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

Clinical and genetic diagnosis and management of rare genetic disorders

by

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Abbreviations:

AFP: alpha-foetoprotein

ASD: atrial septal defect

CGH: comparative genomic hybridization

COX: cytochrome oxidase

DD: developmental delay

FISH: fluorescent in situ hybridization

FMF: fetal medicine foundation

FoA: foramen ovale apertum

IUGR: intrauterin growth retardation

MCA: multiple congenital anomalies

MLPA: multiple ligation-dependent probe amplification

MR: mental retardation

MWS: Mowat-Wilson syndrome

NADH: nicotinamide adenine dinucleotide

OFC: occipitofrontal circumference

SBBYSS: Say-Barber/Biesecker/Young-Simpson syndrome

SNP: single-nucleotide polymorphism

UPD: uniparental disomy

VSD: ventricular septal defect

WES: whole exome sequencing

WGS: whole genome sequencing

Keywords: rare disease, syndrome, congenital anomalies, mental retardation, genetic

I. Introduction

„Rare diseases are rare, but rare disease patients are numerous" – says the motto of the Orphanet, the reference portal for information on rare diseases and orphan drugs. If all of the people with rare diseases lived in one country, it would be the world's 3rd most populous country.

Increasing attention is devoted to this group of patients for several reasons: 1. The recognition of a rare disease – not to mention clinical diagnosis and molecular confirmation may take years due to lack of knowledge of physicians, limited or no access to certain diagnostic tests, and confusing patient routes. 2. Numerous rare diseases are rapidly fatal or devastating, and a considerable ratio of affected individuals die before even receiving a proper diagnosis. 3. For 95% of rare diseases, no approved cure or definitive treatment exists.

The era of subspecialization within the specialty of medicine resulted in increasing knowledge of professionals in their chosen fields but narrowed their field of vision in others, causing a gradual decline in comprehension of the coexisting symptoms as intimately interconnected and explicable only by reference to the whole. Specialization lead to fragmentation of care and discontinuity even for patients with a single disease *(Detsky et al 2012),* and any improvements resulting from having more highly trained specialists deliver specific services can be offset by the quality-eroding and time-consuming effects of such complicacy of health care when it comes to diagnosing and treating patients with multiple, chronic symptoms as is usually the case with sufferers of rare diseases.

According to the data of the Rare Diseases Impact Report, it takes an average of 7 years for a patient with a rare disease to receive a proper diagnosis. On the journey to diagnosis, a patient typically visits up to eight different physicians and receives two to three misdiagnoses. Not surprisingly, patients with rare diseases have an increase in stress, anxiety, worry and feelings of isolation *(Boice 2013).*

Translating these data into Hungarian terms, an estimated 50 000 citizens of the country live with a rare disease, many of them not even knowing the name of their condition or not even being aware of the fact that their diverse and separately treated symptoms can be attributed to a single disease.

The Clinical Genetics Center, operating in the Institute of Paediatrics at the University of Debrecen Medical and Health Science Center has been dedicated its work to the diagnosis and treatment of patients with rare diseases for over 5 years now, and had done so in the past 40 years, before even existing as a center. Known for the cytogenetics laboratory appertained, patients – mainly children – with suspected chromosomal abnormalities and malignant diseases were referred for expert opinions and in many of them, chromosomal etiology was found. Yet, numerous cases remained unsolved, and as the attention of diagnostic medicine and of the public shifted towards rare and extremely rare diseases, as the scope of technology expanded, significant changes occurred in the needs and challenges we faced: patients with an increasingly wide spectrum of morphological anomalies and/or disabilities began to seek medical help, and in the first place: diagnosis.
Given the centralized structure of the molecular diagnosis of rare diseases in a common effort to improve cost-effectiveness and allow expertize to develop, apart from expanding our own profile we made serious efforts to go beyond borders if a patient's problem required so. Molecular cytogenetic tests, numerous molecular genetic tests and often consultations on very difficult cases where national experiences are lacking, are carried out with the help and contribution of foreign countries.

The focus of the present dissertation is the diagnosis and management of rare diseases with known or suspected genetic origin. Applying a factual, one-by-one evaluation and classification of patients seeking medical help at the Clinical Genetics Center in the Inst. of Paediatrics, University of Debrecen in a 5 ½ year-long-period, the author presents the results

of a diagnostic work dedicated to patients suffering from rare genetic syndromes. Some extremely rare conditions are reported, two of which with associating malformations first described in the literature. Reasons for success and shortcomings are discussed, further steps and future goals are delineated.

II. Review of the literature

II./1. Prevalence of rare diseases and their significance in health care

A disease or disorder is defined as rare in Europe when its prevalence is less than 1:2000. To date, approximately 7000 rare diseases affect approximately 350 million people wordlwide and over 30 Million people in the European Union alone, making these conditions a serious public health concern. 80% of rare diseases are genetic of origin, and vice verse: 80% of genetic disorders are rare. The remaining 20% are caused by infections, environmental damage, or are immunological, degenerative and proliferative by nature *(Bavisetty et al 2013; Eurordis- Rare diseases Europe 2012; Yaneva D.M. 2011).* Increasing evidence supports the major role of genetic predisposition in this group of diseases, too. A considerable proportion of rare diseases are dysmorphic syndromes, some of which being ultra-rare, having patient populations of fewer than 100 individuals worldwide. Quintessentially disabling, the patients' quality of life is affected by the lack or loss of autonomy due to the chronic, progressive, degenerative, and frequently life-threatening aspects of their condition.

Rare diseases are characterised by a broad diversity of symptoms that vary not only from disease to disease but also from patient to patient affected by the same disease. Because these diseases are so diverse and complex, there are inherent gaps that exist in patient care and physician resources, leading to misdiagnosis and delay in treatment. Autopsies in the United

States reveal up to 40% discrepancy between the diagnosis provided by physicians and those disclosed by pathologists, one third of which treatable if diagnosed correctly. This astonishing ratio is referred to as „Low-tech autopsies in the era of high-tech medicine" by G.D. Lundberg *(Lundberg 1998).*

The fact that there are often no existing effective cures adds to the high level of suffering and isolation endured by patients and their families. Also the need for appropriate quality health care engenders inequalities and difficulties in access to treatment and care. This often results in heavy social and financial burdens on patients. Lack of sufficient knowledge on most rare conditions, lack of international registries and follow-up studies call for a broader distribution and better sharing of information, encouraging publication of clinical cases, education of health-care providers and beyond borders consultations of patients and professionals *(Carey 2010).*

II./2. Rare diseases in the pediatric population

The significance of rare diseases is especially high in the pediatric population as 50% of rare diseases touch children, presenting often as birth defects or multiple congenital anomalies *(Eurordis- Rare diseases Europe 2012).* 20-30% of all neonatal deaths and 30-50% of post-neonatal deaths are due to genetic disorders *(Hudome et al 1994; Schulpen et al 2006; Stevenson and Carey 2004).* In the 1990's 29.5% of pediatric hospital admissions were for children with genetic disorders and 12% of adult hospital admissions were for genetic causes *(Emery and Rimoin 1990; Kumar et al 2001).* According to a more recent paper, changes in health care delivery in the past 25 years, especially trends towards outpatient care of uncomplicated problems and a greater understanding of the genetic basis of many disorders lead to a much higher proportion of genetic diseases among pediatric inpatient – up to 71% in

the United States - representing a 81% share of the total health care charges *(McCandless et al 2004).*

As a result of the global fall in infant mortality and birth rate, the focus of health policy development is shifting from acute problems towards management of chronic disease and disability, and from vertical programmes towards integrated health care systems. This change is particularly marked in the area of reproductive and child health, because an increasing proportion of infants born with birth defects or potentially disabling conditions, who would previously have died undiagnosed, now survive and require medical and supportive interventions *(Christianson et al 2006).* In many developed countries infant mortality is now lower than the birth prevalence of potentially lethal congenital disorders *(Working group of the Eastern-Mediterranean Regional Office of WHO 1999).* Due to steady improvements in general health care, many rare disease patients now survive into adulthood and require medical help for chronic, age-related symptoms, or become affected by a "second" disease independent from the underlying condition, thus further increasing the socioeconomic burden of rare diseases.

II./3. Causes of birth defects

The causes of birth defects are many and complex. According to Turnpenny and Ellard, chromosomal anomalies visible by G-banding account for an approximate 6% of congenital disorders (including Down syndrome representing half of the cases), an additional 10-14% is caused by submicroscopic copy number changes. According to a more recent report, chromosome abnormalities can be detected in one in every 10 investigated patients with developmental delay *(van Karnebeek et al 2005).* Mendelian disorders represent another 7.5%, and environmental causes can be identified in 5- 10% of cases. In 20-30%, the

underlying genetic cause is multifactorial, and we have no exact data on the prevalence of UPD and imprinting defects in the genetically ill population *(Fig.1)*. We do know, however, that while the prevalence of intellectual disability is 1-3% in the general population *(Shevell et al 2003)*, in an estimated 38-40% of cases the genetic cause remains unknown, especially if the intellectual disability is very severe or mild and is not accompanied by dysmorphic signs *(Miller et al 2010; Turnpenny and Ellard 2005)*. A significant improvement is being achieved in this group of patients with the increasingly widespread use of genome-wide assays.
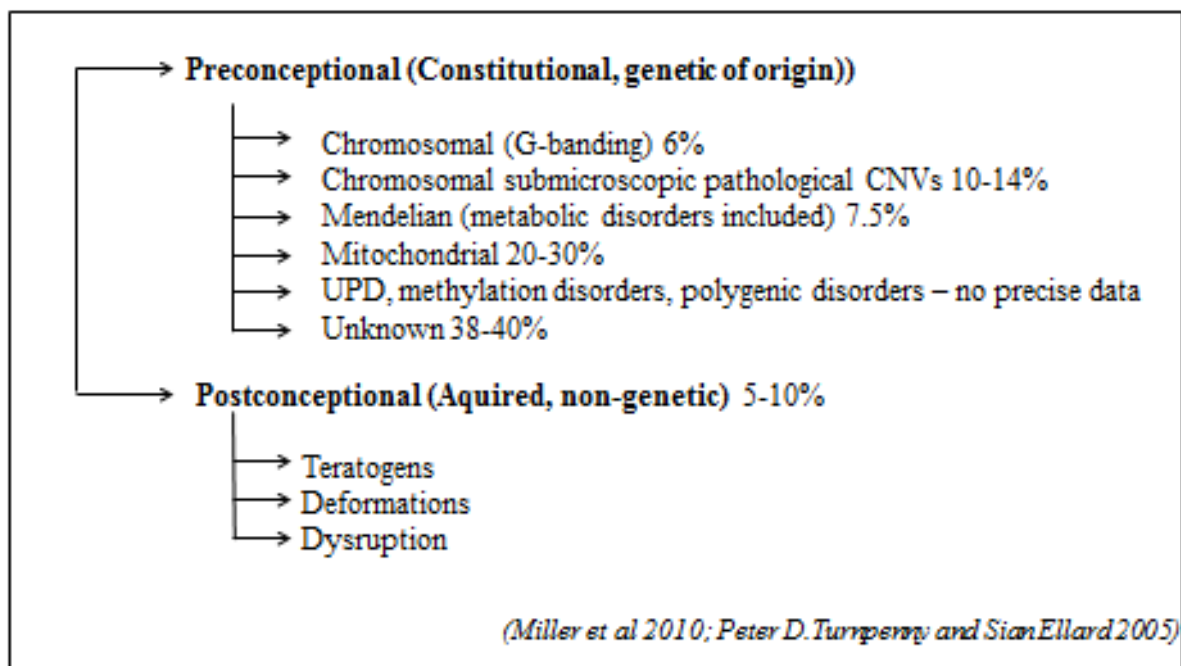
Fig. 1. Causes of birth defects according to Turnpenny and Ellard, 2005.

In Hungary, the total birth prevalence of all isolated major congenital anomalies is about $600/10^4$. These congenital anomalies may cause, per 10^4 livebirths, about 4800 years of life loss, about 37000 years of potentially impaired life and about 4500 years of actually impaired life *(Czeizel and Sankaranarayanan 1984)*. More recent data by Beke A. and Papp Z. report on a 1.4% and 1.78% share of mendelian disorders and chromosomal aberrations in perinatal mortality, respectively, not including other modes of inheritance that are suspected not to cause perinatal deaths. *(Beke and Papp 2001)* Considering the deficiencies of

registration discipline to the National Registry of Birth Defects, the true prevalence of birth defects may be undercounted.

II./4. Dysmorphology and syndromology – history and general perspectives

Due to their low prevalence, the diagnosis of rare diseases is often extremely difficult, pricy and time-consuming. Until the 1990's, genetic testing of rare diseases were restricted to karyotyping, fluorescent in situ hybridization (FISH) testing for the most common microdeletions, multiple ligation-dependent probe amplification (MLPA), biochemical assays and single gene sequencing – methods that are still in use and inevitable even nowadays. In the past 10-15 years, the advent of genome-wide studies, the use of array-based molecular cytogenetics on a routine diagnostic basis and the increasingly widespread application of „panel-testing" with next generation sequencing as well as of whole exome (WES) and whole genome sequencing (WGS) have fundamentally changed today's genetics, largely contributing to the identification of new genes in syndromes previously of unknown origin, of the recognition of new syndromes and of the better understanding of genotype-phenotype correlations. The tremendous amount of information obtained by these tests, however, require a whole new approach and refined interpretation of genetic results. Providing a diagnosis based on phenotypic features without the support of molecular or molecular cytogenetic tests where available is not acceptable any more. On the other hand, choosing the right method with the highest possible diagnostic yield and the lowest possible cost in a given case demands precise phenotyping, detection and evaluation of symptoms, identification of signal signs on a level more advanced than ever before. *„Next generation sequencing requires next generation phenotyping"* says the title of a recent paper of one of today's most acknowledged syndromologist, Prof. Raoul C.M. Hennekam *(Hennekam and Biesecker 2012).* For this, a

profound knowledge on the mechanism of genetic diseases, study of the normal and abnormal human form and function, sufficient clinical experience and complex knowledge in several fields of medicine (embryology, anatomy, endocrinology, neurology, psychiatry) are needed.

„*The study of abnormal form*" as described by the seminal figure of dysmorphology, David W. Smith (1926-1981) has evolved from a small nucleus of clinicians in the 1950s into a recognized and widely practiced discipline, and more recently has incorporated translational research into developmental biology, molecular genetics, and metabolic medicine *(Allanson et al 2009a)*. A pediatrician, endocrinologist, embryologist and clinical geneticist himself, David Smith's chosen specializations were a true example of the complexity of the knowledge dysmorphology demands. In his book entitled Diagnostic Dysmorphology, his former student – acknowledged professional himself John M. Aaase describes: "*As a scientific discipline, dysmorphology combines concepts, knowledge, and techniques from the fields of embryology, clinical genetics and pediatrics*". He nevertheless states that dysmorphology practice cannot be restricted to the consultation rooms of rare diseases – it is present in all fields of medicine. "*As a medical subspecialty, dysmorphology deals with people who have congenital abnormalities and with their families. Whenever any physician is confronted by a patient with a birth defect, he or she becomes, for the moment at least, a dysmorphologist*" *(Aase 1990).*

