SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (Ph.D.)

DETECTION OF SUBTELOMERIC CHROMOSOMAL ABERRATIONS AND ANALYSIS OF THE GENETICAL AND BIOCHEMICAL ALTERATIONS OF SMITH-LEMLI-OPITZ SYNDROME IN MENTAL RETARDED PATIENTS

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The Examination takes place at Department of Ophthalmology, Medical and Health Science Center, University of Debrecen, 21st November, 2013, at 11.00.a.m.

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The Ph.D. Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Medical and Health Science Center, University of Debrecen
21st November, 2013, at 1.00 p.m.

INTRODUCTION

1.1. Definition and prevalence of mental retardation

The mental capacity and intellectual capability of a person is a multifactorially determined state, which is under the influence of varied genetical and environmental factors and settle a special relationship between an individual and its environment. Based on the IQ of a population the typical distribution usually follows the Gauss equation where the right side of the curve represents people of high IQ score with outstanding mental capacity while individuals on the left side of the Gauss curve suffer from mental retardation with low IQ score. Parents of individuals in the latter group usually have lower IQ as well. In these cases specific causes should not be looked for. The causes of mental retardation are to be searched for in cases of moderate to severe forms of mental imparment.

According to *American Mental Retardation Association* the definition of mental retardation (intellectual disability, learning disability, mental retardation) is a special funtional status, which manifests under 18 years of age and is associated with intelligence quotiens (IQ) under 70 and out of 10 adaptive functions at least 2-3 are severely affected.

Based on the IQ scores we distinguish 3 different forms of mental retardation such as mild (IQ: 70-51) moderate (50-35) and severe (35-20) forms. The prevalance of mental retardation is about 2-3 % but that value may vary between 1-10%, depending on both patient selection and investigational methods.

1.2. Etiology of mental retardation

The etiology of mental retardation is extremly heterogenous. Either inherited genetic factors or aquired disorders during the pre-, peri- or postnatal life could be responsible for mental retardation. Factors leading to aquired forms of mental retardation are either endogenous or exogenous in terms of origin such as intrauterin infection, teratogenic noxa in the prenatal period or maternal disorders and/or inborn error of metabolism (e.g. maternal

PKU). Causes of severe brain damage and associated mental retardation are several fold, such as perinatal hypoxia, asphyxia, prematurity related intracranial hemorrhagia as well as perinatal infection (e.g. herpes virus type 2). Central nervous system infections, intracranial bleeding and cranial injuries are the most common postnatal noxa that affect mental development.

Our knowledge about the genetical factors of mental retardation is considerably growing thanks to a tremendous progress seen in cytogenetic and molecular genetical techniques, therefore the aspects of the etiological classification of mental retardation have been continously changing. Despite the enormous development of cytogenetic, molecular cytogenetic and molecular biological techniques, the cause of mental retardation remains unknown in about 60-75 % of moderate/severe mentally retarded patients and in 80 % of those with mild form of mental retardation. Cryptic unbalanced subtelomeric chromosomal aberrations have been recognized as a significant cause of both mental retardation and multiple congenital anomalies. The clarification of the genetical diagnosis enable us to reveal the pathogenetic background and assess the prognosis and most importantly, prevent repetition in the family. Among genetical factors balanced and unbalanced alterations can be distinguished. Unbalanced alterations could be either relatively large chromosomal aberrations such as trisomies, monosomies, duplications, deletions or small, hardly recognizable deletions or gains of the genetical material that can only be detected by refined molecular genetic methods. Balanced alterations are such kind of chromosomal rearrangements (translocations, inversions or insertions) in which either gain or deletion of the genetical material cannot be detected by conventional cytogenetical methods. However, modern, state of the art molecular genetical methods revealed that previously undetectable microdeletions or microduplications exist in breakpoints. And last but not least, alterations within a gene such as nucleotide exchange or change in the nucleotide sequence may result in

mental retardation with or without visible morphological changes. Depending on either the type of the genetical alteration (size, level) or the method sutable for detecting it, different subgroups are distinguished:

- classical chromosomal aberrations,
- cryptic (not detectable with classical cytogenetic analysis) chromosomal aberrations,
- copy number variations (CNVs),
- mendelian and not mendelian inherited gene mutations (monogenic disorders, X-linked mental retardation, mitochondrial disorders),
- genetical mechanisms, resulting in altered gene expression (uniparental disomia, genomic imprinting) and epigenetical alterations.

Numerous genetical disorders such as Down-syndrome, Rett-syndrome or mucopolisaccharidosis, can easily be recognized based on their characteristic phenotypic features, which makes clinical diagnosis easy for an experienced clinical geneticist. What is more challenging is to establish diagnosis for patients with mild-to moderate mental retardation in whom associating phenotypic features are not present, the so-called non-syndromatic mental retardation patients. It is equally difficult to give diagnosis in those with multiple aberrations, possibly associated with a rare syndrome.

2. BACKGROUND

In my thesis I have attempted to present the results of my investigation in two clinically and methodologically distinc patient populations that required different scientific approaches. Furthermore, I have summarized my clinical experience obtained in: 1/. cases with unbalanced subtelomeric chromosomal aberrations and 2/. patients suffering from Smith-Lemli-Opitz (SLO) syndrome.

2.1. Subtelomeric chromosomal aberrations

Aberrations (deletion, duplication, translocation) of the telomeric, subtelomeric regions, a gene-rich area of the chromosome, remains undetected with conventional G-band technique due to their bright (negative) staining (cryptic aberrations). The subtelomeric FISH examination was introduced in 1995 in order to investigate those gene-rich regions of human chromosomes, and in 6.8 % of severe mentally retarded patients as well as in 0.5 % of mild cases subtelomeric chromosomal aberrations were detected.

Subtelomeric chromosomal aberrations are associated with varied clinical appearance. Since the introduction of this method more than 10000 cases have been reported worldwide. It has been revealed that certain subtelomeric chromosomal regions and their rearrangements, such as 1pter, 2qter, Xqter, have more frequently been involved compared to other chromosomal regions in the studied patient population. Numerous genetical syndromes (1p36 subtelomeric deletion, 3q29 subtelomeric deletion) with characteristic clinical signs and symptoms are relatively easy to recognize with experience. The subtelomeric FISH examination is a cost-and time-consuming method, that is why careful patient selection is very important. However, too strict selection criteria may result in unrecognized positive cases (false negative). Different patient selection methods used may explain the reported diversity in related studies. Score systems have been worked out for patients with subtelomeric chromosomal aberrations via utilizing their clinical and anamnestic data to improve patient selection method. It was recognized that intrauterine growth retardation as well as positive family history for mental retardation carry positive predictive value for patient selection. Clinical investigations revealed that the single most important selection parameter is the repetition of mental retardation in a family. Approximately 50% of subtelomeric chromosomal aberrations are of familial origin, which means that at least one of the parents carries a balanced translocation. During meiosis partial deletion or duplications may occur when chromosomes segregate in gametes. The final phenotype is determined by

both the deleted and duplicated genes, however in offspring the deletious phenotype is more penetrant and dominant. Still, clinical features may vary case by case. In the case of unbalanced translocations the participating partner chromosomes might significantly influence the clinical outcome. There are several other techniques for the detection of subtelomeric and other cryptic chromosome aberrations such as Multiplex-Ligation-dependent Probe Amplification (MLPA), Multiplex Amplifiable Probe Hybridisation (MAPH) and array-Comparative Genomic Hybridisation (CGH) techniques. The enormous development in modern molecular cytogenetical techniques led to the recognition of more and more unbalanced translocations in cases that previously remained unsolved. An increasing number of scientific publications highlighted the importance of subtelomeric FISH examination. Until now, a relatively low number of genetical entity can be correlated with subtelomeric rearrangements, that is why in practice all of the chromosome ends (subtelomeric regions) are to be tested, except the short arms of the acrocentric chromosomes, based on the expected frequency of their possible involvement. To recognise of further entities large-scale genotype-phenotype correlation studies should be performed in a relatively large patient population. Ganing new experience in this field may help to refine both patient selection methods and detection efficacy and improve cost-effectiveness as well. Besides the recognition of genetical rearrangements the aim is to determine the exact size and location of deletion/duplication in order to gain further understanding of candidate genes localized and involved in the rearrangements, moreover to get to know more about their potential function and role in the pathomechanism. Based on the above mentioned scientific observations our aim was to perform subtelomeric chromosomal FISH analysis in selected patients with moderate to severe mental retardation, of whom previous cytogentical analysis revealed normal kariotype and other genetical examinations (FraX, microdeletion syndrome, etc.) were not able to establish diagnosis. We examined the frequency of subtelomeric chromosomal

aberrations in a selected patient population as well as their *de novo* or familial origin, and in positive cases we identified the breakpoints and the genes possibly involved. We also tried to establish genotype-phenotype correlations by comparing data with that of the literature.

