status (ages of menstruation and menopause, marital status, age of first and last childbirth during at least two last generations).
2. Place of birth
3. Marital residence (matrilocal, patrilocal)
4. Demography data (average lifespan, gender ratio per generation in accordance with existing genealogy maps)

5. Probable cause of death
6. Anthropometric data for newborns during last generations (length and weight)

Based on the importance of genetic research of the Caucasus (Georgia) in geographical, cultural and religious spheres, as well as the sensitivity of some of the results, we designed the project to be consistent with bioethical guidelines and maintain the privacy of participants in the research. To this end, we developed a special informed consent form for this project.

HIGH PREVALENCE OF K/NRAS G12D MUTATION IN TISSUE SAMPLES OF COLORECTAL CANCER PATIENTS IN GEORGIA

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Establishing K/NRAS mutation status increasingly becomes a standard-of-care molecular test in metastatic CRC patients. Although prognostic values of these markers are still controversial, K/NRAS mutations have firmly established a predictive role for targeted therapy in CRC. K/NRAS positive cancers that are about 35-50% of all CRCs will not respond to anti-EGFR therapy (Cetuximab) warranting decisions for alternative treatments. KRAS exon 2, codons 12 and 13 account for about 80%, and the cumulative frequency of NRAS mutations is up to 10% of all RAS mutations. There is no data on the prevalence of K/NRAS mutations in CRC patients in Georgia.

Hence, using Competitive Allele-specific TaqMan PCR (CastPCR™), we evaluated K/NRAS mutations in the DNA extracted from formalin-fixed paraffin-embedded (FFPE) postsurgical tissue samples from 56 CRC patients. We tested nine hotspot mutations in exons 2 and 3. Specifically, we analyzed KRAS G12D, G12V, G13D, G12C and NRAS G12D, G12C, G13DV, Q61R, Q61K mutations.

Although our study has not covered all hotspot mutations, the overall rate of RAS positive samples at 38% was within the expected range. Interestingly, using the above panel, we found that G12D was the most frequent genetic alteration among both KRAS, as well as NRAS mutations. This mutation represented about 50% of all RAS positives in our study, while no samples were identified with NRAS G13V and G12C mutations. KRAS G12V and NRAS Q61K were the second most prevalent mutations accounting for 30% and 27% of mutations in respective RAS isoforms. Our findings indicate that further studies using extended hotspot mutation panels are required to reveal the complete mutational status of CRC patients in Georgia.

Epidemiology of Familial Mediterranean fever mutations in Georgia

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Introduction

Familial Mediterranean fever (FMF) is an inherited disorder characterized by recurrent episodes of fever accompanied by sterile peritonitis, arthritis, and pleuritis. Many mutations in the MEFV gene

have been identified as causing FMF. However, accompanying epidemiological information remains quite scarce in Georgia, even though Georgia is located near the Mediterranean region (which is known as a risk group).

Materials and Methods

After receiving informed consent from 15 patients diagnosed with FMF their clinical cases were analyzed. Diagnoses were made via sequencing of 2nd and 10th exons of MEFV gene, that is related to Familial Mediterranean fever. Information collected from patients was such: mutations found by genetic testing, age of medical manifestation (when first symptoms appeared in patient's life), current age, everyday dosage of Colchicine (main medication for patients with FMF), amount of fevers per year. Data charts will represent: correlation between mutations and age of disease manifestation, correlation between fevers per year and relative dosage (everyday dosage of Colchicine divided by patient's age) from the view of specific mutations.

Results

Data analysis shows, that M694V is prevalent mutation in Georgian people 63.33% (19/30). Other revealed mutations are M680I (4/30), E148Q (2/30), V726A (3/30), R761H (1/26), E251K (1/30).

Conclusion

Studies showed, that there is correlation between specific mutations and effective dosage of Colchicine, which should be considered by physicians during management of FMF cases.

The Future of Oncology: Liquid Biopsy for Cell-Free DNA (cfDNA)

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Oncology has been celebrating an introduction of a new tool that is thought to make a revolutionizing effect on better management of cancer patients: The liquid biopsy (in other words, fluid biopsy). Instead of tissue, which is sometimes unavailable for the analysis of molecules, the blood is a readily and always-available source for the analysis. In similar to the classical technique, tissue biopsy, liquid biopsy is able to give all the tumor-related information required by the oncologists. Tissue biopsy has some disadvantages. For example, each part/section of a tumor tissue may yield more or less different information. However, a liquid biopsy is thought to represent all sections of a tumor tissue, thereby better information could be available. Moreover, it is non-invasive, possible to repeat it as many as required, helpful to monitorize the progress/relapse of the disease and the response to treatment after each cycle. Tumor evolves: new mutations take place all the time, especially following the treatment. Therefore, it is of immense importance for an oncologist to know which new mutations occurred after the treatment because those new ones may alter the treatment response or even result in drug resistance, which is a dreadful scenario in cancer world. The collection of circulating tumor cells (CTC) is also another aim for liquid biopsy but in this talk cfDNA will mainly be discussed, rather than cfDNA.