In its original Greek meaning the term „dysmorphy" stands for „badness of form". Its first clinical application was recorded in 1954, and gained further content since then. Today the term dysmorphy is used to describe any feature on the human body of which the size, shape, position, or even its presence or absence differs from the anthropological characteristics of the healthy individuals in a given population. Certain anatomical features - e.g.: intercanthal distance, head circumference - are quantitative, their deviation from the normal can be objectively described, parametrized. Significant are those that exceed the healthy control extremes by >2 SD in a given population. Others are quality features - their

presence or absence may denote an underlying genetic etiology. Description of dysmorphic features require the use of specific terms collected in the London Dysmorphology Database (n=683), updated and refined in 2009 by the world's 34 leading dysmorphologists *(Allanson et al 2009a; Carey et al 2012)*. Important it is to note that the presence of isolated dysmorphic signs does not necessarily suggest a genetic disorder – minor anomalies (simian crease, depressed nasal bridge or clinodactyly) may be found in otherwise healthy individuals. Genetic origin may be suspected if single or multiple major congenital anomalies occur with or without mental retardation/developmental delay, or if multiple minor anomalies go together with intellectual deficit. The association of certain dysmorphic features - especially if they are accompanied by developmental delay - may appoint to a common etiology and thus may be parts of a more complex entity called a *syndrome*. With sufficient experience and knowledge on genetic conditions, symptoms that imply to a common origin - called „signal signs" - may be identified and highlighted from the several other, non-mandatory symptoms of a certain disorder. Except for some extremely rare entities, dysmorpic syndromes have *mandatory, frequent* and *occasional* symptoms. *Mandatory* are those that constitute the syndrome – without their presence the diagnosis is invalid or rather questionable (e.g. in Hanhart syndrome the association of hypoglossia and hypomelia are critical fetures of the syndrome - if any of the two is missing, the diagnosis cannot be set). *Frequent* signs are those that often accompany the mandatory symptoms and provide further strong diagnostic clues if present. *Occasionals* are the symptoms that have ever been described in a syndrome beside the mandatory and frequent signs. As such, objective requisits significantly reduce misdiagnoses derived from subjective judgements. An example for a better understanding: patients with Noonan-syndrome may present over 50 dysmorphic signs/functional anomalies (short stature, macrocephaly, sparse, curly and kinky hair, antimongoloid slant of palpebral fissures, low-set ears, thick helices, loose nuchal skin, pectus carinatum, cardiomyopathy, congenital cardiac

anomaly, feeding difficulties, café-au-lait spots or freckles, scoliosis, intellectual deficit – just to mention a few). However, the critical features out of all these are short stature, congenital cardiac anomaly, antimongoloid slant of palpebral fissures and skeletal malformations. By picking out the right tetrad of symptoms, the mandatory and the non-mandatory symptoms together underpin the suspicion of Noonan-syndrome, while the wrong definition of the signal signs may divert our thinking to another entity, which later proves to be incorrect, causing diagnostic delay, unnecessary testing-related costs and uncertain prognosis. The science of syndromology is therefore a more advanced application of dysmorphology, where the right combination and correct identification of signal signs result in the identification of the right syndrome, which then, of course, has to be proved with appropriate genetic tests. According to the definition of Seemanova, *syndromology is a diagnostic method based on the analysis of phenotypic features, by which seemingly separate symptoms that mark a common etiology can be identified, the differential diagnostic spectrum can be narrowed and the true diagnosis is delineated (Seemanova 2002).*

II./5. Structure and strategy of the laboratory diagnosis of rare diseases

The early and accurate diagnosis of rare diseases has gained increasing importance over the past decade. Although their prevalence per diagnosis is low, their cumulative prevalence is high enough to make them a significant health issue. Given the rarity of most conditions, confirmatory laboratory tests are centralized all over the world to ensure expertise and cost-effectiveness in testing. According to the Orphanet database, over 15,000 professionals work in over 5000 accredited expert centers and laboratories worldwide, and operate via networking. No single lab can afford to set the diagnostic tests of all known genetic disorders or gather knowledge over all of them - each develops a spectrum of diseases they test for. In case of dysmorphic syndromes, the baseline test to clarify the underlying problem is comparative genomic hybridization (CGH; resolution 2-120 kB) in most

developed countries - in others conventional karyotyping is still in use (resolution >10 Mb) For obvious chromosomal syndromes (trisomies 13, 18 or 21), suspected balanced reciprocal translocation carrier status or whole chromosomal imbalances in malignant diseases, G-banded karyotyping is the test of choice. For known microdeletion syndromes [1p36 deletion syndrome; Wolf-Hirschhorn (critical region: 4p16.3), Cri-du-chat (5p15.3), Williams (7q11.2), Kleefstra (9q34.3), Prader-Willi/Angelman (15q11q13; hereafter 15q11), Rubinstein-Taybi (16p13.3), Smith-Magenis (17p11.2), apparent Down-syndrome without whole trisomy of chromosome 21 (critical region: 21q22.1), Di-George/Velocardiofacial (22q11.2)] region-specific FISH or MLPA can be used. Syndromes due to loss of heterozygosity or uniparental disomy (certain ataxias, Beckwith-Wiedemann, Prader-Willi/Angelman) are best tested with SNP arrays, where not available, DNA marker analysis using parental blood serve as an option. Methylation defects (Silver-Russel, Beckwith-Wiedemann syndrome) can be tested using bisulfite conversion, methylation-sensitive restriction enzymes, methyl-binding proteins or anti-methylcytosine antibodies combined with massively parallel sequencing at a genome-wide level if necessary. Mendelian syndromes require traditional Sanger-sequencing and/or deletion testing with MLPA. If more than one genes can cause very similar phenotypes, next generation sequencing provides a highly efficient alternative. Syndromes of mitochondrial origin can be proved using sequencing the mitochondrial genome and "numts" (nuclear sequences of mitochondrial origin = fragments of mitochondrial DNA present in the nuclear genome).

Metabolic disorders are tested using tandem mass spectrometry, biochemical assays from plasma and urine or targeted DNA sequencing. In Hungary, tandem mass spectrometry is available since 2007. for 26 treatable metabolic diseases at the University of Szeged and at the Semmelweis University in Budapest, saving an average of yearly 50-60 good quality, often symptom-free lives. Latter also offer testing for 4 lysosomal storage diseases.

Syndromes of teratogenic origin and sequences resulting from intrauterin constraint of the fetus can only be confirmed based on detailed maternal and fetal anamnestic data. Known syndromes of unknown origin or unknown syndromes with normal array CGH profile may be undertaken whole-exome or whole genome sequencing to screen for candidate genes. If a potentially pathogenic mutation is found, parental mutation analysis is suitable to confirm or disapprove de novo origin in the offspring. Considering advances and limitations, the test of choice and further investigations depend on the suspected diagnosis, their diagnostic yield largely determined by the experience and accurate judgement of the clinical geneticist.

II./6. Genetic counseling

If the suspicion of a syndrome is raised and the underlying genetic defect is known, it has to be proved to support the diagnosis and provide a basis for counseling and future prenatal diagnosis. It is no longer acceptable to make a diagnosis from phenotypic features only, unless the genetic cause is unkown. Even in such cases, genome-wide assays (CGH, WES or WGS) are strongly suggested.

It is the responsibility of the syndromologist/genetic counselor to clarify a rare disease or syndrome as much as the existing knowledge and technical power allow, and inform the patient, parents or caregivers about the nature of the disease, its recurrence risk and prognosis. Syndrome atlases, Internet databases, original descriptions, consultation with other professionals, self-help groups or even parental blogs may serve as help in this often enormous work. In desperate cases where the diagnosis is well-founded, inheritance is autosomal recessive or mitochondrial, but no molecular tests are yet able to prove it - the underlying mechanism being unknown - information on the high (25%) or unforseeable recurrence risk has to be transmitted to the parents and a solution should also be offered with it: replacing one of the two gametes (preferably the male gamete) or receiving enucleated donor oocytes into which the nucleus of the genetic mother is transferred and then fertilized

with the genetic father's sperm reduces risk down to the populational background risk. If this is acceptable for the couple, it offers a chance for a healthy offspring.

When counseling families, care should be taken with how to address and interpret the health problem of their loved one. When describing a patient with dysmorphic features, Allanson et al. recommend the avoidance of terms with possibly negative or pejorative meaning- it is not ethical to label a person as „invalid", „dwarf", or „retarded" - instead, neutral terms such as „individual with a genetic condition", „short statured" or „developmental delay" should be used. Careful approach is suggested when declaring features „normal" or „abnormal", as the border between is not a clear line but rather a range of severity *(Allanson et al 2009a)*. Likewise, evaluating a patient in a very early stage of a disease or very early in their lives - especially newborns and premature infants - requires caution as the clinical picture may change over time. Certain features that were previously considered dysmorphic may fade out or diseappear, while others may fade in and become evident. For this reason, regular follow-up should always be provided, and time should be given - for the patients to develop as much as their capabilities allow, and for parents/caregivers to come to terms with the situation they are in. Yet, coping strategies can develop only if a person knows what he/she has to cope with, demanding indulgence and truthfulness from the geneticist at the same time.

„Families pursue genetic counseling in an effort to demystify the mysterious. They seek answers and information. If they did not want to hear it all, they would not bother with genetic counseling. Families want an honest evaluation of what is known and what is unknown, a clear explanation of all possibilities, both good and bad, and a sensitive exploration of all available information with which they can make knowledgable decisions about future family planning" (Bloch et al 1979).

II. /7. Objectives

The aim of my work was to:

- Provide precise clinical and genetic diagnosis to each patient attending to the outpatient clinic of the Clinical Genetics Center.

- Study genotype-phenotype correlation where molecular genetic or molecular cytogenetic tests could clarify the underlying genetic defect and the number of cases allowed to draw such conclusions

- Provide genetic counseling for families, including risk-assessment and prenatal diagnosis where possible.

- Follow-up patients, learn about their development before and after the diagnosis, delineate realistic goals with respect to their condition, refer them for habilitation and rehabilitation, initiate treatment where possible.

- Finally, an important goal was to define in what proportion of patients referred to our clinical genetics center a genetic abnormality/rare disease could be proved, which group of genetic origin they represented and in what ratio– whether the distribution of our diagnoses reflect the international prevalence data and whether the strict policy and limited resources of the Hungarian health care system facilitate or hinder the diagnosis of rare and extremely rare diseases. We also aimed to point out what means would be urgently necessary to step forward in the critical issue of rare diseases.

The following strategy was used:

- All patients with any degree of mental retardation and/or multiple congenital anomalies were undertaken G-banded karyotyping, given that CGH as a first-tier test is not yet available for us and only selected cases can be referred to a molecular cytogenetic center.

- Recognizable chromosomal syndromes (Cri-du-chat, Jacobsen, De Grouchy, trisomies 13, 18, 21, X monosomies and trisomies) could be confirmed by G-banded chromosome analysis. G-banding yielded to surprising results in several other, less obvious cases.

- Chromosomal mosaicism, the origin of small supernumerary marker chromosomes and of derivative chromosomes, known microdeletion syndromes and subtelomeric rearrangements were clarified with FISH.

- Malformation sydromes with suspected submicrospcopic chromosomal origin were referred to CGH testing.

- In cases of clear mendelian disorders, traditional Sanger-sequencing, mutation analysis, or MLPA were performed.

- Methylation defects were detected with methylation specific PCR, uniparental disomies were identified using DNA microsatellite marker analysis.

- Complex dysmorphic syndromes of unknown genetic origin were identified based on phenotypic features, including objective tests such as X-rays, electrophysiology (EEG, EMG, ENG, ERG), anthropometric measurements, MRI, and consultation with qualified practicioners of other medical subspecialties (oto-rhino-laryngology, ophthalmology, neurology, psychiatry) or foreign expert centers. Online databases, textbooks, syndrome atlases, photo documentation of self-help groups were used for search and to compare the phenotype of the patient with those available in the literature or online, diagnosed by others. Where possible, chromosomal origin was excluded with CGH prior to the final diagnosis.

III. Materials ans methods

III./1. Patients and classification system

Data of patients seeking medical help in the genetic outpatient office of the Clinical Genetics Center, Institute of Pediatrics, Medical and Health Science Center, University of Debrecen, were assessed in the time interval of August 01. 2007- March 31. 2013., a 5 ½ year long period. Data were collected by the Dept. of Patient Documentation and Financing and offered for further processing. An overall 6136 visits were recorded in the above time interval, out of which 5432 were handled by the author, representing 2049 patients. Anamnestic data, status, presumed or proved diagnosis and suggested further diagnostic tests were assessed and patients were categorized into one of the 10 categories based on the genetic mechanism of their condition: **1. Chromosomal,** visible by **G-banding**, **2**. **Gains or losses** of genetic material detected with **fluorescent in situ hybridisation** (most common microdeletion syndromes, cases with small supernumerary marker chromosomes, certain cases of mozaicism), **3. Chromosomal submicroscopic** detected with **comparative genomic hybridization**, **4. Single gene disorders**, **5. Uniparental disomies and methylation defects**, **6. Mitochondrial diseases**, **7. Polygenic/Multifactorial**, **8. Phenotypically diagnosed but not molecularly proved**, **9**. Patients with **infertility** without detectable genetic anomalies, **10. Unclarified** conditions with presumably genetic origin.

Patient data are displayed in corresponding tables, two unique cases from each group are described in details in the Results section. Informed consent from parents was obtained to perform genetic tests and use photo material.

The author wishes to remark that access to CGH has been possible only in the past two years in a foreign laboratory, and several molecular tests for monogenic diseases have been available in Western-European countries only. Both require application for cost-coverage

from the National Health Insurance Fund on an individual basis with an average lead time of 3 months.

Technical methods used for diagnosing a syndrome or rare disease were: G-banded karyotyping, region-specific, multicolor and subtelomeric FISH, DNA sequencing (next generation in only one case and traditional Sanger-sequencing in the remaining), CGH, Southern-blot, methylation sensitive PCR, DNA microsatellite marker analysis, and biochemical assays in metabolic disorders. Given the large heterogeneity of the conditions and of their genetic cause included in the present work, the detailed description of the testing of each and every disorder would exceed the frames of this work, therefore a general overview of the applied methods is provided here only.

III./2. Genetic tests

III./2.1. Cytogenetics – G-banded karyotyping (results described and interpreted by Dr. Erzsébet Balogh, Univ. of Debrecen)

Karyotyping based on Giemsa-Trypsin-Giemsa banding (~500 band resolution) was performed on metaphase peripheral blood lymphocytes using standard methods. Karyotype was described according to the ISCN (2009).

III./2.2. Fluorescent in Situ Hybridization (FISH; performed by Dr Anikó Ujfalusi, subtelomeric by Dr. Gabriella P. Szabó, Univ. of Debrecen)

FISH analysis was performed using commercially available probes (or mixtures of probes for subtelomeric testing and multicolor FISH) (Vysis, Illinois, USA) according to the manufacturer's instruction. Fluorescence images were captured with a Zeiss Axioplan2 epifluorescence microscope and analysed by ISIS software (MetaSystems, Germany).

III./2.3. Array Comparative Genomic Hybridization (CGH; performed exclusively by Dr. Alida C. Knegt, Academisch Medisch Centrum Amsterdam, The Netherlands).

Array CGH was performed using a 4 x 180K oligo array, (custom design ID: 023363, Agilent technologies Inc., Santa Clara, CA, USA). Fluorescent labeling of gDNA was performed using the CGH labelling kit for Oligo Arrays (Enzo Life Sciences, Inc. Farmingdale, NY, USA). The array hybridization and washing was performed as specified by the manufacturer (Agilent Technologies). Arrays were scanned using an Agilent G2250C scanner and Agilent Scan Control software using default settings, and analyzed using Feature Extraction software and Genomic Workbench (Agilent). All genome coordinates mentioned in this study are according to human genome build 18 (NCBI 36.1).

III./2.4. Fibroblast culture (performed by the author)

Skin biopsy was taken from the patient under local anaesthesia. All cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) (Sigma-Aldrich, St. Louis, MO, USA) in 37°C incubator with 5% CO_2. Cells were used before 15 passages.

III./2.5. Mutation analysis (performed by Beáta Bessenyei and Dr. István Balogh, Univ. of Debrecen, Dr. Noémi Polgár, Dr. Katalin Komlósi and Dr. Judit Bene at the Univ. of Pécs, and foreign experts if certain cases)

DNA isolation from blood leukocytes was performed using a commercial system (QIAgen Blood Mini Kit, Qiagen, Germany). Exons of the candidate genes were amplified using sequence-pecific forward and reverse primers. For DNA sequencing the PCR products were purified using ultrafiltration microcolumns. Purified PCR products were sequenced.

Removal of unincorporated nucleotides was performed using gel filtration. Purified DNA was undertaken capillary electrophoresis.

III./2.6. Microsatellite marker analysis (performed by Dr. Márta R. Czakó, Inst. of Genetics, Univ. of Pécs)

DNA isolation was performed using the salting out technique of Miller et al. *(Miller et al 1988)*. Primer sequences of the microsatellite markers of interest were constructed based on the Ensembl Genome Browser (www.ensembl.org). Amplification of markers was performed according to the following thermal cycling: denaturing at 94 ℃ for 3 min, 38 cycles at 94 ℃ for 45 sec per cycle, annealing at 55-57 ℃ for 30 sec, synthesis at 72 ℃ for 30 sec, final extension at 72 ℃ for 3 min, cooling at 4 ℃. After cooling, DNA was stored at -20 ℃. Amplification was tested on a 2 % agarose gel with ethidium-bromid dying. PCR products were separated in 8% polyacrlylamide gel and were visualized with silver dye. The alleles of the index patient were compared to his/her parents. Markers for which the parents were homozygous (two alleles with the same size on the gel-electophoresis) were excluded from the analysis. Likewise, alleles that the parents were heterozygous for but the parents-child trio carried the same two alleles were not taken into consideration, as parental origin cannot be defined in such cases. Microsatellite marker analysis was based on the identification of two alleles the parents were heterozygous for and the alleles of the two parents differed.

III./2.7. Methylation sensitive-PCR (performed by Dr. Petra Zeitlhofer, Medgen At., Wien)

Three duplex reactions were used to compare the ratio of the respective methylated and unmethylated alleles of the H19 (promoter), IGF2 (exon 8) and KCNQ10T1 (promoter) genes. Using this approach, methylation defects, duplications and UPD of the region 11p15 could be identified.