2.2. Smith-Lemli-Opitz syndrome

The Smith-Lemli Opitz syndrome (McKusick 270400) is an autosomal recessive severe developmental disorder with multiple congenital anomalies, which is caused by a defect of cholesterol biosynthesis. The syndrome was first reported by Smith and his collegues in 1964, and was characterized by dysmorphic face, microcephaly, hypospadiasis and severe growth retardation. The established incidence of the SLO syndrome in the Kaukasian population is 1: 20 000 and 1 : 40 000 and the carrier frequency is 1 : 50-1 : 70.

The cause of the disease is explained by the defective function of the 7-DHCR enzyme which catalyzes the last step of cholesterol biosynthesis. This enzyme is responsible for the transformation of 7-dehydrocholesterol to cholesterol. In patients with SLO syndrome an elevated levels of 7- and 8-DHC precursors and decreased cholesterol values can be measured. Cholesterol is an important component of the lipid raft of cell membranes, it is an important constituent of myelin, a precursor molecule of bile acids, steroid hormones and neurosteroids and plays an important role in the embryogenesis/morphogenesis as well. In SLO syndrome the accumulated 7-DHC replaces cholesterol in the sterol- and caveolin-rich membrane regions and disturbs cell signalling. It has been suggested that the neurological symptoms in SLO syndrome are associated with the oxysterols that are produced from 7-DHC.

The clinical signs of SLO syndrome are very characteristic: mental retardation, microcephaly, holoprosencephaly, nutritional – and behavioral disorders, facial dysmorphy, genital- and renal anomalies. The phenotypic features show a wide variety due to the

anatomical abnormalities of ten embriologically separated organs (brain, oral region, acral, eye, heart, kidney, liver, lung, bowel and genitals) on the basis of which a score system was designed. Based on the clinical severity scores, patients were assigned to three different groups: mild form (<20), typical (20-50) and severe form (>50) SLO syndrome.

The human *DHCR7* gene, located on chromosome 11q13 region, spanning 14,100 base bairs (bp) of genomic DNA and containing nine exons, was cloned and sequenced in 1998. According to Human Gene Mutation Database 164 different mutations have been identified to date: missence/nonsence mutation: 142, splicing: 6, small deletion: 8, small insertion: 2, small index: 1, large deletion: 5.

Until recently, the only therapy of SLO syndrome has been supportive. In the 90's the results of multicenter studies suggested that cholesterol substitution with or without bile acids can improve the biochemical parameters as well as the clinical state of SLO patients. Initial experimental treatment protocols for SLO syndrome recommended 50 mg/kg/day cholesterol or even higher doses up to 300 mg/kg/day. It has been reported that statins – by decreasing 7-DHC levels and increasing the residual activity of 7-DHCR – also has a beneficial effect in the treatment of SLO syndrome. In accordance with that, Stark and her collegues observed clinical improvement in patients on simvastatin therapy. Still, serious doubts have been raised concerning the efficacy of the above mentioned treatment options in recent years. On the basis of such observations no significant improvement was reported in patients on specific therapy.

3. AIMS

1. The aim of my work was to select eligible patients with mental retardation, who were diagnosed and followed in the Clinical Genetic Outpatient Center of the Pediatric Institute at the University of Debrecen, for subtelomeric FISH examination when indicated, based on international selection criteria (Lam et al. 2006). My major

objective was to introduce subtelomeric FISH analysis in order to detect and identify rearrangements located on the terminal parts of chromosomes. Furthermore, I intended to work out a precise evaluation process and apply a selection protocol based on international standards and integrate subtelomeric FISH examination in a new diagnostic algorithm.

2. We intended to refine our previous results obtained by locally available methods, using Comparative Genomic Hibridization (CGH), in collaboration with others. By obtaining exact genetical diagnosis we aimed to seek genotype-phenotype correlations via checking genomic database for affected genes located in the targeted region. We tried to make correlation between gene function or dysfunction and observed clinical symptoms.
3. Another important project was to establish clinical, biochemical and genetical diagnostic approach of SLO syndrome via setting up a a special team in our center. We wished to gain further experience in the follow-up and treatment of such patients.
4. An intriguing question for us was, whether there was any correlation between phenotypic features and biochemical parameters measured in patients diagnosed with SLOS in Hungary. What is the prognostic value of the initial cholesterol - or DHC levels?
5. Finally last but not least, we decided to follow up and support SLO patients and their families in several ways. Mutational analysis data gave us a chance to inform parents about possible prognosis and more importantly, prenatal diagnostic approch enabled us to prevent repetition in the same family.

4. PATIENTS and METHODS

4.1. Analysis of subtelomeric aberrations

4.1.1. Patients

149 subtelomeric FISH examinations were performed in the given time-period in the Clinical Genetic Center and Genetic Laboratory Institute of Pediatric of Medical and Health Science Center of Debrecen University. Out of the 149 subjects 125 were mentally retarded patients (male:female=73:52; mean age: 9,8 years, range: 4 years- 27 years) and 24 were healthy family members.

Informed consent was obtained from all family members for sample collection, storage and performing genetic analysis. The research protocol had been reviewed and approved by the Ethics Committee of the Medical and Health Science Center of Debrecen University. Patients' selection was performed according to the protocol recommended by Lam et al. The following inclusion criteria were used for subtelomeric studies: major criteria: 1. normal karyotype, 2. moderate/severe mental retardation (IQ<50) minor criteria: 1. facial dysmorphism, 2. congenital anomaly, 3. abnormal growth, 4. behavioural disorder, 5. family history of mental retardation, 6. family history of miscarriages or perinatal death. Two major and three minor criteria indicate subtelomeric FISH testing.

4.1.2. Methods

4.1.2.1. *Subtelomeric FISH examination*

For FISH analysis peripheral blood samples were taken, the chromosomal preparations were fixated in methanol: acetic acid 3:1 mixture. FISH analysis of subtelomeric regions was performed on blood lymphocytes using ToTelVysion Multi-color DNA Probe Mixtures (Abbott Molecular/Vysis Inc., Des Plaines, IL) according to manufacturer's instructions. The probe-kit include various combination of 15 "ready-to-use" directly labelled probe mixture, which are specific to the p and q arm telomeric regions of each of the chromosomes (except

the p arm of acrocentric chromosomes 13, 14, 15, 21 and 22). LSI and CEP probes in the probe mixture serve as an internal control for the identification of chromosomes. Patient as well as further linear relatives were examined directly with FISH targeted to specific regions of chromosomes 10pter (D10S2488), 21qter (D21S1446), 8pter (RH65733), 12pter (D12S158), CEP10 (D10Z1), CEP8 (D8Z2) (Poseidon RF, Kreatech Diagnostic, Vysis) according to the manufacturer's instructions. For detection of the deletion of 1p36.33 region subtelomeric region-specific probe (D1S2217, Kreatech) was used. FISH specimens were viewed using a fluorescence microscope *Axioplan 2* (Zeiss) and analyzed with *MetaSystems* image software (*Altussheim, Germany*). 15 metaphases were analyzed with each probe mixture.

4.1.2.2. *Array-Comparative Genomic Hybridization (CGH)*

On a diagnostic basis array CGH was performed (Agilent 180K oligo-array (Amadid 023363); 13kb overall median probe spacing; NCBI Build 36.1). For the labelling and hybridisation standard methods were used. Array CGH profiles were analyzed using Agilent software (DNA analytics).

4.2. Diagnostic approach of the Smith-Lemli-Opitz syndrome SLOS)

4.2.1. Patients: Selection of Smith-Lemli-Opitz syndrome patients

The aim of our work was to establish a scientific group for studying the clinical, biochemical and genetical characteristics of SLOS and gaining experience with available therapeutic approaches. The diagnosis of SLOS of the very first patient in the program was confirmed by biochemical and molecular genetic analysis in collaboration with professor Libor Kozak (Molecular Genetic Center, Brno) who performed the mutational analysis. The efficacy of cholesterol-and simvastatine combined treatment was monitored by UV-spectophotometry

via measuring 7-DHC and cholesterol levels. The success of our initial effort triggered an ambitious project with participating clinical genetic centers nationwide, in which we collected clinical data of patients, of whom the presumed diagnosis was SLOS but detailed genetical analysis was yet to be done. Eventually, the Genetical Center of the Pediatric Department of Debrecen University in collaboration with the Department of Molecular Genetics of Laboratory Medicine established the Hungarian SLOS Diagnostic and Therapeutic Research group. We managed to collect and analyze the clinical data and genetical samples of 15 SLOS patiets. The diagnosis of 15 SLOS patients (age: 0,1-18 years; male:female = 8:7) were initially made based on their phenotypic features, then biochemical and molecular genetic examinations were performed to confirm the diagnosis. The severity of SLOS was determined according to phenotypic features based on severity score system of Kelley and Hennekam published in 2000. The patients enrolled into the mild group are still alive (n=4; age when the diagnosis was set up, 0,5-18 years). In the typical SLOS group (n=7; age at diagnosis 0,1-7 years), two children passed away before the age of two, while five children are still alive. All patients with severe SLOS died in the newborn period (n=4, age<2 months).