III./2.8. FMR1 Southern blot and PCR (postnatal tests performed by Beáta Bessenyei, prenatal diagnosis by Dr. Veronika Karcagi, National Inst. of Environmental Health)

Until the end of 2011 FMR-1 gene analysis was performed by chemiluminescence Southern-blot analysis. DNA samples were digested with two restriction enzymes (EcoRI, NruI, Thermo Fisher Scientific Biosciences, Fermentas, Canada). For hybridization and detection pFxa1NHE probe (Fragile-X Chemi DNA probe, Millipore Corporation, Billerica, MA, USA) and the Sure Blot Chemi Hybridization a Detection kit (Millipore Corporation, Billerica, MA, USA) were used. The analysis was performed according to the manufacturer's instructions. From 2012 PCR-based analysis has been introduced for the detection of CGG triplet expansion. The first-line test is a PCR analysis followed by agarose gel-electrophoresis, which detects the normal and the premutated alleles. In the absence of amplification a second-line test is performed which is able to detect full mutation based on fragment analysis (AmplideX FMR1 PCR Kit, Asuragen Inc., Austin, TX, USA).

III./3. Methods for phenotyping

III./3.1. Magnetic Resonance Imaging (analyzed solely by Dr. Ervin Berényi)

Neuroimaging including single voxel localized proton spectroscopy (1H-MRS) and diffusion tensor imaging (DTI) was performed on 1.5 T Excite Magnetic Resonance Imaging (MRI) scanner (GE Healthcare, Milwaukee, WI, USA). High resolution 3D T1 weighted gradient echo (Time of relaxation (TR):30 ms; Echo time (TE):7 ms; voxel size: 0.68x0.68x1.1 mm) and T2 weighted fast spin echo imaging (TR:5640 ms; TE:95 ms; Echo train length (ET):16; voxel size: 1x1x3 mm) were prepared for basic anatomic evaluations. Single voxel proton MR spectroscopy (TR:1500 ms; TE:144 ms; voxel size:1.5x1.5x1.5cm) was performed, voxels were placed to the deep grey matter of the basal ganglia [Kugel et al. 1998; Thayyil et al. 2010]. The diffusion tensor measurement (TR:7500 ms; TE:106 ms; b factor: 1000; voxel

size:1x1x3.3 mm; Motion probing gradient, MPG:25) was quantified by calculating secondary grayscale images that allow visual inspection and analysis of diffusion-related parameters. For this purpose, we calculated parametric images that are generally available in all DTI processing software packages: fractional anisotropy (FA) maps and longitudinal diffusivity images were generated. Evaluation of diffusion imaging involved placing regions of interests (ROIs) bilaterally to depicted areas of the frontal white matter that presented altered structure. Tract visualization was carried out using a fibertracking algorithm *(Westin et al.2002)*. Fibers were taken from the entire white matter of the frontal and parietal areas.

III./3.2. Anthropometric measurements (performed by the author)

Anthropometric data were given according to the growth and development curves of Hungarian children using references of the Demographic Research Institute, Hungarian Central Statistical Office *(Joubert K. et al 2009)*.

III./3.3. Morphological evaluation of patients (performed by the author)

Morphological evaluation and description of patients was performed using terms of the Elements of Morphology series, based on the consensus of the world's leading experts *(Allanson et al 2009a; Allanson et al 2009b; Biesecker et al 2009; Carey et al 2009; Hall et al 2009; Hennekam et al 2009; Hunter et al 2009; Klinger and Merlob 2009)*. Syndrome identification was pursued using the following sources: Orphanet - The portal for rare diseseases and orphan drugs; Winter-Baraitser Dysmorpholgy Database and Baraitser-Winter Neurogenetics Database as part of the London Medical Databases; D.W. Smith & Kenneth L. Jones: Smith's Recognizable Patterns of Human Malformations; R.C.M. Hennekam, I.D. Krantz, J.E. Allanson: Gorlin's Syndromes of the Head and Neck; H.R. Wiedemann, J. Kunze, H. Dibbern: Atlas of Clinical Syndromes – a Visual Aid to Diagnosis; and A.Schinzel: Catalogue of Unbalanced Chromosome Aberrations in Man *(Orphanet - The portal for rare*

diseases and orphan drugs 2013; Baraitser and Winter 2012; Hennekam et al 2010; Kenneth and Smith 2005; Schinzel 2001; Wiedemann et al 1991).

Although not a site for professionals, but even YouTube (www.youtube.com) was often helpful in desperate situations, because video-records and behavioral characteristics of syndromes can often be found there, its disadvantage is that it cannot be made sure that the there presented case was confirmed molecularly or the diagnosis is rather just a suspicion.

Other than evaluating patients by their visible morphological features, hidden aberrations were sought with the involvement of professionals from other subspecialties. Those who showed unique expertize, interest, and dedication in the diagnosis of rare diseases were asked to continue performing examinations and tests on all further patients thereafter.

Consultation with foreign experts were performed in the most challenging cases.

III./4. Currently used diagnostic protocol

In general, we applied the following diagnostic protocol: After a careful record of anamnestic data, survey of the pedigree and exclusion of teratogenic or intrapartum origin, all patients with any degree of mental retardation (+/- major congenital anomalies) were undertaken chromosome testing. In addition, FMR-1 molecular biological analysis was requested if the patient was a male or the pedigree suggested so in female patients. If the suspicion of a known microdeletion syndrome was raised, region specific FISH was applied – even as a first-tier test prior to karyotyping if the diagnostic clue was strong. All patients with unexplained mental retardation and normal karyotype were undertaken brain MRI imaging to reveal anatomical anomalies that may suggest a specific genetic origin. Patients with multiple congenital anomalies and at least moderate mental retardation were tested for subtelomeric chromosomal rearrangements. Major and minor congenital as well as functional anomalies

were precisely characterized and objectivized by X-rays, ophthalmological, orthopedic, cardiological, orthodontic, oto-rhyno-laryngological examinations, auditory screening. The type and degree of intellectual disability and behavioural problems were assessed by neurodevelopmental experts and psychiatrists, neurological disturbances were described by neurologists and electrophysiology experts. For suspected metabolic disorders, tandem mass spectrometry for the available 26 metabolites were repeated, if negative, other specific tests were requested. Mitochondrial and storage diseases were attempted to be proved from tissue biopsies (muscle, liver, bone-marrow, skin), and if the findings supported the original suspicion, the corresponding biochemical or genetic tests were requested. If any features suggested a mendelian disorder, molecular genetic test of a single gene or next generation sequencing of all known responsible genes were requested regardless of their availability in Hungary, applying for individual cost coverage if necessary to a foreign laboratory. If the underlying genetic condition remained unknown, syndrome feature search, literature check, consultation with home or foreign experts followed, and further tests were performed according to their suggestions. For consultations, photo and video-material subtitled in English, case histories, electronic imaging studies were summarized, often requiring 2-20 hours of preparation prior to sending the material. If the patient did not have a chromosomal anomaly visible by G-banding, did not fit into mendelian disorders, mitochondrial or metabolic disease, array CGH was requested often as last step. *(Fig. 2.)* This protocol was consistent with the guideline of Shevell et al. *(Shevell et al 2003)*, but contradicted some most recent others that suggest CGH to be the first-tier test *(Miller et al 2010)*. Nevertheless, to a certain extent we had to compromise with national possibilities. Whole exome sequencing was never done, the first correspondence on it I started this year, after the astonishing results of the first sequencing project on a diagnostic basis in the Netherlands *(de Ligt et al 2012)*.
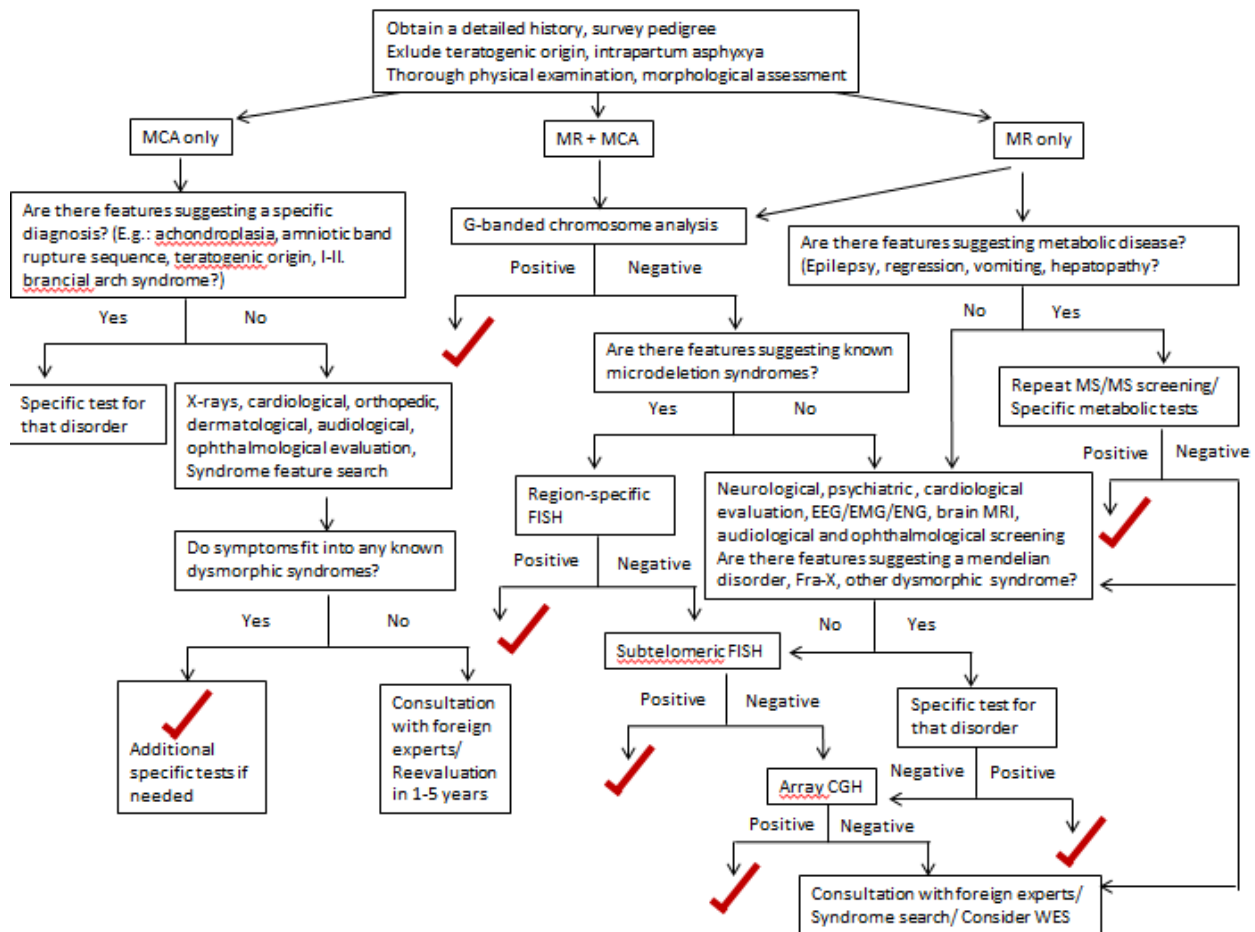
Fig.2. *Algorithm for evaluation of the child with mental retardation and/or multiple congenital anomalies, adapted to the possibilities of the Hungarian health care system. Note that international guidelines recommend array CGH at a much earlier stage of the diagnostic procedure.*

IV. Results

IV./1. Chromosomal abnormalities detected by G-banding

220/2049 patients (10.7%) were proved to have a chromosomal abnormality, 188 (85.4%) of them having numerical or structural aberrations of the autosomes (chromosomes 1-22), 31 (14.1%) had abnormalities of the gonosomes (chromosomes X and Y), and one (0.5%) had both. *(Table 1.)* 105 patients had Down syndrome (5.1% of the total number of patients and nearly half of all chromosome aberrations) – 95 were free trisomy 21, five patients had a mosaic form, and five patients carried an apparently balanced translocation in addition to the extra chromosome 21. Trisomy 13 was diagnosed in one patient, surprisingly he is still alive (aged 6 years) in spite of his severe mental retardation and multiple congenital

anomalies. Full trisomy 18 was diagnosed in one patient who did not survive the perinatal period, and in mosaic form in a further patient. 28 patients were clinically healthy individuals carrying balanced reciprocal (robertsonian included) chromosomal translocations, seven were apparently balanced reciprocal translocation carriers with abnormal phenotype (three of them were consulted for primary infertility; four patients had major congenital anomalies suggesting submicroscopic gains or losses along the breakpoints). The elucidation of the underlying genetic cause in this group of patients requires the application of CGH. Large (>10 Mb) deletions were seen in 16 patients, two out of these had it in a mosaic form. Duplications/partial trisomies were detected in 14 patients. All patients with duplications and deletions presented with intellectual deficit and multiple congenital anomalies. Ring chromosomes were detected in two patients (chromosomes 10 and 21), pericentric inversions (one breakpoint on the short arm and one on the long arm, possibly disturbing crossing over and recombination in the offspring) were shown in two clinically healthy individuals (one of them produced an unbalanced offspring). Chromosomal breakage was found in one patient having Dubowitz syndrome. A small supernumerary marker chromosome was seen in nine cases, their origins were identified by Prof. Thomas Liehr (Uniklinikum Jena, Germany) using molecular cytogenetics, excepted two cases. Complex autosomal anomalies (deletion and duplication together) was seen in one case.

Gonosomal aneuploidies accounted for 14.1% (31/220) of all chromosomal anomalies, 1,5% of all patients. 45,X Turner syndrome was found in eight patients, two had trisomy X (47,XXX). In four patients 45,X/47,XXX/46,XX mosaicism, in two 47,XXX/46XX, and in another one 47,XXX/45,X was detected. Deletion of the short arm of the X chromosome was found in one patient. 47,XXY Klinefelter syndrome in six, 47,XXY/46,XY mosaicism in one patient. Klinefelter variants with 48,XXXY and 49,XXXXY karyotype were identified in one patient each. A double Y (47,XYY) was found in one patient. 46,XY gonadal dysgenesis with

female external genitalia was found in two patients. Combined autosomal and gonosomal aneuploidy was present in one patient.

| Chromosomal aberrations found in patients referred to genetic counseling between 01.08.2007-31.03.2013 (Total of 220 patients)			
		Mechanism	N^{o}. of cases
Aberrations of the autosomes	**Numerical**	Trisomy 13 (Patau)	1
		Trisomy 18 (Edwards)	1
		Trisomy 18, mosaic	1
		Trisomy 21, free (Down)	95
		Trisomy 21, mosaic	5
		Trisomy 21, translocational	5
		sSMC	9
	Structural	Deletion	16
		Duplication	14
		Complex (deletion and duplication)	1
		Ring chromosome	2
		Balanced reciprocal translocation, robertsonian included	28
		Apparently balanced reciprocal translocation with abnormal phenotype	7
		Inversion (pericentric only)	2
		Breakage	1
Aberrations of the gonosomes	**Numerical**	49,XXXXY (Tetra X, Klinefelter variant)	1
		48,XXXY (Klinefelter variant)	1
		47,XXY (Klinefelter)	6
		47,XXY/46,XYmosaic	1
		47,XXX	2
		47,XXX/46,XX mosaic	2
		45,X (Turner)	8
		45,X/46,XX	1
		47,XXX/45,X mosaic	1
		47,XXX/45,X/46,XX mosaic	4
		47,XYY	1
		46,XY female	2
	Structural	del(X)(p)	1
Combined	**Numerical**	47,XX,+21/46,X,+21	1

Table 1*. Chromosomal aberrations in patients referred to genetic counseling in the reported time interval*