4.2.2. Methods

4.2.2.1. *Biochemical examination (Detection of 7-DHC)*

For the determination of cholesterol levels a cholesterol oxidase enzymatic method was applied (CHOD-PAP, Roche Diagnostics, Modular P). The rapid determination of 7-DHC in serum was performed by the modified UV spectrophotometric method and it was compared to the GC/MS method. Humatrol normal serum (Human, Germany, Cat. No. 13511) was used as negative control. The detection limit of this method is about 10 mg/L and it is linear in the range of 10-400 mg/L. During extraction 0,2 mL serum and 1,6 mL of c-hexane:i-propanol

(3:1) mixture was vortexed for 20 seconds and after centrifugation (10 min, 900g) the absorbance of clear supernatant was measured at 282 nm against solvent blank. The 7-DHC concentrations were compared to those obtained by a published GC/MS method. Ten serum samples were analysed in parallel by GC-MS and UV spectroscopy. The same samples proved to be negative (n: 6) and positive (n: 3) as measured by both methods. The only difference was observed in the case of a carrier's serum showing a slightly elevated 7-DHC level by GC-MS (0, 49 mg/L), which was not detectable by UV spectroscopy, being under the detection limit of UV spectroscopy. The serum levels of lipids and lipoproteins were determined by photometry (Modular-P, Roche), with exception of the ratio of VLDL-cholesterol (agarose gelelectrophoresis, Sebia). For measuring the serum vitamin-D_3 level a chemiluminescent immunoassay (Modular E-170, Roche) was used.

4.2.2.2. *Molecular genetic examination of the DHCR7 gene mutations*

- *PCR/RFLP analysis*

Genomic DNA was extracted from 5-10 mL of EDTA anticoagulated blood by using a salt precipitation method. A rapid PCR-based DNA analysis was used to identify or confirm eight prevalent DHCR7 mutations (W151X, L157P, V326L, IVS8-1G>C, L109P, R446Q, R352Q and C380Y) of our population that create or abolish the given natural and amplification-created restriction sites.

- *Sequence analysis*

Exons with flanking intronic sequences were analyzed using the Thermo Sequenase Primer Cycle Sequencing Kit (Amersham Biosciences, UK) on an automated sequencer ALFexpress II (Amersham Biosciences, UK) according to the manufacturer's instructions. PCR products were sequenced on both forward and reverse strands.

4.2.2.3. *Statistical analysis*

Statistical analysis was performed by using Microsoft Office Excel 2003 Analysis ToolPak Add-In (Regression Tool). The P values in each selected time point proved to be significant ($p < 0.05$).

Statistical comparison of cholesterol, 7-DHC and α-lipoprotein levels among the three groups was carried out by Kruskal-Wallis test. For the cholesterol and α-lipoprotein levels that showed Gaussian distribution the Bonferoni test was applied for pairwise comparisons.

5. RESULTS

5.1. Results of the subtelomeric study

149 subtelomeric FISH examinations were performed in the given time-period at the Genetic Laboratory of the Clinical Genetic Center of Medical and Health Science Center of Debrecen University. Among the 149 subjects tested, 125 were mentally retarded patients (male:female=73:52; mean age: 9,8 years, range: 4 years- 27 years) while 24 were healthy family members Among the 125 mentally retarded patients of unknown origin, previously unidentified subtelomeric chromosomal aberrations were identified in 13 patients. In terms of origin, 4 cases out of the 13 proved to be *de novo*, while nine other cases turned out to be familial. ***De novo* aberrations were as follows**: **ish del(3)(qter-); ish del(1)(pter-); ish del(22)(qter-). *Familial aberrations:* ish der(8)t(8;12)(pter-,pter+); ish der(21)t(10;21)(pter+,qter-); ish der(4)t(4;8)(pter-;qter+); ish der(3)t(3;8)(pter-;pter+); ish der(10)t(10;17)(qter-;qter+).** 24 healthy family members were also examined in order to clarify the origin of the chromosome aberrations, and seven of them turned out to carry a balanced translocation. Full analysis, all of the 41 subtelomeric chromosomal regions were checked in 81 cases (81/125) while only targeted FISH examinations were performed in 44 (44/125) cases.

We have carried out targeted FISH analysis in all linear relatives (parents, siblings, grandparents, aunts, uncles, cousins) of the carriers of subtelomeric chromosomal aberrations or in those cases where characteristic phenotypic features raised high suspicion for a specific subtelomeric aberration, as well as in every chromosomal aberration suspect case. Varied forms of subtelomeric chromosomal aberrations were detected in 9 cases out of the 8, in whom a full analysis was performed, while in 4 other cases (4/44) targeted FISH was employed successfully. Unfortunatelly, in familial cases preventive efforts (prenatal diagnosis with subtelomeric FISH) were only possible after the birth of a 2nd and a 3rd child, since introduction of the subtelomeric FISH anaylysis was delayed until 2008.. Indication for subtelomeric analysis was based on the selection criteria by Lam in patients with idiopathic mental retardation. The 1p36 deletion syndrome is one of the most common subtelomeric chromosomal aberration with moderate/severe mental retardation, typical facial dysmorphy, hypotonic muscles, obesity and epilepsy.

The clinical presentation of 1p36 commonly overlaps with the phenotypic features of Prader-Willi syndrome. Based on the similarity between the two phenotypes we have screened 12 patients for 1p36 subtelomeric deletion, of whom the PW-specific FISH examination targeting the 15q11.2-region were negative. We excluded the possibility of uniparenteral disomy only in 4 cases. Interestingly, 2 patients out of the 12 P-W suspect cases turned out to carry the 1p36.33 deletion (ish subtel del(1pter))with *de novo* origin in both cases. Further sets of investigation are in progress in 6 additional patients for UPD and methylation profile.

5.1.1. Case reports

5.1.1.1. *Familial unbalanced translocations*

ish der(8)t(8;12)(pter-,pter+)

Family 1.: Siblings were examined in the affected family. The clinical signs and the genetic aberrations were the same. Phenotypic features of the patients are as follows: hypotonia, postaxial polydactyly, facial dysmorphy (flate midface, hypertelorisms, ptosis, low-set small ears, downturned corner of mouth). Opthalmological examination revealed retinitis pigmentosa, nystagmus, declorated papilla.

Subtelomeric FISH analysis detected a single 8pter signal and three 12pter signals in all affected patients as a result of the deletion of 8pter and duplication of 12pter region. The unbalanced translocation caused by these subtelomeric rearrangements and FISH results were interpreted as follows: **ish der(8)t(8;12)(pter-;pter+).** During FISH analysis of the family, the father of the proband proved to be the carrier of the balanced translocation of 8pter and 12pter regions: **ish t(8;12)(pter-,pter+;pter-,pter+)dn.**

ish der(21)t(10;21)(pter+;qter-)

Family 2: Siblings and their cousin were examined. Dysmorphic features and the severity of mental retardation were similar in all three cases. Phenotypic features of the patients are as follows: ptosis with remarkable asymmetry, midface hypoplasia, long philtrum, malformed ears (mild prominence of antihelix stem, prominent antitragus, large upturned earlobe), high narrow palate, thin upper lip, micrognathia. Atrial septal defect, scoliosis and kyphosis were detected. Further anomalies, such as atrophy of the thenar and hypothenar muscles, hyperflexibility of joints and habitual patellar luxation were detected in the cousin. He also had atrophy and moderate muscle weakness. Electromyographic examination detected moderate myogen lesion.