**Case report – Patient 1**. **Autosomal aneuploidy**

Patient (female) was born from healthy, non-consanguineous Caucasian parents as their second child, with intrauterine retardation. She had a healthy older sister. Birth weight was 3 pc, length and OFC were normal for gestational age. Dysmorpic features were apparent at birth: long face, high frontal hairline, prominent forehead, broad and flat nasal root, telecanthus, proptosis, long philtrum, tented upper lip, retrognathia, low-set and posteriorly rotated ears. *(Fig.3A.)* In addition, generalized hypotonia, apparently severe mental retardation and congenital heart defect (ASD, pulmonary stenosis, cor diatriatum dextrum) were noted. G-banded chromosome testing revealed a derivative chromosome 11. *(Fig. 3B)* Parental testing proved a 46,XX,t(9,11)(q22;q23) balanced reciprocal carrier status *(Fig.3E)* in the father, based on which the extra bands on chromosome 11 in the propositus were identified to be derived from chromosome 9, resulting in partial trisomy of chromosome 9q (46,XX,dup(9)(q22q34.3) *(Fig.3C).* Patient died at 6 months of age. Four members of the family including the father, the father's brother, the paternal grandmother and the healthy sister were proved to be balanced reciprocal carriers *(Fig.3D)*. Given the 0.8% chance to produce an unbalanced offspring with similar partial 9q trisomy and a 7% risk for a child with partial 11q trisomy, all carriers were offered prenatal diagnosis in case of future pregnancies. (Specific risk of an unbalanced offspring is different for each chromosome involved in translocation, falling in the range from 0-30%. Risk figures for a liveborn aneuploid child are summarized by Gardner et al. *(Gardner and Sutherland 2004).*
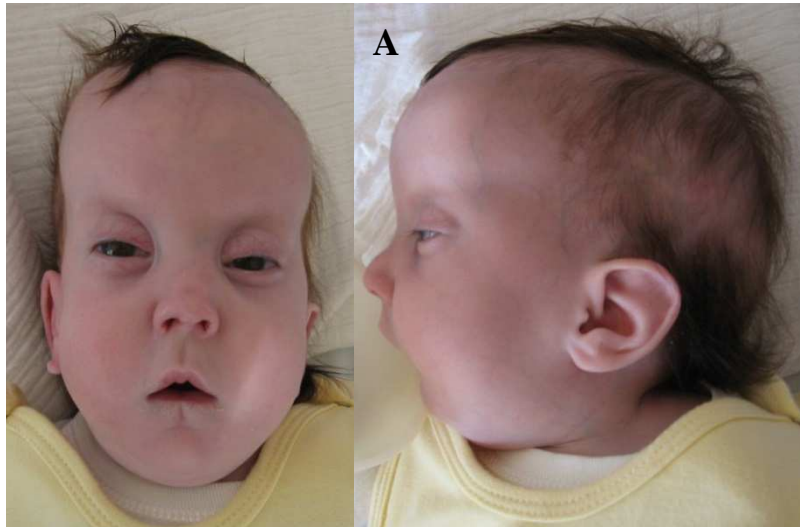
Fig. 3A. *Facial appearance of patient with partial 9q trisomy: Long face, prominent forehead, high anterior hairline, telecanthus, broad nasal root, low-set and posteriorly rotated ears, long philtrum, retrognathia, vertical groove under the lower lip.*
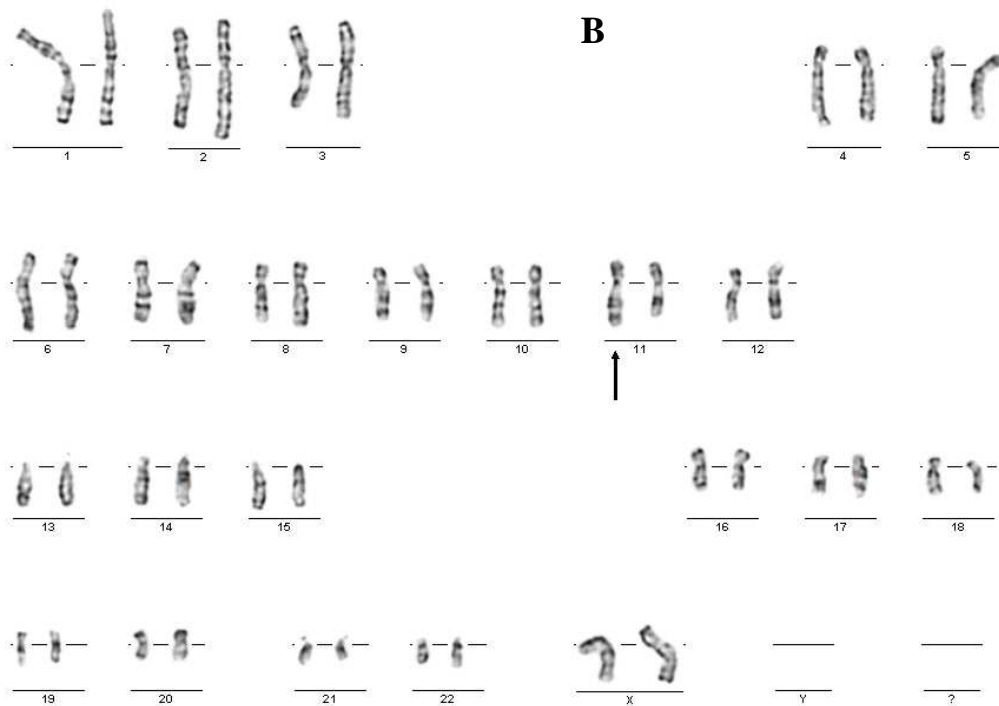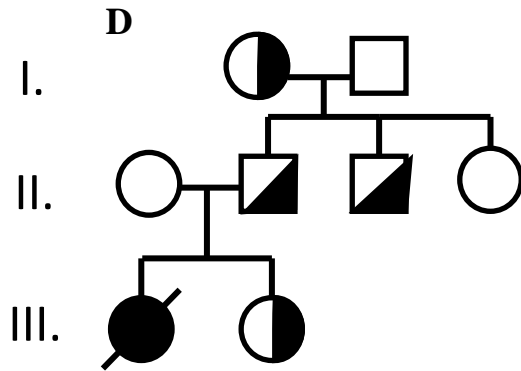

Fig.3B. *Karyogram of patient with partial trisomy 9q. A derivative chromosome 11 is shown (arrow) with extra bands originating from chromosome 9.*
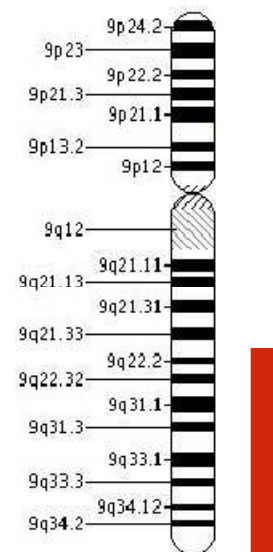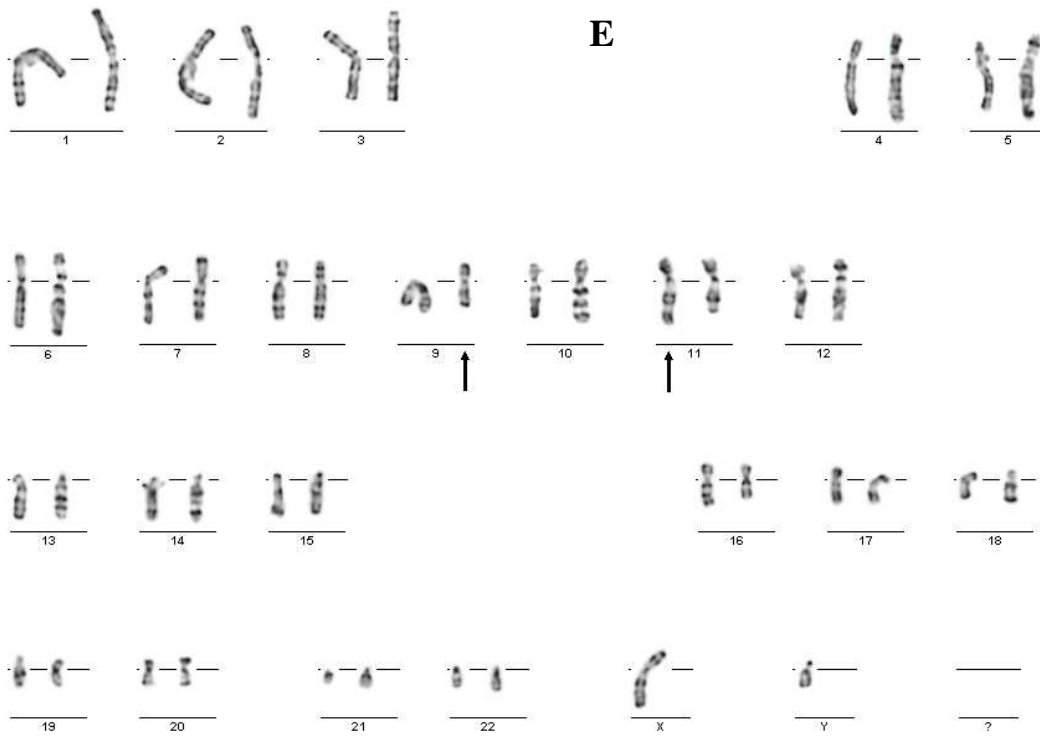
Fig. 3C. *Ideogram of chromosome 9. The duplicated region in case 1 is marked with a red coloumn.*

D

Fig. 3D. Pedigree of patient showing balanced carrier status for t(9,11)(q22;q23) in four individuals

E

Fig.2E. Karyogram of the carrier father showing balanced reciprocal translocation of the long arms of chromosome 9 and 11.

Case report – Patient 2. Gonosomal aneuploidy

The patient was born to term with intrauterine growth retardation (IUGR). Birth parameters were: weight 150g<3pc), length 2cm<3pc, OFC 10pc. Due to poor sucking he was bottle-fed. Hypotonia and microgenitalia were obvious at 3 months *(Fig.4A)*, radioulnar synostosis and consequent movement limitation of the elbows were described during regular orthopedic screening. Developmental delay and otherwise good social skills were apparent by

9 months of age. By 20 months, speech delay, agressive behavioral changes developed. Prader-Willi syndrome was considered, but chromosome testing revealed 49,XXXXY syndrome *(Fig.4B)*. Later in life severe myopia, short stature, the progression of the cubital joint contracture and moderate mental retardation is to be expected. Authors of a recent paper concluded that each extra `chromosome` X causes a 10-15 points loss from the IQ *(Tartaglia et al 2011)*. The patient was referred to early physiotherapy, ophthalmology and endocrinology, testosteron therapy is suggested to enhance not only physical but also cognitive development *(Samango-Sprouse et al 2011)*.

Fig. 4A. *Patient with 49,XXXXY syndrome, showing round face, short forehead, low anterior hairline, mild obesity, limited movements of the elbows, and hypogenitalism due to hypergonadotrop hypogonadism.*
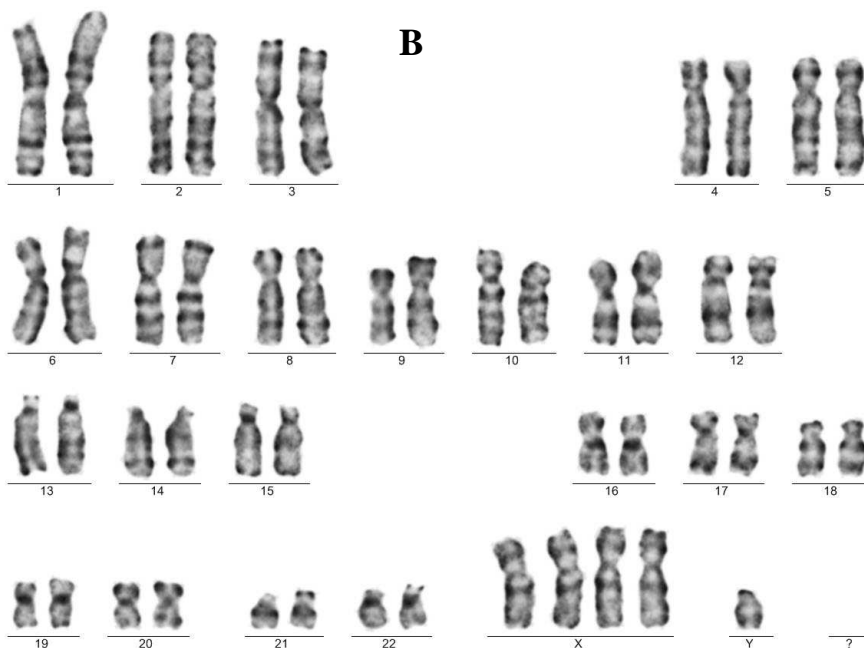
Fig.4B. *Karyogram of patient showing 3 extra X chromosomes.*

49,XXXXY syndrome is an very rare entity with a prevalence of 1/100.000. It results from nondisjunction of the X chromosome during both meiosis I and meiosis II. Thus, an aneuploid oocyte ($X_m X_m X_m X_m$) is fertilized with a normal male sperm (Y_p).It is most likely a one-off event and known to be sporadic, recurrence risk is very low.

IV./2. Gains or losses of genetic material detected with fluorescent in situ hybridization

Positive FISH results were obtained in 62 cases. In 40 of them (2% of all patients), G-banded karyotyping provided no diagnostic clue for the underlying problem, hence FISH provided a diagnosis alone. In 22 cases, G-banded karyotyping indicated the presence of derivative or small supernumerary marker chromosomes, or suggested mosaicism, but chromosomal origin and true percentage ratio could only be identified by FISH. In the latter group, karyotyping and FISH were both needed to conclude a diagnosis. (To avoid double-counting patients, they were listed in the chromosomal group only).

Result in chromosomal order are displayed in *Table 2.*

Our laboratory possesses FISH probes for the following microdeletion syndromes: 1p36 deletion syndrome, 3q29 (3q subtelomeric deletion syndrome) 4p16.3 (Wolf-Hirschhorn syndrome), 5p15.3 (Cri-du-chat syndrome), 5q35 (Sotos syndrome), 7p11.2 (Williams-Beuren syndrome), 9q34 (Kleefstra syndrome), 15p11.2 (Prader-Willi/Angelman syndrome), 17p11.2 (Smith-Magenis syndrome), 21q22.1 (Down-syndrome), 22q11.2 (DiGeorge/Velocardiofacial syndrome), 22q13.3 (Phelan-McDermid syndrome), and subtelomeric FISH probes for the long arms and short arms of each chromosome except for the short arms of the acrocentric chromosomes (chromosomes 13, 14, 15, 21 és 22). Recently, 16p13.3 (Rubinstein-Taybi syndome) was also obtained, and we have just started its first clinical use on 4 patients' samples who are suspect of the syndrome. Other than these, centromere and arm specific probes are availabe for all chromosomes.

Microdeletions/duplications and other chromomosomal inblances detected by FISH in 2007-2013.			
Diagnosis/Syndrome	**G-banding**	**Chromosomal region**	**N° of cases**
1p36 deletion	No abnormalities on G-banded karyoptype	1p36	2
3q subtelomeric deletion		3q29	1
Wolf-Hirschhorn		4p16.3	3
Cri-du-chat		5p15.3	1 (2 further cases visible by G-banding)
Sotos		5q35	0; 1 case proved with MLPA of the NSD1 gene
Williams-Beuren		7p11.2	11
Kleefstra		9q34	0, 1 case proved with CGH
Prader-Willi		15p11.2	3
Angelman		15p11.2	6
Smith-Magenis		17p11.2	1
Down		21q22.1 microduplication	1
Di-George/Velocardiofacial		22q11.2	6
Phelan-McDermid		22q13.3	1
Subtelomeric		Subtelomeric	4 (3 combined deletion and duplication, 1 deletion)
Identification of chromosomal origin of derivative or marker chromosomes	Abnormalities found on G-banded karyotype	Multicolor and subtelomeric	7
Mosaicism (percentage of cells with normal and abnormal karyotype)		Centromer-specific probes	15 (5 mosaic trisomy 21, 10 gonosomal)

Table 2. *Summary of cases with submicroscopic chromosomal origin of congenital anomalies and mental retardation detected by FISH*

Below are the two rarest and most challenging cases:

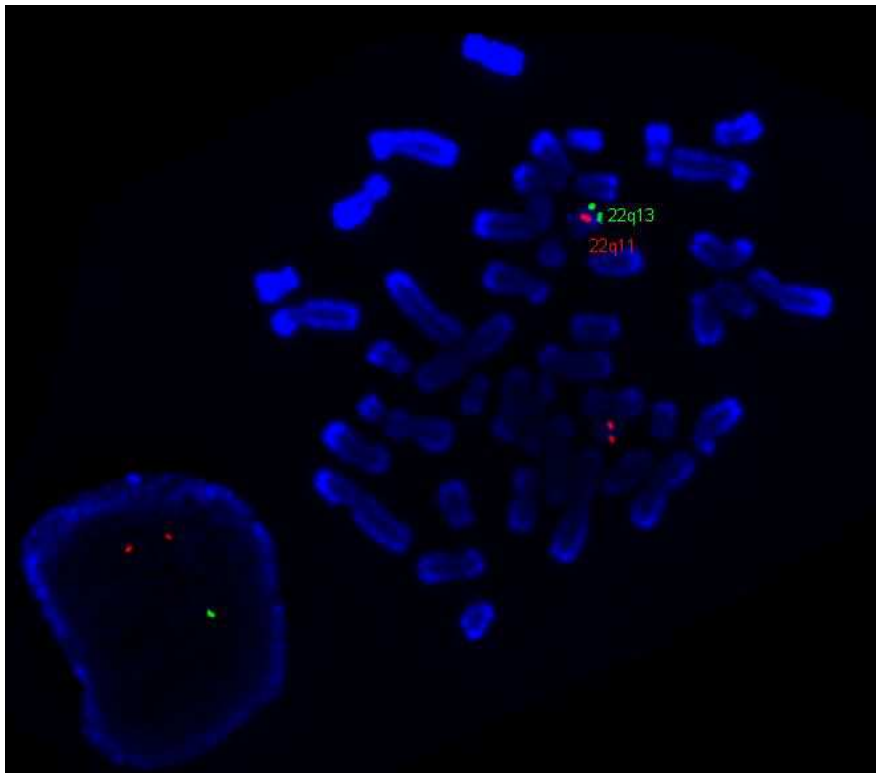**Case report 3. Microdeletion syndromes – Phelan McDermid syndrome**

Patient was born to term from healthy, non-consanguineous parents with normal parameters. Perinatal period was uneventful, although a constant stridor was present due to laryngeal hypoplasia. Right hypoplastic and non-functioning kidney, contralateral pyeloureteral stenosis, hydronephrosis and megaloureter were noted at 6 months on occasions of a urinary tract infection. Global developmental delay was obvious by 1 year of age: patient aquired sitting at 2 years, crawling at 3 years, unaided walking has not yet been achieved (6 years). Speech is completely absent, poor understanding of commands, mouthing, and difficult to manage stubbornness were part of the clinical picture. Upon physical examination, macrosomia, macrocephaly (weight, length and OFC >90 pc), hypotonia and severe intellectual deficit were noted. Other dysmorphic features were: antimongoloid slant of palpebral fissures, broad nasal base, large, open mouth, everted lower lip, large, fleshy hands with deep palmar creases. *(Fig. 5A)*

Karyotyping showed a normal male karyotype. Sotos syndrome was considered, but the mental retardation and speech delay was much more severe than usually seen in Sotos syndrome.