FISH and array-CGH examinations confirmed the same genetic alterations. Subtelomeric FISH analysis detected one 21qter signal and three 10pter signals in all affected patients as a result of the deletion of the 21qter and duplication of 10pter region. The unbalanced translocation caused by these subtelomeric rearrangements and FISH results were interpreted as follows: **ish der(21)t(10;21)(pter+;qter-).** All linear relatives were analysed by FISH using 10pter and 21qter specific FISH probes. The mother, sister, aunt and cousin of the proband all proved to be carriers of the balanced translocation of the 21qter and 10pter regions: **ish t(10;21)(pter+,qter-;pter+,qter-).**

Array profile confirmed the subtelomeric FISH result. The distal duplication of chromosome 10 is of 6.7 Mb size within the p15.3-p14 region (410 oligo's) while the distal deletion of chromosome 21 is of 5.6 Mb in the q22.2-22.3 region (412 oligo's). The breakpoint on the short arm of chromosome 10 is between bp 6873275 and 6885392, those on the long arm of chromosome 21 are between bp 41244147 and 41270119. The array karyotype is as follows: **arr10p15.3-14(138206-6873275)x3,21q22.2-22.3(41270119-46920225)x1**.

ish der(4)t(4;8)(pter-;qter+)

Family 3: The patient with the chromosomal aberration inherited it from her father, who carries the unbalanced translocation. Phenotypic features of the patients are as follows: severe somatic retardation, microcephaly, hypotonia, equinovarus on the both side, facial dysmorphy: (asymmetric frontal and occipital bossing, hemifacial microsmia, thin palpebral fissures, small nose, hypoplastic a'la nasi, bulbosus tip of the nose, small philtrum, retrognathia, downturned corner of mouth.

FISH analysis detected one 4pter signal and three 8qter signals in affected patient as a result of the deletion of the 4pter and duplication of 8qter region. The unbalanced translocation caused by these subtelomeric rearrangements and FISH results were interpreted as follows:

ish der(4)t(4;8)(pter-,qter+). The FISH analysis of the parents with using specific probe mixture (Mix4; Mix8) showed that the father of the proband carried a balanced translocation of 4pter and 8qter regions: **ish t(4;8)(pter-,qter+;pter-,qter+).** In order to clarify the origin of the translocation, the grandparents of the father should have been checked but without their consent it was not done.

ish der(10)t(10;17)(qter-;qter+)

Family 4: Siblings were examined in the affected family. Phenotypic features of the patients are as follows: proportionated nanosomy, facial dysmorphy (wide nasal bridge, hypertelorisms, prominent nasal tip, low-set ears), mild form of scoliosis and spina bifida.

Subtelomeric FISH analysis detected one 10qter signal and three 17qter signals in both patients as a result of the deletion of 10qter and duplication of 17qter region. The unbalanced translocation caused by these subtelomeric rearrangements and FISH results were interpreted as follows: **ish der(10)t(10;17)(qter-;qter+).** The FISH analysis of the parents with using specific probe mixture (Mix9; Mix10) were carried out and the father proved to be the carrier of the balanced translocation: **ish t(10;17)(qter-,pter+;qter-,pter+).** FISH analysis of the grandmother and uncle were not done without their consent.

Array profile confirmed the subtelomeric FISH result. A distal deletion of chromosome 10q26.3 is of 2,9 Mb size (410 oligo's) while a distal duplication of chromosome 17q25.3 is of 4 Mb (412 oligo's) were detected. The breakpoint on the long arm of chromosome 10 is between bp 132555396 and 132539691, those on the long arm of chromosome 17 are between bp 77049814 and 77038050. The array karyotype is as follows: **arr10q26.3(132555396-135474787)x1,17q25.3(77049814-81045222)x3**.

5.1.1.2 *De novo* cases

ish subtel del(1pter)

Targeted FISH examinations were performed in two cases, with characteristic Prader-Willi-like phenotypic features, for detection 1pter subtelomeric deletion. The phenotypic features showed significant overlap with symptoms of Prader-Willi syndrome: hypotonia, hyperflexible joints, obesity, cryptorchism, mental retardation, small hand and foot, facial dysmorphy (flat face, brachydactyly, small nose, short philtrum, pointed chin, straight eyebrows, low set ears). The classical cytogenetic analyis, Fra-X test and Prader-Willi FISH test were all negative. Due to the characteristic features deletion of the 1p36.33 region were examined with using region-specific probe (D1S2217, Kreatech). As expected, the FISH test detected a deletion of the 1p36.33 region. FISH results of the parents proved to be negative, suggesting *de novo* origin of the chromosomal aberration.

ish subtel del(3qter)

We identified a 3q29 subtelomeric deletion in a case with characteristic phenotypic features. Phenotypic features are as follows: microphthalmia, convergated strabismus, scoliosis, coxa valga, V. clinodactyly, long-tapering fingers, facial dysmorphy (long, narrow face, short philtrum, hyperthelorism, prominens nasal bridge, bulbosus nasal tip) and rare ophthalmic anomaly (uvea coloboma). Subtelomeric FISH have shown the deletion of 3qter (D3S4560) region: ish del(3)(qter-). FISH results of the parents proved to be negative suggesting, *de novo* origin of the chromosomal aberration.

5.2. Clinical and genetical diagnosis of the first child with SLO syndrome

Our therapeutic approaches

Based on the characteristic phenotype observed at birth the diagnosis of SLO was suspected in a patient, whose clinical symptoms at birth were as follows: facial dysmorphy (ptosis, epicanthal folds, micrognathy, wide nasal bridge and tip with anteverted nares, submucosus cleft palate, low-set deformed ears), microcephaly, cryptorchism, hypospadiasis, syndactyly of toes 2-3 and atrial septum defect, during infancy the patient had feeding problems and muscle hypotonia. According to SLO severity score system our patient had a severity score of 25 (typical group: 20-50). Diagnosis of SLO syndrome was confirmed by the detection of low serum cholesterol level (2, 77 mmol/L), and high level of 7-DHC (102 mg/L, ref: < 0, 15 mg/L). Since abnormalities of serum lipid, lipoprotein and vitamin-D_3 levels have been reported, we measured these biochemical parameters as well. In our case the apolipoprotein B-100 (0,38 g/L) was low compared to the age-matched reference range but other lipid parameters (TG, HDL-, LDL-, VLDL-cholesterol, Lp(A), apolipoprotein-A1) remained in the reference range. Vitamin D_3 level (118 nmol/ L) was found at the upper limit of the reference range (28-118 nmol/L) corresponding with the fact that 7-dehydrocholesterol is the precursor of vitamin-D_3. Molecular genetic examination was carried out in the patient as well as in other members of the family. Compound heterozygosity – c.964-1 G>C/c.1097 G>T (p.G366V) - was detected in the patient. The c.1097 G>T mutation, locating in the exon 9 of the *DHCR7* gene, affects the 4th cytoplasmatic loop, is a novel, previously unpublished, pathogenic mutation. This mutation is associated with a guanine-thymin nucleotide change, and results in an exchange of glycin to-valin aminoacids in the 7-dehydrocholesterol reductase enzyme. The c.964-1 G>C mutation, which was revealed in the mother and the sister of the patient, is a characteristic genetic aberration of SLO syndrome. The c.1097 G>T

mutation was detected in the father. No specific mutation was found in the proband's twin-brother.

Cholesterol substitution therapy was carried out according to the published protocol by Irons et al. (Cholesterol Module, 100 mg/kg/day, Nutricia; product code number 18,012) in addition simvastatin treatment was introduced as described by Jira et al., (0,2 mg/kg/day). Combined cholesterol-simvastatin therapy was well tolerated by the patient, without side effects. During the 25-months follow-up we experienced a significant increase in cholesterol level (from 2.77 mmol/L to 3.2 mmol/L) and almost a twofold decrease in 7-DHC (from 102 mg/L to 53 mg/L) (Figure 2., 3., Table 2.). The patient was checked regularly, every two months and we measured the following parameters: body length, body weight, chest and head circumferences and nutritional state. Moreover, serum triglyceride, cholesterol, HDL, LDL, 7-DHC, GOT, GPT, CK, AP, γGT values have been regularly measured. Assessment of the behaviour state and psychomotor development of the patient was performed by applying two methods: the parental interviews and the Vineland Adaptive Behaviour Scale. The parents found the patient less irritable, they experienced less autoagressive behaviour, improved sleep pattern and better appetite. Changes in the adaptive functions were determined by the Vineland Adaptive Behaviour Scale. In two distinct domains, in Daily Living Skills (personal subdomain) and in Motor Skill (gross subdomain) a moderate improvement was observed upon pharmacotherapy. The patient's DQ (Brunet-Lèzine) score did not change.