Macrosomia, hypotonia, mental retardation and speech delay were considered signal signs, and using these in the Orphanet syndrome feature search, the possibility of Phelan Mc Dermid was raised and was proved with region specific FISH. *(Fig. 5B)* Similarity to another case accessible on the Internet was striking. Prevalence is lower than 1/1 000 000, the here presented case is the first one identified in Hungary.

Fig.5A. *Photos of patient (left) with Phelan-McDermid (22q13 deletion) syndrome. Note macrocephaly, high forehead, hypotonic face, large mouth and deep palmar creases. Parents declined the use of full facial photograph. On the right a photo of a patient from the Internet. Source: http://parentingspecialneeds.org/article/69*


Fig.5B: *FISH indicating monosomy 22q13 in patient 3. (the red signal is the DiGeorge critical region (22q11.2, as control probe), the green is the Phelan-McDermid critical region.*

Case report – Patient 4. **Microdeletion syndromes – DiGeorge syndrome, recurrence in a family**

A 3-month-old girl was referred to our genetic outpatient office for minor anomalies (unusual facial gestalt, small and low-set ears, hypertelorism), and mild developmental delay. Birth was complicated with threatening asphyxia. Assisted walking was achieved by 1 year of age, social behaviour and cognitive skills appeared normal to age. Dysmorphic features were mild: round and flat face, flat supraorbital ridges, depressed nasal bridge, tented upper lip, small and low set ears with overfolded helix and Darwinian tubercle *(Fig.6).*The voice was somewhat hypernasal. No cardiac defect was found, no history of hypocalcaemia or recurrent infections was reported.

Chromosome analysis with G-banding showed a normal karyotype, and as the intellect was satisfactory, follow-up and reevaluation in 6 months was proposed. It was strange, however, that the grandmother always escorted the mother to consultations, as if for personal comfort, and that the mother had a „stifled" voice with incorrect pronunciation of wovels, somewhat similar to the altered speech of deaf people. She had normal hearing though, normal intellect, her highest degree of education was regular high-school diploma, and worked as an office administrator. She also had hypertelorism and did not have cardiac anomaly. The father was reported to be healthy, a craftsman by profession. Based solely on the mother's voice suggesting insufficient power of the pharyngeal muscles, and the child's mild developmental delay, the suspicion of DiGeorge snydrome was raised, and the mother was undertaken FISH testing first. Haploinsufficiency of the 22q11.2 region was found, and it was a straightforward diagnosis that the child had the same. The FISH results provided explanations for the symptoms. Based on the knowledge of the diagnosis and the mother's favourable status, a similarly favourable outcome for the child could be predicted (keeping in

mind that breakpoints might change as a result of crossing over of the homologue chromosomes), and prenatal diagnosis in future pregnancies can be offered.
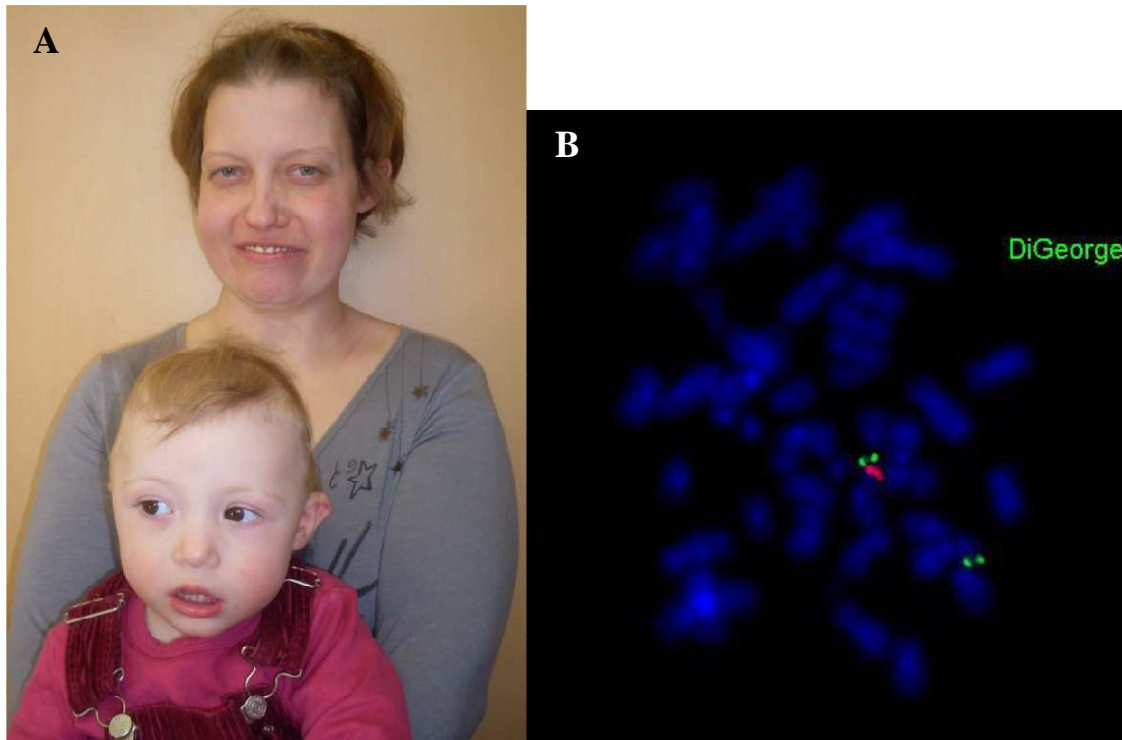
Fig.6A) Mother and child affected by Di-George syndrome, B) their FISH image showing haploinsufficiency of the 22q11.2 chromosomal region. Note flat face and hypertelorism in both, and downturned corners of the mouth in the mother. The green signal on the FISH is the control probe (22q13) in the mixture, the red signal is the Di-George critical region (22q11.2).

IV./3. Chromosomal submicroscopic copy number changes detected with comparative genomic hybridization

If the association of symptoms do not match any of the well-known microdeletion syndromes, but suggest chromosomal origin, array CGH is the test of choice.

We started using CGH for diagnostic purpose in the last two years, with foreign help and individual financing of the Hungarian National Health Insurance Fund. In certain cases, parents volunteered to take the financial burden of the test. In spite of its enormous clinical need and utility, and in spite of international guidelines that suggest CGH to be the baseline test and not G-banded karyotyping, our present financial situation does not allow to replace first-tier karyotyping with first-tier CGH. However, the Institute of Laboratory Medicine and

the Center of Clinical Genomics and Personalized Meicine, Institute of Biochemistry and Molecular Biology in collaboration plans to set the method on a diagnostic basis in the near future and is currently doing preparations for this.

To date, six patients requesting medical genetic help were proved to be affected by a microdeletion, none had microduplication *(Table 3.)*.

Submicroscopic chromosomal aberrations detected by CGH in patients referred to genetic counseling between 2010-2013.			
Region of deletion	**Size of deletion**	**Key symptoms**	**Relevant gene contributing to the core phenotype**
1p36.2	10.3 Mb	Proportinate growth retardation, midface hypoplasia, blepharophimosis, moderate mental retardation	64 genes involved
3p25.3	3.4 Mb	Severe mental retardation, multiple major congenital anomalies: heart defect, deep facial creases, thin lips, micrognathia, cryptorchism, Von Hippel Lindau disease	A large number of genes involved, 6 with morbid OMIM reference including VHL (von Hippel Lindau)
3q29	Not reported	Mild mental retardation, impaired hearing, micrognathia, crowded teeth, resembling Treacher-Collins phenotype	20 genes deleted including PAK2 (protein activated kinase 2) and DLG1 (Discs large), autosomal homologues of X-linked mental retardation
9q34.3 (Kleefstra syndrome)	958 kb	Moderate mental retardation, brachycephaly, perivenrticular white matter changes, heart defect, remarkable speech delay, long and curly eyelashes	EHMT1 (Eucromatic histone methyltransferase)
12p12.1	254 kb	Severe mental retardation, autism, behavioural problems, normal motor performance	SOX5 (SRY-box 5)
21q22.1	308 kb	Moderate mental retardation, friendly disposition, downward slant of palpebral fissures, emotional shock from certain objects of everyday use	GART (Glycinamide phosphoribosyltransferase)

Table 3. *Pathological copy number variations in our patients detected by CGH in 2010-2013.*

Case report – Patient 5. Submicroscopic pathological copy number changes – monosomy 3p25.3

First child of healthy parents, born with IUGR (weight 100g<3pc; length 6cm<3pc, OFC 3pc). Remarkable dysmorphic features were visible already at birth: unusually light blue iris, deep facial creases, low-set ears, thin lips with dowtured corners of the mouth, pointed chin, proximally displaced thumbs, branchial fistule on the neck, bilateral cryptorchism. *(Fig.7.)* Cardiac ultrasonography revealed dilated right ventricle, FoA (5 mm), small perimembran VSD, grade I. mitral and tricuspidal insufficiency, patent ductus arteriosus. Mental retardation was severe. After years of unsuccessful testings and syndrome search, the patient was referred to CGH testing based on a consultation with Prof. Raoul Hennekam, and was proved to have a 3.4 Mb large deletion on the short arm of chromosome 3, including the Von Hippel Lindau (VHL; OMIM 608537) gene (arr 3p25.3p25.2(8835685-12264574)x1. Genetic counseling could now predict a poor outcome and a low recurrence risk for the parents' future pregnancies. Regardless of the fact that the diagnosis of Von Hippel Lindau was an accidental finding, information about the nature of the disease was diclosed to the parents and the follow-up of the patient will be adjusted to this knowledge.

Fig. 7. Photo of Patient 5 at 2 months and at 7 months. Note deep facial creases, mild proptosis, long and featureless philtrum, thin lips and retrognathia. Severe intellectual deficit is evident.

Case report –Patient 6.. **Submicroscopic pathological copy number changes – monosomy 21q22.11**

Patient was born to term with intrauterine retardation (weight 350g<3pc, length and OFC not recorded). Mild dysmorphic features were apparent at birth (hypertelorism, antimongoloid slant of palpebral fissures). G-banded chromosome analysis showed a normal female karyotype. Gross motor development was delayed, sitting was aquired at 10 ½ mths, walking at 2 ¼ yrs, sphincter control at 6 yrs. Speech was delayed but developed to a satisfactory conversational level. At 10 years moderate mental retardation was described. The mother reported a strange aversion from certain objects: the sight of a curling iron or the insertion of earrings resulted in immediate fainting and loss of conscience. Chewing of solid food was never achieved. On physical examination proportionate growth retardation (weight 1kg<3pc, length 6cm<3pc, OFC 10-25 pc), hypertelorism, downward slant of palpebral fissures, epicanthus, ptosis, strabism, prominent palatinal ridges, and prominent, cranially displaced upper incisors were noted along with widely spaced nipples, broad thumbs, small feet, and exaggerated lumbar lordosis. *(Fig.8.)* CGH proved a 308 kb loss on the long arm of chromosome 21, (arr21q22.11(34863258-35171348)x1, including the GART (Glycinamide phosphoribosylglycinamide, OMIM 138440) gene, known to play a role in purin synthesis. Latter might be responsible for the mental retardation. Testing of the parents to prove de novo origin was postponed by the Hungarian Special College of Genetics, with reference to advanced maternal age and thus a very small likelihood of the need for prenatal diagnosis.

It is noteworthy, that this patient waited 10 years for a diagnosis, the one presented as Patient 5. waited 6 years.

Fig.8. *Photo of patient with a 308 kB loss on chromosome 21q22.11. Friendly disposition, moderate intellectual deficit, downward slant of palpebral fissures, prominent maxillary incisors.*

IV./4. Mendelian disorders

In 110/2049 cases (5.4%) a single gene disorder was found and proved molecularly. Two of these were metabolic disorders proved biochemically ***(Table 4.).*** Some syndromes were relatively frequent and easily recognizable such as neurofibromatosis I or Apert syndrome, others were challenging and required months of repeated attempts of syndomological evaluation and search.

Mendelian disorders confirmed in our patients with congenital malformations and/or neurological deficits		
Diagnosis group	Corresponding gene(s)	N^o of cases
Craniosynostoses	FGFR2, FGFR3, (Fibroblast growth factor receptors), TWIST	7
Fragile X	FMR1	12 incl. female carriers
Neuromuscular	Dystrophin, POMT1 (Protein O Mannosyltransferase) CHRNE (Choline Receptor, nuclear, Ɛ subunit), SMN1 (Survival of motoneurons)	15 incl Duchenne female carriers and Myasthenia gravis carriers
Neurofibromatosis	NF1, NF2	9
Osteochondrodysplasias	FGFR2, FGFR3	8
Marfan	FBN1 (Fibrillin 1)	7
Rett	MECP2 (Metly CpG-binding protein)	2
Metabolic/ Neurodegenerative	NPC1,2 (Niemann-Pick C), IDUA (α-L iduronidase), GAA (α-glucosidase), GAMT (Guanidino-acetate methlytransferase) deficiencies, ATM (Ataxia teleangiectasia	18 (incl. carriers, 3 cases proved by other institutes but treated here on long-term)
Single cases of dysmorphic syndromes	NSD1 (SET-Domain Protein 1), KAT6B (Lysine-acetyltransferase 6B), TCOF1 (Treacher-Collins syndrome), PTPN11 (Protein tyrosine phosphatase, nonreceptor type), RAF1 (V-RAF1 murine leukaemia viral oncogen homologue), EXT1,2 (Exostosin), ZFHX1B (Zinc-finger E box-binding homebox 2), ALSM1 (Alström Syndrome), NIBPL(Nippled B-like), HP1 (Hermansky-Pudlak)	13 All tests performed in foreign labs
Others		19

Table 4. *Mendelian disorders proved in patients with MR/MCA in the reported period.*

Case Report – Patient 7. **Say-Barber/Biesecker/Young-Simpson syndrome (SBBYSS)**