5.3. Relation between biomarkers and clinical severity in patients with Smith-Lemli-Opitz syndrome

The age of patients at the time of diagnosis established varied in a wide range (0,5-18 years). In the mild-type SLO group (n=4; score: <20), the mean level of serum cholesterol was 2,37 ±0,8 mmol/L and the mean of 7-DHC was 0,38 ± 0,14 mmol/L (147 ± 55 mg/L). In the group

of typical SLOS, diagnosis was established earlier (age of 0,1-7 years) (n=7; score: 20-50); the mean serum cholesterol and 7-DHC levels were 1,47 ± 0,7 mmol/L, and 0,53 ± 0,20 mmol/L (202 ± 77 mg/L) respectively. Those patients who died at the newborn period (at the age less than 2 months) were enrolled into the severe SLOS group (n=4, score: > 50). Their 7-DHC level (0,47 ± 0,14 mmol/L; 181 ± 52 mg/L) was similar to that of the typical SLO group with great scatter, but their cholesterol level (0,66 ± 0.27 mmol/L) was significantly lower than the corresponding values in the mild SLOS group (2,37 ± 0,8 mmol/L). The initial cholesterol/7-DHC ratio showed a similar weak inverse relationship with clinical scores (r=0,669). A significant difference could be observed between the cholesterol/7DHC ratios of the group as well (Kruskal-Wallis test, p= 0,004). Lipoprotein gel electrophoresis detected decreased percentage of α-lipoprotein in severe SLOS (7 ± 5 %) compared to the age-matched control group 25,4 ±1,6% (n=5; 0-3 years), to the typical (31,6 ± 9 %) and mild SLOS (33 ± 6 %). Bonferoni test proved that the ratio of α-lipoprotein in the severe SLO group was significantly lower than in the typical (p=0,003) and mild SLOS group (p=0,004).

DISCUSSION

The etiology of mental retardation is heterogenous. Exogenous factors such as perinatal infections, asphyxia or intrauterin teratogenic noxa (e.g. fetal alkoholic syndrome or maternal PKU) may be responsible for mental retardation, however the vast majority of the cases are explained by either inherited- or aquired genetical impairment. In our everyday practice differentiation of aquired forms of mental retardation, without associated genetical abnormality, from those where definitive genetical abnormality can be detected is a common difficulty. It is not surprising since consequences of morphological or functional lesions of the central nervous system are often similar to overlapping clinical features like epilepsy, mental retardation, behavioural dysfunctions just to mention a few. Skeptical mindset often

undermine the effort of a geneticist as the importance of establishing diagnosis for mentally retarded patients is queried saying that extensive, cost-and time consuming investigations may be senseless since there are no therapeutic consequences in the lack of specific treatment options, furthermore, developmental support and general medical approach to these patients highly overlap, regardless of the origin. Still, in my opinion we have to try to unravel these cases as much as possible for several reasons:

- Modern and progressively developing genetical methods enable us to exactly specify affected genes that are involved in a certain genetic alteration, and by knowing the function of those genes genotype-phenotype correlations may be recognized.
- From the practical point of view there are several advantages of knowing the exact genetical diagnosis:
    - The exact genetical diagnosis and ethiology help to determine and recognize syndrome-specific clinical features and somatic symptoms.
    - Precise knowledge of the genetical abnormality enable us to reveal early inevitable disease symptoms, which may be obscured at the time of diagnosis, but recognition their is helpful to plan ahead both preventive and therapeutic interventions.
    - A given genetical abnormality define the type, severity and prognosis of mental retardation. That information guides us in many ways, from selecting the optimal treatment for the patient to design early developmental and preventive programs.
    - It is obvious, the raising, nursing and caring of a child with mental retardation is a tremendously demanding job, a lifetime commitment for both the family and the society, which comes with inevitable psychological and financial difficulties. The most effective preventive effort is to establish an exact genetical diagnosis, which

enable us to carry out prenatal diagnosis in order to prevent the recurrence of the same disease in subsequent pregnancies in the same family.

Consequently, there is no doubt that every effort should be made to try to clarify the etiology of mental retardation and establish genetical diagnosis whenever it is possible.

6.1. *Diagnostic tools in the diagnosis of mental retardation, assessment of genetical alterations*

Our knowledge about genetical factors is growing rapidly as cytogenetic- and molecular biological techniques improve. Despite our broadening methological toolkit in diagnosing genetical diseases, only 60-75% of severe mental retarded patients have exact diagnosis, while in 80% of those with mild mental retardation the etiology remains unknown. That is why I aimed to introduce a relatively new genetical diagnostic method in our laboratory, which gave us the opportunity to obtain exact genetical diagnosis in some of those cases where previous investigative efforts failed to establish diagnosis The subtelomeric FISH examination is an efficient method for the detection of subtelomeric chromosomal rearrangements. The introduction of the subtelomeric FISH analysis opened a new possibility to clarify the etiology of mental retardation in selected patient population. In addition, data of 15 Hungarian patients with Smith-Lemli-Opitz syndrome (SLOS), a rare monogenic genetical disorder, are presented, among them the very first SLOS patient, whose genetical diagnosis was made in Hungary. Our workgroup (SLO group) managed to collect clinical and biochemical data of 15 newly diagnosed Hungarian SLOS patients where correlation between mutational analysis data and biochemical as well as clinical parameters have been made.

6.1.1. Subtelomeric chromosomal rearrangements

The aberrations (deletion, duplication, translocation) of the telomeric, subtelomeric regions, gene-rich areas of the chromosome, remains undetected with conventional G-band technique due to their bright (negative) staining (cryptic aberrations). Deletions, duplications of these gene-rich areas may result in unbalanced copy number variations with associated severe clinical syndromes. Subtelomeric chromosomal aberrations are of familial origin, which means that at least one of the parents carries a balanced translocation.

125 mentally retarded patients and 24 healthy family members were tested at the Genetic Laboratory of the Clinical Genetic Center Institute of Pediatric of Medical and Health Science Center of Debrecen University. Among 125 mentally retarded patients of unknown origin, previously unidentified subtelomeric chromosomal aberrations were identified in 13 patients. Four cases out of the 13 proved to be *de novo*, while nine other cases were familial The detection rate of the subtelomeric FISH examination was 10.4%, which value turned out to be higher than similar values in the literature. The subtelomeric FISH examination is a cost-and time-consuming method,that is why careful patient selection is very important, however too strict selection criteria may result in unrecognized positive cases (false negative). Indication for subtelomeric analysis was based on the selection criteria by Lam in patients with idiopathic mental retardation. Based on our experience, the single most important selection parameter is the repetition of mental retardation in a family.

The results of several thousand subtelomeric examinations, moreover the critical review and refinement of previously published cases provided further understanding of associated syndromes and helped to descibe new entities. It has been revealed that rearrangements of 1pter, 2qter, Xqter are more frequently seen compared to other chromosomal regions. That is why we frequently indicated subtelomeric chromosomal

analysis based solely on phenotypic features, especially in case of 1p36 deletion. Certain phenotypic characteristics of 1p36 partially overlap with those of Prader-Willi syndrome.
The 1p36 deletion syndrome is one of the most common subtelomeric chromosomal aberration with moderate/severe mental retardation, typical facial dysmorphy, hypotonic muscles, obesity and epilepsy.

Based on the similarity between the two phenotypes we have screened 12 patients for 1p36 subtelomeric deletion, of whom the P-W-specific FISH examination targeting the 15q11.2-region were negative. Before indicating 1p36 subtelomeric FISH, the following additional P-W specific tests were performed: detection of deletion of the specific region (15q11.2) by FISH, checking the methylation pattern for detecting gene inactivation and analysis of maternal uniparental disomy (UPD), which is due to the duplication of the inactive maternal allel. In none of the cases was deletion of 15q11.2 region shown. UPD examination was performed in 4 cases with negative results.

Two patients out of the 12 Prader-Willi suspect cases carried the 1p36.33 deletion (ish subtel del(1pter)), both had *de novo* origin.

The most common leading clinical feature of both Prader-Willi and the 1p36 deletion syndromes is the generalized hypotonia in infants, which is termed as „floppy-baby" sign. Based on our experience, we strongly suggest performing both P-W and 1p36 FISH tests in every infant with muscle hypotony for the detection of disease-specific deletions.

6.1.2. SLO syndrome

The clinical, biochemical and genetical diagnosis of my first patient presented in the thesis triggered the formation of our diagnostic and therapeutic team that has provided a complex investigation for such patients in a national diagnostic center for SLO, based in Debrecen. In our first case of SLOS we discovered a previously unidentified novel mutation in combination

with a known mutation in compound heterozygosity form, which resulted in disease manifestation. Based on the phenotypic features diagnosis of SLOS was raised at the age of six month of the patient, but it took years to clarify the biochemical and genitical background, until the introduction of UV-spectrophotometry and molecular genetical analysis was created. As biochemical and genetical diagnosis became available we managed to follow the effect of simvastatin and cholesterol substitution treatment. The efficacy of the therapy was assessed by monitoring changes in cholesterol and 7-DHC levels as well as by clinical symptoms.

Molecular genetic examination was carried out in patient as well as in other members of the family. Compound heterozygosity – c.964-1 G>C/c.1097 G>T (p.G366V) - was detected in the patient. The c.1097 G>T mutation, locating in exon 9 of the *DHCR7* gene, which affects the 4th cytoplasmatic loop, is a novel, previously unpublished, pathogenic mutation. This mutation is associated with a guanine-thymin nucleotide change, and results in an exchange of glycin to-valin aminoacids in the 7-dehydrocholesterol reductase enzyme. The c.964-1 G>C mutation, which was revealed in the mother and sister of the patient, is a characteristic genetic aberration of SLO syndrome. The c.1097 G>T mutation was detected in the father. No specific mutation was found in the proband's twin-brother.

Following the first molecular genetic examination that was performed abroad, we managed to collect and analyze the clinical data and genetical samples of 15 SLOS patients. Afterwards, the Genetical Center of the Pediatric Department of Debrecen University in collaboration with the Department of Molecular Genetics of Laboratory Medicine established the Hungarian SLOS Diagnostic and Therapeutic Research group. The presented case is the first Hungarian patient in whom mutational analysis was performed.

Shortly after the introduction of 7-DHC detection by UV-spectrophotometry, it became possible to confirm new cases and monitor simvastatin- and cholesterol substitution treatment efficacy. The genetical analysis of *DHCR7* gene performed by SLO workgroup

enabled us to detect family-specific mutations of new cases and most importantly prenatal diagnosis could be carried out.

Cholesterol substitution and simvastatin therapy was carried out after confirmation of the diagnosis. Combined cholesterol-simvastatin therapy was well tolerated by the patient, side effects were not observed. During the 25-months follow-up we experienced a significant increase in cholesterol level (from 2.77 mmol/L to 3.2 mmol/L) and a significant decrease in 7-DHC (from 102 mg/L to 53 mg/L). Improvement was detected in both antropmetric parameters and behavioral patterns of the patient, even though the therapy was only started at the age of 6 , after biochemical and genetical confirmation of the diagnosis was made.

Unfortunately, impairments in the SLO syndrome have already developed in early embrionic period, so the effectivness of the therapy introduced early is highly questionable.

An inverse correlation was observed between clinical severity and the initial cholesterol levels in 15 Hungarian SLO patients. Our findings suggest that the initial level of serum cholesterol fundamentally determines the severity and life expectancy of SLO patients. Significantly lower initial cholesterol levels were measured in the severe SLO group compared to the mild SLO group. We pondered that the ratio of Cho/7-DHC and α-lipoprotein has additional prognostic value in SLO syndrome.

6.2. Genotype-phenotype correlations

In case of subtelomeric chromosomal aberrations, phenotypic features are essentially determined by genes involved in deletion or duplication. The array-CGH is a useful technique for the determination of both the size of deleted-and duplicated regions and the localization of the breakpoints. Exact knowledge of the breakpoints enable us to investigate the genes involved and establish possible genotype-phenotype correlations. When the genotype is known one can predict clinical outcome and prognosis as well as the appearance

of corresponding symptoms in time. Array-CGH was performed in the members of two affected families.

ish t(8;12)(pter-,pter+;pter-,pter+)

Both members of the siblings inherited the derivated chromosome 8 from their father in the first family. In the subtelomeric region of the derivated chromosome 8 we detected part of chromosome 12, which resulted from 8pter deletion and 12pter duplication. 8pter deletion syndrome is associated with mental- and somatic retardation, cardiac anomaly and behavioral problems. Two 8pter deletion cases with size <5,1 Mb were reported in the literature, both were associated with mild mental retardation, behavioral disorders without cardiac anomaly. The authors detected that *GATA4* gene, which is supposed to be associated with cardiac anomalies, was not deleted because of the proximal position of the <5,1 Mb size deletion. In both siblings of the first family examined phenotypic features typical of dup(12p) syndrome, most importanly facial dysmorphy, were seen. Most of the published cases of dup(12p) syndrome patients are of familial origin. Despite the fact that phenotype is considerably influenced by both the size of duplication and the presence of partner chromosomes in the rearrangement, facial dysmorphy in dup(12p) syndrome is revealing, just like in our cases. Features like flat face, prominent frontal bossing and chin, short nose, downslanting corner of mouth, hyperthelorism, posteriorly rotated ears were observed.

ish der(21)t(10;21)(pter+,qter-)

In all three members of the second family we detected a familial subtelomeric unbalanced translocation between the 10p15.3-14 and 21q22.2-22.3 chromosomal regions, which was associated with moderate mental retardation, delayed speech development, muscle hypotonia, facial dysmorphy, cardiac-and orthopedic abnormalities. Until now, only four

cases of submicroscopic trisomy 10p have been reported. Comparing the phenotypic features of our patients with those previously described in the literature we found the highest similarity to the patients of Stone. In the presented cases the size of the 10p subtelomeric duplication (6,7 Mb) proved to be smaller than that published in previous reports. Stone and her collegues descibed two members of a family where an unbalanced translocation between 10p and 9p chromosomes was detected. The chromosome breakpoint was localized in the 10p14-15 region and the size of the duplication was 9 Mb. A patient with a 6,9 Mb 10p subtelomeric duplication (10pter-p15) and a subtelomeric 18q deletion (18q23-qter) was reported by others. Phenotypic features were as follows: psychomotor retardation, cardiopathy, strabism, umbilical hernia and facial dysmorphism.

Partial monosomy 21q-syndrome is a rare chromosome abnormality associated with varied clinical appearance. Depending on the size and location of the deleted region, and the partner chromosomes involved in the translocation, different phenotypic features have been reported. For example, holoprosencephaly (HPE) was reported in a case with pure partial monosomy 21q22.3. Based on the findings of two patients with partial monosomy 21q22.3 caused by an unbalanced translocation the region of HPE1 was defined as the most telomeric 4 Mb of 21q (chr21:41521879-46944323, based on UCSC 2006 hg18 assembly). The genotype-phenotype correlation has, however, not yet been completely delineated. Previous case reports of partial monosomy 21q mentioned five critical regions involved in the manifestation of major phenotypic features of the monosomy 21q, regions that are all located proximal to *ETS2*. According to publications the proximal limit of the terminal deletion is located between *HMG14* and *MX1*. However, in another study a 7,9 Mb terminal 21q deletion with inclusion of the *ETS2* gene was associated with a rather mild phenotype such as thin marfanoid build, facial anomalies, mild mental retardation. In our case, where *ETS2, HMG14* were not deleted and the breakpoint is proximal to the *MX1* gene, we detected moderate

mental retardation, speech delay, hypotonia, facial dysmorphism (ptosis, midface hypoplasia, micrognathia, long philtrum, malformed ears, thin upper lip), cardiac anomalies, scoliosis and kyphosis. The deletion of chromosome 21q found in our patients overlaps with the deletion described by Muenke, being the HPE1 critical region (UCSC 2006 hg18 assembly). Overlapping features with patient of Muenke are mental retardation, developmental delay and patella anomaly.

In our cases a duplication of 6.7 Mb of chromosome 10p15.3-14 and a deletion of 5.6 Mb of chromosome 21q22.2-22.3 were detected by array-CGH. In the duplicated region of the chromosome 10p15.3-14 as many as 21 genes are located. According to the OMIM database some of the genes involved have gene-phenotype correlation: *AKR1C2* (OMIM ref.: 600450, HGNC ID: 385), *IL2RA* (OMIM ref.: 147730, HGNC ID: 6008). Fifty-four genes are located in the deleted region of chromosome 21q22.2-22.3, of which some have been correlated with gene-phenotype relationship. Truncating and missense mutations of *COL6A1* and *COL6A2* gene cause Bethlem myopathy, an autosomal dominant inherited benign myopathy. These patients have moderate weakness and atrophy of the muscles of the trunk and the limbs with electromyography demostrating a myopathic pattern. In our case we observed muscle atrophy and weakness, scoliosis, kyphosis, joint hyperflexibility and habitual patellar subluxation, symptoms which may correlate with the loss of function of *COL6A1* and *COL6A2* genes as a consequence of haploinsufficiency of these genes. However, currently this correlation is only speculative, because exact verification would only be possible if gene products and their function were known.

3q29 microdeletion syndrome

3q29 microdeletion syndrome is a well described genetic disorder, which was first reported in 2001. A 1,5 Mb size deletion of the 3q29 region was detected in six cases. The characteristic

features of the phenotype are the following: mild-moderate mental retardation, long-narrow face, short philtrum, high nasal bridge, autistic behavoral, ataxia, chest-wall deformity, long-tapering fingers, microcephaly, cleft lip, cleft palate, horse kidney, hypospadiasis, joint hyperflexibility, abnormal skin pigmentation, reccurent ear infections. Additional phenotypic features such as vertebral anomaly, joint contactures, nasal voice, congenital cataracta and other ocular anomaly, cleft thumb have been reported by others. In our observed case, besides the characteristic signs, a rare ocular anomaly, uvea coloboma occured.

1p36 deletion syndrome

1p36 deletion syndrome was first described in 1997. It is the most common subtelomeric chromosomal aberration with an estimated incidence of 1: 5000-1:10000, a clinical entity with quite characteristic appearance and well recognizable features.