The patient was born from unrelated healthy Hungarian parents in the 39th week of gestation as their second child. She has an unaffected brother. Birth weight was 75 pc, length 25-50 pc. She was referred to genetic counseling due to failure to thrive, generalized

hypotonia, cleft palate and developmental delay. On clinical evaluations she had round face, bilateral blepharophimosis, ptosis, small ears, stenotic external ear canals, hypertelorism, depressed nasal bridge, long philtrum, thin vermilions and dental malformations. *(Fig.9A).* The proximal phalanges of the thumbs were long *(Fig.9B).* No visceral malformation could be detected by ultrasound examinations. Primary hypothyroidism with serum TSH 13.39 mU/L (Ref.: 0.3-4.2 mU/L), fT4 19.14 pmol/L (Ref.: 12-22 pmol/L), fT3 7.13 pmol/L (Ref.: 2.4-6.3 pmol/L) required L-thyroxin treatment. Hearing impairment found by auditory steady state responses (ASSR) was mild. Motor and intellectual development was severely delayed – the patient began to sit at 15 months, and made a few steps with assistance at 27 months. Her IQ according to the Brune-Lezine scale was 38, consistent with severe intellectual disability. Brain MRI disclosed delayed myelination and disruption of tracts in the periventricular white matter adjacent to the anterior horn of the right lateral ventricle. At 4 years, the bony islands of the patellae have not yet appeared according to patellar X-rays, and unaided walking has not yet been achieved. Chromosome analysis showed a normal female karyotype (46,XX), subtelomeric FISH and array-CGH were normal. *(Szakszon et al 2011)*
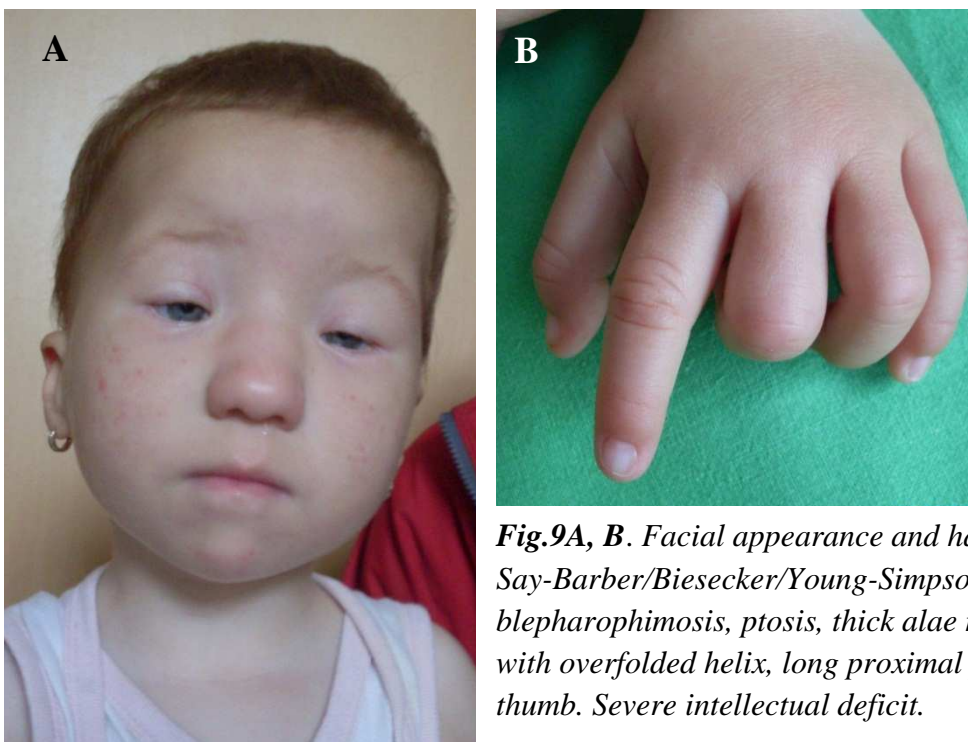
Fig.9A, B. *Facial appearance and hand of patient with Say-Barber/Biesecker/Young-Simpson syndrome: blepharophimosis, ptosis, thick alae nasi, low-set ears with overfolded helix, long proximal phalanx of the thumb. Severe intellectual deficit.*

At the age of 6 months conventional T1- and T2-weighed brain MRI did not show anatomical abnormalities. *(Fig.10A, B).* However, a delayed myelination was visible in the pons, the anterior horn of internal capsule and the splenium of the corpus callosum. Diffusion tensor imaging revealed a circumscribed 6-8 mm large area of decreased anisotropy in the frontal white matter adjacent to the frontal horn of the right ventricle.
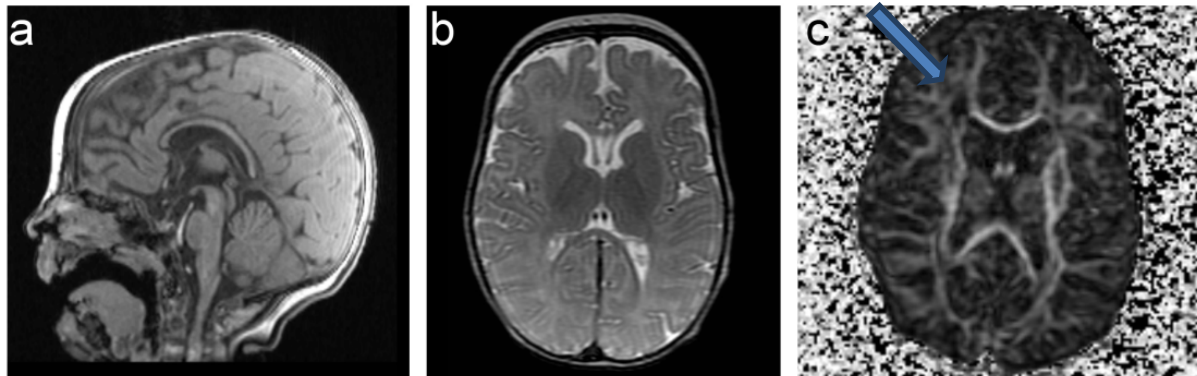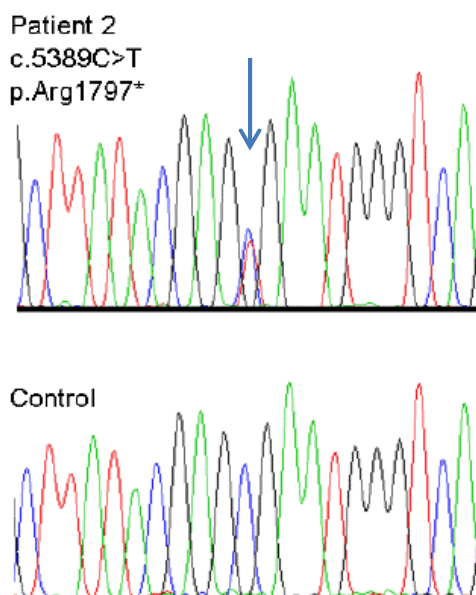

Fig. 10. MRI and fractional anisotropy map of the proband at age 6 months. A) Sagittal T1-weighted image. Note the thin corpus callosum. B) Axial T2-weighted image. Note the hypointense internal capsule, and thin and hypointense corpus callosum. C) Axial section of the fractional anisotropy map derived from diffusion tensor imaging. Note the decreased isotropy (arrow) in the right frontal white matter adjacent to the anterior horn of the right lateral ventricle.

Fibertracking analysis identified disrupted tracts within this region *(Fig.10D)* particularly affecting the connections of the frontal cortex. Region of interest analysis on secondary diffusion images revealed decreased anisotropy (Mean value, left side: 0.267 ± 0.1; right side: 0.226 ± 0.1) and decreased longitudinal diffusivity (Mean value, left side: 1.72 ± 0.6; right side: 1.62 ± 0.4) in the frontal white matter of the right side compared to those of the left. No cortical dysplasia was found. Single voxel proton MR spectroscopy did not detect pathological accumulation of lactate in the brain.
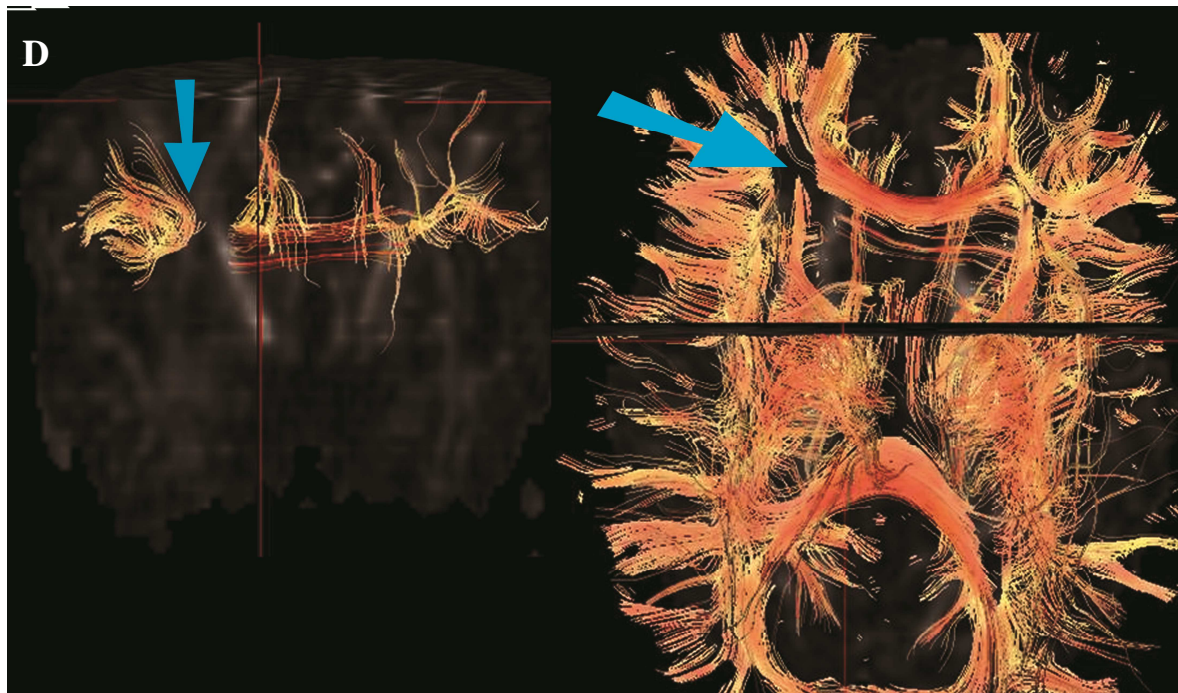
Fig.10 D. Fibertracking analysis of the proband at age 6 months. Note the disrupted tracts in right frontal areas and reduced connectivity of the right frontal cortex (arrow).

In 2012., our work-group identified a de novo heterozygous nonsense mutation c.5389C>T (p.Arg1797*) in the KAT6B gene (Lysine-acetyltransferase 6B; OMIM 605880) in this patient *(Fig.11.) (Szakszon et al 2013)*. This was the first confirmatory study following the discovery that mutations in the KAT6B gene are responsible for the SBBYS phenotype. The p.Arg1797* mutation was previously reported in another affected individual, and in both instances it arose de novo, suggesting that its recurrence is due to independent mutation events at a hypermutable CpG dinucleotide.

Fig. 11. Sequence chromatogram showing part of KAT6B exon 18 with a heterozygous nonsense mutation in the patient (arrow).

Case Report – Patient 8. **Mowat-Wilson syndrome**

First and only child of healthy, Caucasian parents. Normal size at birth. In the perinatal period (2 weeks of age) ileus developed, due to Hirschsprung's disease - latter was proved histologically. Several surgical events followed, the last was at 3 years of age. Epilepsy developed at 2 years and requires combined antiepileptic treatment. Cardiological follow-up is suggested due to ASD. Patient was referred to genetic counseling because of delayed motor and intellectual development. According to the opinion of neurologists, latter could have been due to long lasting hospitalizations, bedridden periods, and lack of stimuli when being is hospitals in spite of the efforts of a very caring mother.

Upon physical examination, severely impaired motor and intellectual performance was seen along with complete absence of speech and stereotypic behavior. Patient sat at 4 years of age and began to walk with assistance at 6 years. Somatic growth was normal to age. The face was unusual: large and deep-set eyes, strabism, antimongoloid slant of palpebral fissures and a pointed chin were noted, the ears were uplifted and cup-shaped. *(Fig. 12A, B)* Brain MRI showed normal anatomical structures, karyotyping, subtelomeric FISH and mutation analysis of the RET (Rearranged during transfection protooncogene, OMIM 164761) gene suspected to be responsible for the Hirschsprung-disease gave negative results. Search with numerous combination of the key-symptoms was performed and accessible photos and video-records on the Internet were matched. The association of mental retardation, epilepsy, absent speech and Hirschsprung disease finally resulted in a very strong diagnostic clue of **Mowat-Wilson syndrome (MWS)** Reevaluation of the patient based on the phenotypic criteria and frequent features of the syndrome strengthened the diagnostic suspicion, and a request for the molecular test of the **ZFHX1B** gene (OMIM 6058802) was kindly accepted by the Wessex Regional Genetics Laboratory, Salisbury. A heterozygote point mutation in exon 7, c.823C>T; (p.Gln275X) was found, confirming the diagnosis. This case was the first, and to

my knowledge, to date the only confirmed case Hungary; prevalence is <1/1 000 000. Finding out that the condition of the child was due to a de novo mutation, the mother was encouraged to engage in further pregnancies, but the child's irreversible and heavily burdening status caused the father to leave the family.

Fig.12A. Patient affected by Mowat-Wilson syndrome: hypotonia, Hischsprung-disease, severe motor and intellectual deficit, epilepsy.

Fig. 12 B, C. Facial appearance of patients with MWS: large eyes, downward slant of palpebral fissures, long columella, uplifted earlobes, long and pointed chin. Our patient is on the left (B) the two patients on the right (C) are shown to highlight facial similarity. Source: http://joannawongmowatwilsonsyndrome.weebly.com.

Considering that metabolic diseases represent a special group of single gene disorders, a unique case from this entity is described here.

Case report - Patient 9. Niemann-Pick C disease

First and only child of healthy, non-consanguineous parents, uneventful perinatal period, normal parameters at birth. Early childhood development was normal – the patient seemed to be an even unusually bright toddler. Head control, walking, sphincter control were achieved earlier than average. At 18 months, mild uncertainty in walking was noted. ENG, EMG, brain and whole spine MRI showed no anatomical abnormalities, however, the parents were informed about degenerative sings of unknown origin in the white matter of the brain. Follow-up was suggested. At 2 years more obvious balance problems and spasticity developed, physiotherapy was introduced. At 4 years patient lost her ability to walk, but she fancied sitting at a little bench with desktop and draw – intellect was still untouched. Retrospectively (based on photos), vertical supranuclear gaze palsy appeared around this time. At 5 years, speech became slurred, but comprehension was maintained. Presenile dementia developed, already aquired skills were lost gradually. *(Fig.13.)* Previous, detailed investigations in other institutes to prove neurometabolic or mitochonrial origin, or spinocerebellar ataxias, resulted in negative findings. On occasion of the first physical examination in our genetics outpatient office (age 5 years) no dysmorphic features were seen apart from loss of muscle mass and full cheeks. Functional anomalies were striking: the patient repeated nursery rhymes after the mother, but could not recite one on her own; she had vertical supranuclear gaze palsy and advanced stage dystonia. The first diagnostic suspicion was Niemann-Pick C, followed by leukodystrophies if tested negative for NPC. Hepato-splenomegaly was absent. A bone marrow biopsy (evaluated by Dr. Szabolcs Szakál, Inst. of Pathology, University of Debrecen) revelaed the presence of foamy macrophages. Molecular genetic test of the NPC1 gene (OMIM 607623) followed (performed by Dr. István Balogh,

Institute of Laboratory Medicine, University of Debrecen). First, cDNA from cultured fibroblasts was extracted and reverse transcribed using random hexamer oligonucleotides. Sequencing revealed a c.2983T-C point mutation in exon 20 (p.F995L). The mutation was previously not described, its pathogeneicity is proven by the critical position and phylogenetic conservatism of two neighbouring aminoacids – a glycin and a methionin 3 aminoacid positions away as well as the aminoacid in the 995. position. However, the presence of a mutation in a heterozygote form does not prove the diagnosis of an autosomal recessive condition and does not correlate with the severe clinical symptoms. Therefore, parental genomic DNA was extracted, and in the maternal sample a c.2196-2197insT (p.P733fsX9) mutation was found in exon 14. The mutation was not decribed previously, its pathogeneicity is evident by causing early degradation of the mRNA from this allele, a mechanism called nonsense-mediated mRNA decay. Latter is a surveillance pathyway existing in eukaryotes, its main function is to reduce errors in gene expression by eliminating mRNA transcripts that contain premature stop codon and would otherwise result in the translation of a deleterious, aberrant protein.

At age 7 years, the patient lost the ability to swallow, required tube feeding and became wheelchair-bound. By age 8 years, coma and vegetative status developed, and a spontaneous bone fracture occured most likely due to the occupation of the bone marrow by storage material and thinning of the cortical layer. At 9½ years, the patient died. Miglustat therapy was attempted, but by the time the previously off-label drug was approved as the only therapeutic mean for NPC, she was in an advanced stage of the disease and neurological deficits could not be reversed. In addition, the known side-effect of miglustat, diarrhoea resulted in progressive weight loss and the parents requested cessation of the therapy.

Fig. 13. Series of photos showing disease course and regression in Patient 9. suffering from Niemann-Pick C disease, from age 9 months till age 7 years.

IV./5. Uniparental disomies, methylation defects

Uniparental disomies (UPD) occur when an individual receives two copies of a whole chromosome or part of a chromosome from one parent and none from the other. If the two chromosomes inherited from the same parent are non-identical (originating from both grandparents as a meiosis I error), the phenomenon is called heterodisomy and is most commonly benign. If, however, two identical copies of a chromosome is inherited (a duplication of a chromosome of just one grandparent (meiosis II or mitotic error)), it is described as isodisomy. UPD may subsequently result in the duplication of recessive genes, or loss of function/gain of function of genes (isozygosity) subjects to imprinting. UPD should be

suspected in an individual manifesting a recessive disorder, where only one parent is a carrier. *(Gardner and Sutherland 2004)*.

In the years 2007-2013 UPD was found to be the pathogenetic mechanism responsible for the phenotype of four of our patients (0.1%). All were entire chromosomal UPDs. One patient had Prader-Willi syndrome (maternal UPD of chromosome 15), three had Angelman. Case 10 respresents a rare entity with isodisomic paternal UPD of chromosome 15 causing Angelman syndrome.

Methylation defects were found in four cases – one Russel-Silver and three Beckwith-Wiedemann syndromes. A case of Russel-Silver syndrome is presented from this category.

Case report – Patient 10. Angelman syndrome with paternal isodisomy 15

The patient was born from healthy parents as their first child. Perinatal anamnesis was uneventful. At 4 months, a grand mal type epileptic seizure occurred, carbamazepine was introduced. Torticollis was noted in infancy, developmental delay at approximately 18 months. Brain MRI did not show any abnormality. Neurohabilitation was started at 2 years, but it was noted that the severity and frequency of seizures did not explain the degree of mental and motor impairment. Previous chromosome analysis showed a normal male karyotype. Genetic counseling was requested, upon which the patient presented severe intellectual deficit, delayed motor skills (he could crawl at age 2, but could not walk independently), and stereotypic hand-flapping. Dysmorphic features included plagiocephaly, strabism, myopia, large mouth with thick lips and widely spaced teeth *(Fig.14.).* The muscle tone was spastic, and the movements were overall dysharmonic. Attention deficit and mouthing was observed when being approached with toys.

The triad of epilepsy, mental retardation and characteristic behavior suggested Angelman syndrome. DNA microsatellite marker analysis was requested after the 15q11 region specific FISH proved a normal signal pattern, and it revealed paternal isodisomy of chromosome 15. Differential diagnosis prior to receiving the results included Pitt-Hopkins syndrome: hyperventillation was often seen, the morphology of the mouth and teeth and the mental status would have been in accordance with Pitt-Hopkins.
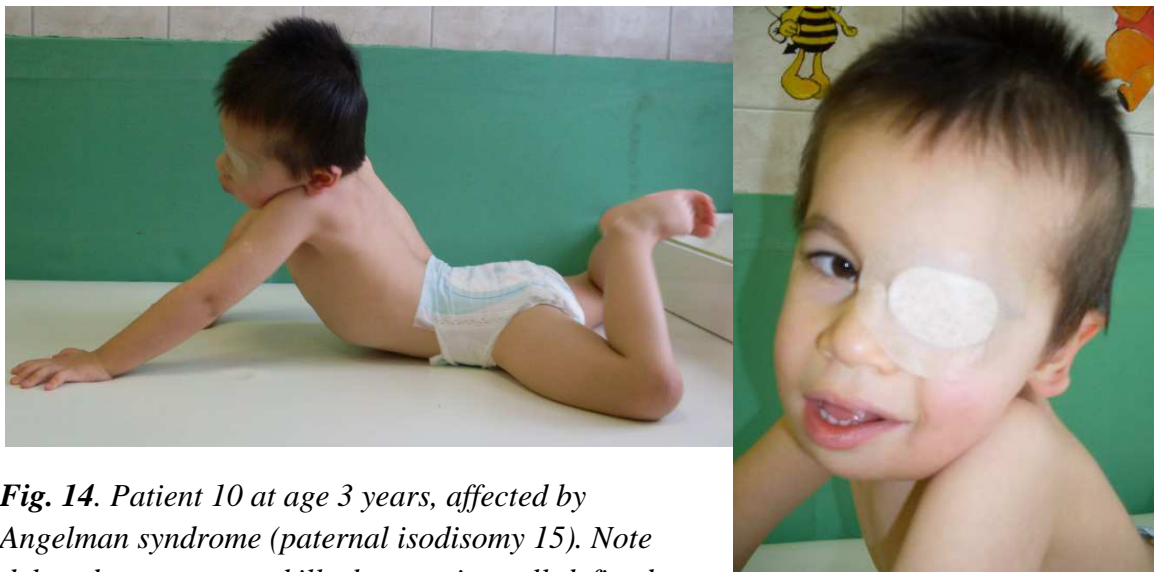
Fig. 14. Patient 10 at age 3 years, affected by Angelman syndrome (paternal isodisomy 15). Note delayed gross motor skills, hypotonia, well-defined eyebrows, strabism, wide mouth.

Considering that the prevalence of Angelman syndrome is 1-9/100 000, and most frequently (65-75%) it is due to microdeletion of 15q11, UPD is responsible for 2-5% of cases, the below presented case has an estimated incidence of 0.5-1.8/ 1000 0000.

Case report – Patient 11. Russel-Silver syndrome

Patient was born as the first child of healthy, nonrelated parents, with IUGR (weight 600g<3pc, length 4.5cm<3pc, OFC 25-50pc). Ectopic kidney was noted on prenatal ultrasonographies, postanatally it proved to be hypoplastic but functionally normal. Brain ultrasonography revealed dilated lateral ventricles, cardic UH showed a small FoA. Motor

development was mildly delayed – patient learnt to sit at 9 months and walked with assistance at 15 months. The reason for the parents' seeking help in genetic counseling was lack of weight gain rather than retarded longitudinal growth. At 15 months, weight was 2600g<3pc, length 1cm<3pc, OFC 50-75 pc – weight was more affected than length and the head was normal to age but inproportionately large for the rest of the body confering pseudohydrocephalic appearance. The skull had a triangular shape with prominent, broad forehead, sparse, straight hair and a small chin. *(Fig. 15.)* An overall gracile built was seen with loss of subcutaneous fat tissue. The intellect was normal, speech was fluent with rich vocabularly, voice was strikingly hypernasal. A methylation specific PCR (performed in Wien by Dr. Petra Zeitlhofer, Medgen At GmBH) proved the hypomethylation of the H19 promoter and that of exon 8 of the IGF2 gene in the 11p15 region. Prevalence is 1-9/1 000 0000.

Fig.15. 15 months old patient with Russel-Silver syndrome. Inproportionate growth retardation with weight more affected than length, pseudohydrocephaly, triangular face, receding chin. Intelligence is normal.

IV./6. Mitochondrial diseases

Between 2007-2013, 3/2049 patients were proved to have a mitochondrial disease based on molecular genetic examinations, and one based on MRI spectroscopy. Two patients had mitochondrial depletion syndrome, one of them presenting with the classical features of MNGIE (mitochondrial neurogenic gastrointestinal encephalopathy) although the onset was unusually early. Both patients died. One patient had Leigh's syndrome with T9176C mutation in the mitochondrial genome, brief description is presented below.

Case report – Patient 12. Leigh disease

The patient was born to term with normal parameters, perinatal period was without complains. Inguinal hernia was noted in early infancy and was planned to be undertaken surgical intervention at 4 months of age. Routine laboratory examinations prior to anaesthesia revealed high lactate levels (4-7 mmol/l) and normal ammonia. Repeated metabolic screening gave negative results. Decreased muscle tone was noted on occasion of a physical examination, and patient was referred to a neurologist and clinical geneticist. The association of high lactate and mild hypotonia raised the possibility of a mitochondrial disease. Muscle biopsy was performed and the remnants of the muscle tissue from histological processing was used for DNA isolation. The histology provided evidence for a mitochondrial disease – intracellular lipid droplets and uneven, granular distribution of mitochondrial enzymes (COX and NADH) were seen. Ultrastructural investigations with electronmicroscopy did not further support the diagnosis, so sequencing of the mitochondrial genome from muscle biopsy followed. A T9176C mutation proved Leigh syndrome. Soon after receiving the molecular results, encephalopathy developed, the patient presented alternating coma and slightly more alert episodes and needed ventillation support. *(Fig.16.)* Brain MRI at 18 months revealed multiple lacunar damage of the white matter and basal ganglia, consistent with the finding of

subacute slerosing encephalopathy. The patient has been on mechanical ventillation ever since and shows only very low level of consciousness. The mutation could not be detected from maternal blood. Medication through involvement in a study was attempted, but mechanical ventillation was exclusion criteria.

Prevalence is <1/1 000 000.

Fig.16. Photos of Patient 12. with Leigh-syndrome at 5 months and at 23 months showing evolution of a progressive, devastating disease.

IV./7. Polygenic/Multifactorial disorders

This group of diseases consist of congenital malformations of a heterogenous nature with genetic or partially genetic origin arising from the interaction of a large number of small effect genes and environmental factors, each contributing to the phenotype only to a small extent. Polygenic traits without provoking environmental factors manifest as continouos features (intelligence, height) rather than as diseases, while the sum of predisposing genes and environmental effects exceeding a threshold result in abnormal phenotype.

22/2049 (1.1%) patients in the reported time interval showed phenotypic features that were considered to have arisen as polygenic or multifactorial traits. Eight of them were referred because of subnormal intelligence – mild mental retardation or borderline IQ. In all families, one or both parents had the same, their highest degree of education was elementary school 8th grade. Neither the parents, nor the patients showed major dysmorphic features. This, of course, does not exclude the possibility of inherited pathological copy number variations, but the severity of their condition did not call for urgent molecular cytogenetic investigations. In 14 patients, isolated or multiple congenital anomalies (hip dislocation, anus atresia, scoliosis, spina bifida, clubfoot, cleft palate – *Fig.17.)* were seen, all were sporadic cases. In this group of patients, the intelligence seemed normal, and with surgical or orthopedic treatment a fairly good quality of life could be achieved.

Photos without detailed histories are presented, regarding that the anomalies were isolated.
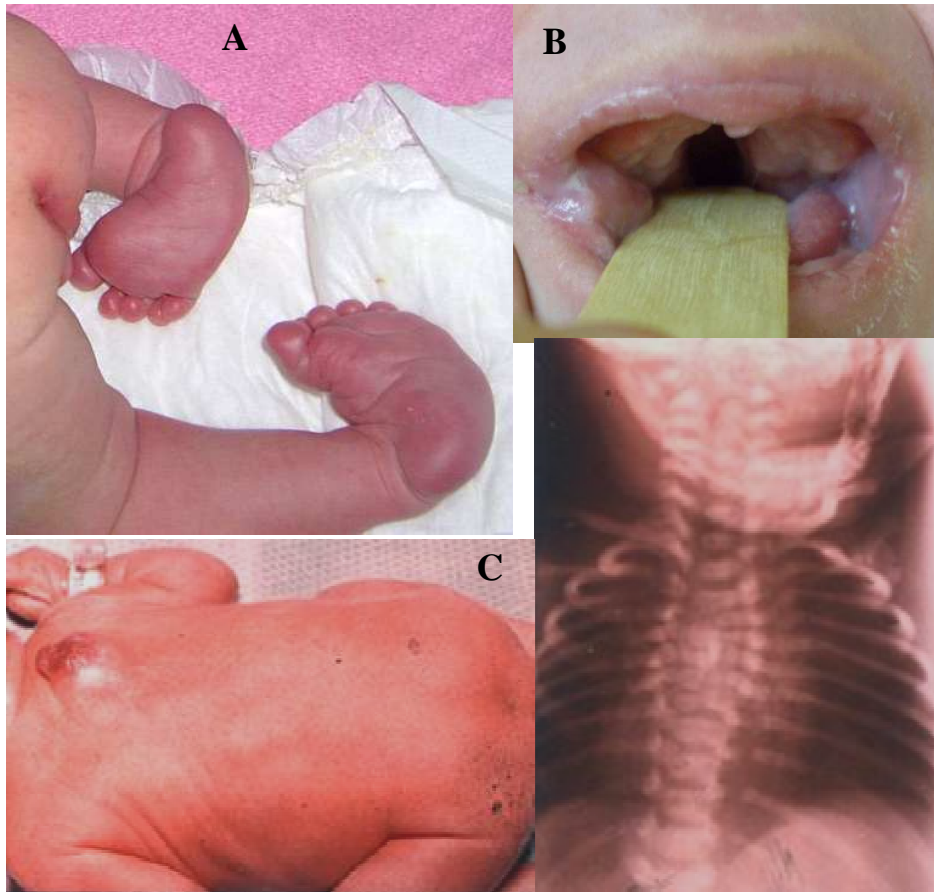
Fig. 17.

A) *Clubfoot*

B) *Cleft palate, prominent palatinal ridges*

C) *Spina bifida with long, complex fusion defects of the vertebrae and spinal cord*

IV./8. Phenotypically diagnosed but not molecularly proved syndromes

The number of syndromes with unknown genetic mechanism is gradually decreasing with the application of genome-wide studies. The advent of comparative genomic hybridization, whole exome and whole genome sequencing have contributed to the understanding of rare genetic disorders to a great extent. Yet, the genetic basis of a number of sydromes and rare diseases remains to be clarified, or confirmatory studies are still in progress to validate the results of genome-wide approaches.

In the reported time interval 249/2049 (12.2%) patients received a diagnosis based on phenotypic features and not molecular or molecular cytogenetic tests. *(Table 5.)* Latter (CGH) would be indicated in almost all of them according to present standards but is very unlikely to be made in the near future due to limitations of technical and financial resources.

In 30 cases (e.g. Acrodysostosis, Coffin-Siris, Klippel-Trenaunay-Weber syndrome), the underlying genetic mechanism was not known at the time of diagnosis (for Acrodysostosis and Coffin-Siris syndrome the corresponding genes were identified very recently), some of these patients would make good candidates for whole exome sequencing. 18 cases fulfil most of the clinical criteria of Marfan syndrome and are planned to be tested, since the molecular genetic test for fibrillin-1 (FBN1; OMIM 13497) has recently become available in our center. Yet, the size of the gene and the capacity of the personnel does not allow mass testing. In 12 cases, molecular testing was done in other centres and patients were asked to go there instead of just sample shipping, they are out of our sight by now. In 30 cases, molecular or molecular cytogenetic tests are currently in progress and results are expected to arrive soon. In three cases, testing was declined by parents or decision-makers. In 100 cases, further molecular genetic tests would be absolutely necessary. In 11 cases teratogenic cause could be identified (maternal alcohol consumption, retinoic acid treatment for acne during pregnancy).

In 33 cases, no confirmatory molecular tests were suggested because the suspected syndrome shows sporadic appearance with no known genetic mechanism (Poland-anomaly, hemihyperplasia, oto-auriculo-vertebral defects). In three cases, where inheritance is autosomal dominant, the patient has not reached his/her fertile years and parents declared not to opt for further. For five patients, no further genetics tests can be offered with the syndrome being evident but confirmatory tests negative. Other molecular mechanisms can be suspected in these cases but no countries offer further tests at present (e.g. a Nicolaides-Baraitser patient without SMARCA2 mutation).

Phenotypically diagnosed cases without confirmatory molecular genetic or molecular cytogenetic tests between 2007-2013 (Total of 249)	
Status	**No of cases**
Genetic basis unknown at the time of diagnosis(e.g. Acrodysostosis, Klippel-Trenaunay-Weber, Cerebro-facio-thoracic dysplasia)	34
Marfan syndrome	18
Presently in progress	30
Performed in other national institutes (Neurofibromatosis I, oncogenetics)	12
Mild morphological features, good quality of like, sporadic appearance (e.g.: Hemihyperplasia, Goldenhar syndrome , Poland anomaly)	33
Confirmatory tests are needed	100
Declined by parents or decision-makers (Beckwith-Wiedemann, Cornelia de Lange)	3
No risk of recurrence - patient not yet in fertile years, parents opted not to engage in further pregnancies (Hypohydrotic Ectodermal dysplasia, Frontonasal dysplasia)	3
Teratogenic cause (fetal alcohol syndrome, retinoic acid embryopathy)	11
No further tests can be offered (e.g. Nicolaides-Baraitser syndrome with no detectable mutation of the SMARCA2 gene, SMMCI)	5

Table 5. *Summary of phenotypically diagnosed patients without the support of confirmatory molecular tests.*

As the table shows, confirmatory diagnosis of 30 patients is presently in progress with additional 120 whose route is now clear. From the phenotypically diagnosed group, the 100 patients without confirmatory molecular tests at present need the most attention. In a number of them, pathological copy number variations (CNVs) might be the genetic cause of symptoms involving major genes in a deletion, requiring CGH testing in most of them. In a smaller number of patients, sequencing and deletion testing of responsible genes are needed in collaboration with foreign laboratories and individual financing from the Hungarian National Health Insurance Fund. This is an extremely costly and time-consuming procedure, but

progress is made over time – an average of yearly 40 cases are handed into the National Institute for Quality and Organisational Development in Healtcare and Medicine for judgement of cost coverage with involvement of the Hungarian Special College of Genetics, and 99% of the requested tests finally gain financing. However, with each case solved, several new, unsolved ones step in their places, making the diagnosis of rare diseases an infinite and challenging task.