Phenotypic features are as follows: mental retardation, brachycephaly, muscle hypotonia, small hands and foot, brain anomaly, epilepsy, cardiac- and ophthalmic anomaly, obesity and hypothyreodism. The high-resolution cytogenetic analysis was shown to be a useful method to establish genetical diagnosis in cases associated with >5 Mb deletion and in complex aberrations. However, if the size of the aberration is smaller than 5 Mb, especially in cases of subtelomeric aberration or interstitially localized deletion, subtelomeric FISH and array-CGH methods should be employed. Hence from the aspect of differential diagnosis Prader-Willi syndrome, Angelman syndrome and Rett syndrome are to be considered. The 1p36 microdeletion syndrome shows significant overlap with Prader-Wili syndrome, that is why several scientific reports have been released about patients with „Prader-Willi like" phenotype. There was a correlation found between Prader-Willi phenotype and the 1p36 deletion syndrome, although others queried it. Epilepsy is a typical phenomenon of 1p36 deletion syndrome with reasonable clinical variety among patients. The pathomechanism of

epilepsy is not completely understood in these patients, however clinical outcome largely depends on the size of deletion of the terminal region of 1p, an otherwise gene rich region. For 1p36 syndrome there are two known genes responsible for epilepsy, which are located on the deleted segment: *KCNAB2*, a voltage-dependent potassium channel β–unit gene and *GABRD*, a human γ-butyric-acid-A receptor delta-unit gene. Such observations strongly support the idea that array-CGH examination should be employed in subtelomeric syndromes associated with epilepsy to gain further information about the genes involved and to clarify pathomechanism.

Smith-Lemli-Opitz syndrome

Expected incidence of Smith-Lemli-Opitz syndrome is ranging from 1:15900 to 1:17000, while in our northern neighbouring countries, the Czech Republic and Slovakia, similar values are ranging from 1:10000 to 1:20000. No corresponding data is available in Hungary. In order to estimate the incidence and carrier frequency in the Hungarian population it was required to introduce molecular genetic and biochemical diagnostic approaches, and to collect suspected SLO cases in Hungary. It is our pleasure that our Debrecen-based SLO team set up and has continuously participated in this project. The SLO phenotypic spectrum is broad and varied. Numerous research groups have studied the genotype-fenotype associations of SLO syndrome. Some of them established correlation between certain mutations and the severity of related clinical symptoms. For example, both W151X nonsense mutation and I251N mutations were reported to be associated with a severe form of SLO syndrome. In contrast, the IVS8-1G>C/V326L genotype seems to cause only mild symptoms. Anyhow, other authors pointed out that there was no clear correlation between mutations and phenotype, suggesting that the degree of severity and the prognosis might be influenced by other factors as well. The severity of SLO syndrome was found to negatively correlate with

plasma cholesterol, but not with 7-dehydrocholesterol, confirming previous observations. Our study group validated the inverse correlation between the severity of SLO and the initial level of serum cholesterol. The most prevalent DHCR7 mutations with relative frequencies over 4 %, are as follows: IVS8-1G>C (27,3 %), T93M (10,4 %), W151X (5,7 %), V326L (4,8 %) and R404C (4,5 %). These five mutations account for 50-60 % of the reported mutant alleles. Other less common mutations include R352W (2,8 %), E448K (2,8%), G410S (1,9 %), R242C (1,6%), S169L (1,6%) and F302 (1,5%), respectively. The importance of our patient's genotype is that c.1097 G>T mutation, in compound heterozygosity form, is a novel pathogen mutation, which has not been previously published.

This mutation is located in exon 9 of the *DHCR7* gene, it involves the 4th cytoplasmatic loop, and it is associated with guanine-thymin nucleotide exchange, which results in a glycine-valin aminoacid exchange in the 7-dehydrocholesterol reductase enzyme. Several multicentric studies have been published about possible therapeutic approaches in SLO syndrome. It seemed obvious that the most sensible approach was to correct the low cholesterol, a leading biochemical abnormality in SLOS. Treatment with cholesterol improves the sterol abnormalities seen in patients with SLO syndrome. The introduction of statins in the treatment was based on the fact that inhibition of HMG-CoA reductase results in a decrease in the precursors such as 7-DHC or 8-DHC. Moreover, upregulation of 7-DHCR activity was detected in human fibroblasts cultured *in vitro* in cholesterol-deficient medium supplemented with lovastatin.

Furthermore, simvastatin is able to enhance the expression of the deficient 7-DHCR. The increase in cholesterol synthesis is due to the increased expression of a mutant allele with a residual function. Dietary cholesterol therapy seemed to improve sterol profile in peripheral, but not CNS tissues. Treatment of Dhcr7 mice (T93M/Delta 3-5) with simvastatin decreased the 7-DHC levels in both peripheral and brain tissues. We observed significant improvement

in both biochemical parameters and behavioural patterns of the patient after the introduction of cholesterol substitution at 6 years of age. .

Recently, the effectivity of treatment of SLO syndrome has been queried. Current studies issued in the last couple of years concluded the lack of significant clinical improvement after treatment, raising serious doubts about previous observations. That has drawn attention to the fact that intrauterine cholesterol deficiency in early embryonic life presumably causes severe and irreversible damage in many organs without the chance of correction. Since maternal metabolism is not capable of compensating cholesterol deficiency like in other metabolic disorders, genetic diagnostic approaches, especially prenatal genetical analysis are of great importance.

6.3. Utilization of the results in medical practice

- ***Elaboration and development of current diagnostic algorithm***

According to the current recommendations by the International Standard Cytogenomic Array (ISCA) Consortium (2010), array techniques should be favoured in the evaluation of mental retardation, which indeed is a standard procedure nowadays in the United States and in Western European countries. In Hungary array techniques are not routinly used, mostly because of the lack of accessibility and high cost, that is why Hungarian clinical geneticists are „forced" to costumize international protocols to the circumstances in order to provide the best possible care for their patients. In all cases where mental retardation is associated with multiple anomalies conventional cytogenetic analysis is performed as a first step. Although conventional chromosomal analysis does not provide information about the underlying molecular abnormality at molecular level, positive result is satisfactory from the clinical point of view, because it clarifies both the genetical anomaly and the origin of mental retardation, besides it gives us a tool to prevent recurrence of the same problem in the family by doing

prenatal examination. In the case of normal karyotype or uncertain result (e.g. marker chromosome) additional molecular cytogenetical examinations are to be performed such as microdeletion FISH, subtelomeric FISH or multicolor FISH. When monogenic syndrome is suspected like SLO syndrome, Rett syndrome or Fragile-X syndrome special sets of molecular genetic analysis should be done. In case previous attempts are all negative and/or subtelomeric FISH examination turns out to be positive, array-CGH analysis is indicated to detect the chromosomal breakpoints. Recognition of affected genes and their functions enable us to determine clinical – genetical associations and gain further information about the possible pathomechanism of the disease, the appearance of related symptoms, eventually we get to know more about the prognosis and outcome. Genotype-phenotype correlations are helpful to clarify possible associations between a subtelomeric deletion and related clinical appearance, which in turn enable us to describe new, specific entities, may improve patient selection protocols, help to refine indications for FISH examination and to select from among available probes. These factors together might shorten the time required for the diagnosis and improve cost-effectiveness. Besides, improved patient-selection criteria enable us to refine detection rate and decrease the number of unnecessary examinations.

Hopefully, as more experience in molecular genetical techniques becomes widely available we face a decreasing number of mentally retarded patients with unclarified diagnosis and the clinical geneticist is helpless in prenatal risk assessment in ongoing pregnancies. Obviously, recurrence of the same problem in familial cases, where parents or related family members carry an unbalanced translocation or heterozygotic mutation can be prevented by clarifying the genetical background. It is needless to emphasize the importance of genetical investigation as it is beneficial for the patients, affected families and eventually for the whole society.

New results of the PhD

1. Most importantly, in accordance with international protocols and patient selection methods we integrated subtelomeric FISH analysis into our diagnostic algorithm that was specifically worked out for mentally retarded patients of unknown origin and /or with multiple congenital abnormalities with consideration to available resources. Using subtelomeric FISH examination we managed to detect subtelomeric chromosomal aberrations in 9 familial and in 4 *de novo* cases, our detection rate was 10.4% (13/125).
2. In collaboration with others we determined the size of deletion/duplication and investigated affected genes, providing possibility to clarify genotype-phenotype correlations of genes involved in translocations (COL6A1 and 2 genes).
3. In a SLO patient we described a previously unknown pathogenic mutation (p.G366V) in compound heterozygosity form. We have gained experience in cholesterol substitution and simvastatin therapy in SLO syndrome. In one case we observed improvement in both biochemical parameters and in certain clinical symptoms as it was concluded by using parental interviews and the Vineland Adaptive Behavioural Scale. We established a diagnostic and therapeutic workgroup in the Pediatric Institute in collaboration with the Institute of Laboratory Medicine.
4. Our SLO group collected the data of 15 Hungarian SLO patients. An inverse correlation was observed between clinical severity and the initial cholesterol levels. Besides the initial cholesterol level, 7-DHC and α-lipoprotein levels seem to have predictive value in the prognosis of SLO syndrome.
5. Lastly, by clarifying the exact genetical diagnosis and detecting family-specific mutations we managed to prevent repetitions in the same family, which effort resulted in the birth of five healthy newborns in „SLO families".

SUMMARY

The etiology of mental retardation is extremly heterogenous. Either inherited genetic factors or aquired disorders during the pre-, peri- or postnatal life could be responsible for mental retardation. Despite the enormous development of cytogenetic, molecular cytogenetic and molecular biological techniques, the cause of mental retardation remains unknown in about 60-75 % of moderate/severe mentally retarded patients and in 80 % of those with mild form of mental retardation. The aim of my work was to apply new genetic methods in our laboratory to either confirm or establish genetic diagnosis in those patients with idiopathic mental retardation in whom previous methods failed to reveal adequate diagnosis. The subtelomeric FISH is a useful method to detect subtelomeric chromosomal rerrangements of chromosomal segments located at gene-riche subtelomeric regions. Besides chromosomal structural abnormalities, monogenic disorders are common causes of mental retardation. In my thesis I present the first Hungarian SLO patient whose diagnosis was confirmed by mutational analysis. In 13 out of 125 mentally retarded patients of unknown origin subtelomeric chromosomal aberrations were identified. Among them four proved to be *de novo* origin while nine another cases turned out to be familial. ***De novo* aberrations were as follows**: **ish del(3)(qter-); ish del(1)(pter-); ish del(22)(qter-). *Familial aberrations:* ish der(8)t(8;12)(pter-,pter+); ish der(21)t(10;21)(pter+,qter-); ish der(4)t(4;8)(pter-;qter+); ish der(3)t(3;8)(pter-;pter+); ish der(10)t(10;17)(qter-;qter+).**

Both biochemical and molecular genetic examinations were introduced to confirm the diagnosis of SLO syndrome. A novel, previously not published, pathogenic mutation was detected in a compound heterozygosity form: **c.964-1 G>C/c.1097 G>T** (p.G366V) – in a patient diagnosed as the first one in Hungary with SLO syndrome. Our findings suggest that the initial level of serum cholesterol fundamentally determines the severity and life expectancy in SLOS and the ratio of Cho/7-DHC and α-lipoprotein has additional prognostic

value. Detection of genetic aberrations allows us to prevent the recurrence of further affected offspring in the family, while analysis of the genotype-phenotype correlations may help to understand pathogenesis and assess the prognosis.

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Item Number:
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Candidate: Gabriella P. Szabó
Neptun ID: BAFBXD
Doctoral School: Doctoral School of Clinical Medicine

List of publications related to the dissertation

1. Oláh, A.V., **P. Szabó, G.**, Varga, J., Balogh, L., Csábi, G., Csákváry, V., Erwa, W., Balogh, I.: Relation between biomarkers and clinical severity in patients with Smith-Lemli-Opitz syndrome.
   Eur. J. Pediatr. 172 (5), 623-630, 2013.
   DOI: http://dx.doi.org/10.1007/s00431-012-1925-z
   IF:1.907 (2012)

2. **P. Szabó, G.**, Knegt, A.C., Ujfalusi, A., Balogh, E., Szabó, T., Oláh, É.: Subtelomeric 6.7 Mb trisomy 10p and 5.6 Mb monosomy 21q detected by FISH and array-CGH in three related patients.
   Am. J. Med. Genet. A. 158A (4), 869-876, 2012.
   DOI: http://dx.doi.org/10.1002/ajmg.a.35236
   IF:2.304

3. **P. Szabó, G.**, Oláh, A.V., Kozak, L., Balogh, E., Nagy, A., Blahakova, I., Oláh, É.: A patient with Smith-Lemli-Opitz syndrome: Novel mutation of the DHCR7 gene and effects of therapy with simvastatin and cholesterol supplement.
   Eur. J. Pediatr. 169 (1), 121-123, 2010.
   DOI: http://dx.doi.org/10.1007/s00431-009-0987-z
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4. **P. Szabó G.**, Bessenyei B., Balogh E., Ujfalusi A., Szakszon K., Oláh É.: Szubtelomerikus kromoszómaátrendeződések kimutatása idiopathiás mentális retardációban.
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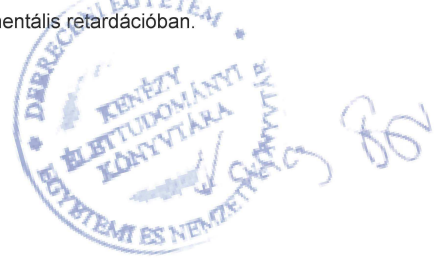

List of other publications

5. Balogh, I., Koczok, K., **P. Szabó, G.**, Török, O., Hadzsiev, K., Csábi, G., Balogh, L., Dzsudzsák, E., Ajzner, É., Szabó, L., Csákváry, V., Oláh, A.V.: Mutational spectrum of smith-lemli-opitz syndrome patients in Hungary.
Mol Syndromol. 3 (5), 215-222, 2012.
DOI: http://dx.doi.org/10.1159/000343923

Total IF: 5.855

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The Candidate's publication data submitted to the Publication Database of the University of Debrecen have been validated by Kenezy Life Sciences Library on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

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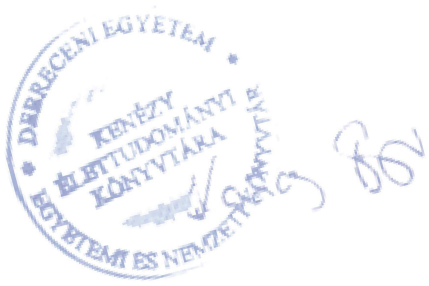

List of presentations related to the dissertation

P. Szabó G., Oláh A., Balogh E., Nagy A., Oláh É.: **Terápiás lehetőségek Smith-Lemli-Opitz szindrómában**, MGYT Fiatal Gyermekgyógyászok III. Konferenciája, Szeged, 2004. ápr.3.

P. Szabó G., Bessenyei B., Szakszon K., Ujfalusi A., Balogh E., Oláh É.: **8p; 12p szubmikroszkópikus kromoszóma átrendeződés kimutatása szubtelomerikus FISH vizsgálattal egy testvérpárban**, MGYT Országos Nagygyűlése, Eger, 2009. jún.18.

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Magyar Humángenetikai Társaság Konferenciája, Debrecen, 2010.09. 2-4.
Magyar Gyermekgyógyász Társaság Konferenciája, Esztergom, 2010.09.23-25.

P. Szabó G., A.C. Knegt, Boczán J., Mechler F. Oláh É.: **Bethlem myopathia előfordulása 10;21 szubtelomerikus kromoszóma aberrációban. A genotípustól a fenotípusig,** Magyar Klinikai Neurogenetikai Társaság Konferenciája, Budapest, 2011.dec.2-3.

P. Szabó G., Ujfalusi A., Balogh E., Szakszon K., Bessenyei B., A.C. Knegt, Oláh É.: **Szubtelomerikus kromoszóma átrendeződések kimutatása mentális retardációban,** Magyar Humángenetikai Társaság Konferenciája, Szeged, 2012.08.22-24.

G. P. Szabó, A. C. Knegt, E. Balogh, K. Szakszon, A. Ujfalusi, É. Oláh:
Subtelomeric study of 65 Hungarian patients with idiopathic mental retardation, facial dysmorphy and/or congenital anomalies, 8th European Cytogenetics Conference, Porto, 2011.07. 2-5.

Other presentations

P. Szabó G., Balogh E., Bessenyei B., Lengyel Zs., Karcagi V. Oláh É.: **Fragilis X szindróma halmozódása egy családban „Mit rejthet egy fénykép”**, MGYT Fiatal Gyermekgyógyászok IV. Konferenciája, Budapest, 2005. márc.20.

P. Szabó G., Balogh E., Ujfalusi A., Princzinger Á., Oláh É.: **Ritka citogenetikai eltérések mentális retardációban**, MGYT Észak-Kelet Magyarországi Területi Szervezetének Tudományos Ülése, Nyíregyháza, 2007.jún.8-9.

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A. Ujfalusi, E. Balogh, A. C. Knegt, G. P. Szabó, K. Szakszon, É. Oláh: **Rare *de novo* duplication of 9p24.3-9p13.1 in two Hungarian cases**
8th European Cytogenetics Conference, Porto, 2011.07. 2-5.