In Case 13, a patient is presented with Solitary Median Median Maxiallary Central Incisor Syndrome (SMMCI) – an extremely rare malformation syndrome where phenotypic features prove the diagnosis, but the molecular basis is still unknown. The case was published in the European Journal of Medical Genetics *(Szakszon et al 2012)* and according to the reviewers' comments, to date she is the only known patient in the literature with the unique association of a solitary median incisor and panhypopituitarism. Her data and photos were requested by the Editorial Board of the London Dysmorphology Database to be included in it.

Case report – Patient 13. Solitary Median Maxillary Central Incisor Syndrome (SMMCI)

Patient (female) was born preterm from healthy, non-consanguineous parents in the 36th week of gestation. Birth weight was 10 pc, length 50 pc, considered normal for gestational age. Both parents are of Caucasian race and normal stature (mother 155 cm, father 170 cm). Perinatal period was uneventful, so as the early childhood. Early motor development was normal.

Growth delay was first noted at 5 years of age, however, parents abstained from inpatient admission and laboratory examinations. Concern over growth retardation grew and medical help was requested as the patient reached 7 years of age. At physical examination extreme growth retardation was noted with a height of 77 cm (35cm<3pc) and weight of

9,5kg (9kg<3pc). IQ was 49 according to the Budapest-Binet test. Bone age was 6 months according to Greulich and Pile. Sellar surface on a skull X-ray was 10 mm2, consistent with the finding of empty sella, confirmed by cranial and sellar MR as well. Neither the neurohypophysis nor the adenohypophysis could be distinguished. Suprasellar structures (including the stem of the hypophysis, chiasma, all four ventricles, falx and corpus callosum) were anatomically intact. No signs of holoprosencephaly was found.

Laboratory investigations proved growth hormone (GH) deficiency (< 0,15 µg/L; < 0,384 mU/L), insulin and L-DOPA provocation tests could not stimulate GH secretion, growth hormone levels remained <0,5 µg/L. Thyroid function tests revealed central hypothyroidism (fT4 8 pmol/L, sTSH 0,4 mU/L). Abdominal and cardiac ultrasonography showed no structural problems, whereas pelvic ultrasonography could identify only a short, partially agenetic vagina and could not distinguish the uterus and appendages.

Cytogenetic investigation showed a normal female karyotype. Comparative genomic hybridization detected an overall normal profile - deletion 2q21.2 (132972039-133141083)x1 (169kB) and duplication 20p12.1 (14163751-14322366)x3(158.6 kB) were of maternal origin, who has a normal phenotype, and were therefore concluded not to have clinical significance. SHH mutation analysis as described by Nanni et al. (p.I111F) and Garavelli et al. (p.V332A) were negative *(Garavelli et al 2004; Nanni et al 2001)*.

Levothyroxin (1.5 μg/kg body weight, gradually raised to 3μg/kg) and growth hormone replacement (Somatropin 0.025 mg/kg/die) were introduced, upon which thyroid hormone levels reached the normal range and a reasonable (4,8cm/year) longitudinal growth was achieved. Regular follow-up was maintained.

At age 14, as no signs of puberty appeared, patient was referred to genetic consultation. At physical examination a strikingly short but proportionate stature, muscular

build with loss of subcutaneous fat tissue, shield chest, and the absence of puberty was seen along with a friendly disposition and mild mental retardation *(Fig.18A)* The external genitalia appeared to be normal to gender. Follicle stimulating hormone (FSH) was 0.103 IU/L, luteinizing hormone (LH) was < 0.1 IU/L, prolactin (PRL) 0.424 μg/L, testosterone (T) was 0.159 nmol/L consistent with the previous finding of empty sella. Visible dysmorphic features included long face, mandibular prognathism, hypotelorism, convergent strabism, and a single maxillary incisor precisely in the midline of the upper dental arch with a symmetric crown *(Fig.18B),* suggesting SMMCI. Pelvic MRI was performed to clarify the status of the internal genitals, and showed a short, 4cm x 0,86 cm hypoplastic vagina, conjoined with a 1.5 cm x 1.5 cm small, rudimentary, horn shaped uterus. No ovaries could be identified. Considering that SMMCI is a midline defect, and the ovaries are not midline organs, this finding was controversial. To confirm or disapprove it, a diagnostic laparoscopy was performed, during which both ovaries were found, and a small, hypoplastic uterus could be distinguished. *(Fig.18C)*

Estradiol (0,5 mg/die) replacement was introduced in addition to growth hormone and thyroid hormone replacement with a good compliance. At age 18, the patient's height was 144.1 cm (10cm<3pc) weight 37.9 kg (8kg<3pc), but the epiphyseal plates were still open, giving a chance for further longitudinal growth. Sexual maturity was A I+ , M I-II, P I-II according to Tanner's staging *(Marshall and Tanner 1968)*. Patient has completed 9th grade of special school, manages well in everyday tasks, and does household work.
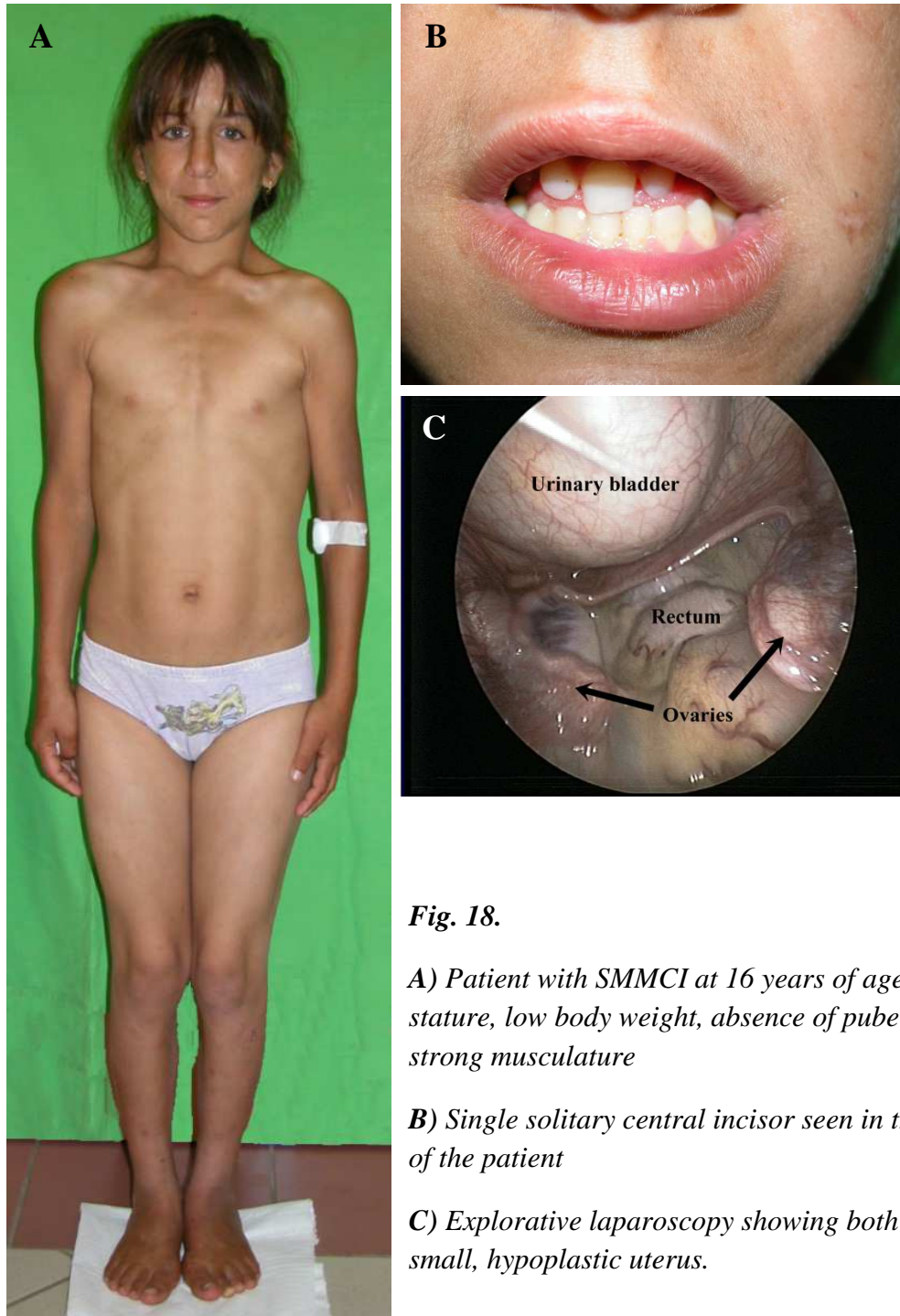
Fig. 18.

A) Patient with SMMCI at 16 years of age, presenting short stature, low body weight, absence of puberty, shield chest, strong musculature

B) Single solitary central incisor seen in the maxillary arch of the patient

C) Explorative laparoscopy showing both ovaries and a small, hypoplastic uterus.

Case 14. Coffin-Siris syndrome

Patient was born as the 7th living child of parents out of the 8th, unplanned pregnancy in the 32nd week with normal somatic parameters. The mother had no knowledge about the

pregnancy unil the second trimester, she had regular periods. In the first trimester, she smoked and did not receive medical attendance. On the 3rd postnatal day a loud heart murmur was noted – haemodinamically significant atrial and ventricular septum defects, patent ductus and pulmonary hypertension was diagnosed requiring surgical correction at 6 months of age. Due to poor sucking, bottle feeding was introduced. Genetic counseling was requested at age 13 months- reasons for referral were major congenital anomalies and developmental delay. Physical findings included moderately retarded somatic growth, hypotonia, and mild cognitive deficit. The face was broad and slightly asymmetric – the right half was apparently smaller. Other dysmorphic feautures included hypertelorism, vertically narrow palpebral fissures, long philtrum, thin vermilions, delayed dentition and the absence of the distal phalanges of the 5th finger on all four limbs. *(Fig. 19.)* X-ray revealed the absence of the distal phalanges of the 4th toes as well, while there were small additional bony islands conjoined with the proximal phalanges of the 2nd and 3rd digits.

Brain MRI was normal, karyotyping proved normal female karyotype.

The association of the visible anomalies of the 5th fingers, cardiac anomaly, developmental delay and retarded somatic growth are consistent with the diagnosis of Coffin-Siris syndrome. Photodocumentation was shown and diagnosis was approved by Prof. Raoul Hennekam. The syndrome is reported to follow both autosomal dominant and autosomal recessive inertitance, the underlying genetic defect is not known and <50 cases have been reported so far in the literature. Prevalence is <1/1 000 000.

Fig. 19. *Coffin-Siris syndrome in a 1-year-old girl. Note broad and asymmetric face, flat supraorbital ridges, thin vermilions, hypoplasia of the 5th fingers and toes.*

IV./9. Patients with infertility with or without detectable genetic anomalies

This group of patients (285/2049 patients; 13.9%) constitute the research field of a colleague in the cytogenetics lab. Their data and cytogenetics findings are included in his already accepted thesis *(Mokánszky et al., 2013.)*

IV./10. Unclarified conditions with presumably genetic origin.

365/2049 (17.8%) of all patients and 35.7% of phenotypically abnormal patients could not be diagnosed during the time interval of the present report. In at least 249 cases – patients

showing major congenital anomalies associated with any degree of mental retardation – CGH would be necessary to narrow the possible diagnostic spectrum, in 79, X-linked mental retardation can be suspected based on male gender, autism spectrum disorder, behavioural problems, other affected males in the family and absence of major dysmorphic features. A considerable proportion of patients in these categories are likely to have detectable genetic defects. In 42 patients, whole exome sequencing could possibly prove the origin of their conditions, but again, only in the knowledge of a negative CGH array profile. And even if all these extended tests were carried out in all patients where needed, a certain proportion would probably still remain undiagnosed and the mechanism through which their conditions evolved could not be clarified.

It is noteworthy, that 235 patients have not returned to the regular yearly follow-up for over 2 ½ years, which we indicate even without concrete future diagnostic plan to avoid forgetting about the patient or losing information about his/her current status – principally his/her mental and somatic development. These patients could either not wait any longer for a diagnosis and got tired of unprofitable medical visits, or some of them might have continued to catch up with peers, some might have tried to seek help in other institutes, and some might have received a definitive diagnosis there. The remaining 130 patients keep trying and show up as requested. In 36 patients, just by assessing their data for the present thesis, strong clues for certain diagnoses arose and their further diagnostic management is now delineated. One patient already proved positive for the suspected diagnosis (DiGeorge syndrome). In conclusion, not only today's genetic diagnostic tests develop rapidly, but the clinicians experience on phenotypes is increasing with every case, and more unsolved cases mean more solved cases.

Case report – Patient 15. Unclarified syndrome, suspected DOOR syndrome

The patient was born from healthy, non-consanguineous Caucasian parents as an intrauterine retarded child in the 36th week of gestation, weight was 3pc, length 4cm<3pc, OFC 3-10pc. The pregnancy was associated with toxaemia, and the mother received L-thyroxin and methyldopa to avoid severe complications. Dysmophic features (clubfeet, microcephaly, unusual face) were noted right after birth, cardiac ultrasonography proved subvalvular aortic stenosis and grade I. mitral insufficiency with normal ventricular function, and a muscular angulation protruding into the left ventricle causing partial obstruction of the left ventricular outflow tract. Further detailed investigations showed mild pyelectasia, hiatal hernia (fundoplication performed at age 5 months), muscular hypotonia, hypospadiasis, atretic external ear canal on the left and normal on the right side but with a 30dB hypacusis (conductive; BERA) here. Beta-blocker therapy was introduced. At 17 months of age an open heart surgery was performed with extracorporal perfusion and the heart anomaly was corrected as much as possible. Ophthalmological investigations revealed bilateral blepharophimosis with atretic nasolacrimal duct. Skull X-ray showed no signs of craniosynostosis. No choanal atresia was noted. Swallowing and oral feeding was never achieved, although upper gastrointestinal tract endoscopy revealed no anatomical anomalies. Pharyngeal reflex and other deep tendon reflexes were described to be diminished.

Gross motor milestones and speech were delayed. Standing was achieved at 2 ½ yrs, taking steps at 3 yrs, manipulation with hands were normal to age, attention was said to be fairly good. In his own environment patient walked unaided at 3 ½ yrs. Speech developed to a few dozen understandable words. Main laboratory parameters: mild iron deficient anaemia, normal hepatic function, blood glucose and renal function, mild hypothyreosis (sTSH:5.6 mIU/L, Ref: 0.3-4.2mIU/L), hyperuricaemia (uric acid: 484 umol/l, Ref<340 umol/l), hyperparathyreosis (PTH: 94 pg/ml, 105, 150, Ref: 10-65), normal serum calcium.

Patient receives physiotherapy, speech therapy and supportive care, as a result parents notice a slow but steady improvement in his mental status. Sleep disturbance is remarkable. The patient has a healthy younger brother.

On physical examination at 3 7/12 years body weight was 3-10 pc, length 3cm<3pc, OFC: 4cm<3pc. Major dysmorphic features included: bilateral frontal bossing, vertically short, wave-shaped palpebral fissures, telecanthus, mild facial asymmetry, flat nose, long and featureless philtrum, thin vermilions. No remarkable dental anomalies were found. Preauricular tags are present bilaterally. The distal phalanges of the right 2nd and left 2nd and 5th fingers of the hands were hypoplastic (confirmed by X-ray), nail could not be distinguished here. The same phenomenon could be seen on the 4th-5th toes on both feet. *(Fig.20.)* The patient is fed through gastric tube, nutritional status is normal, mental retardation is moderate and behavior is respectful and friendly behavior. Understanding of speech and perception is much better than expressive language. No history of seizures.

Karyotype, subtelomeric FISH, serum 7-Dehydrocholesterol, brain MRI were negative. Array CGH is presently in progress, DNA sample was shipped to Amsterdam, if negative, DOOR (Deafness-Onychodystrophy – Onycholysis Retardation) syndrome was suggested by Prof. Raoul Hennekam, and biochemical and molecular testing of oxyglutarate dehydrogenase (OGDH; OMIM 613022) was offered generously by Dr. Guntram Borck, Institute of Human Genetics, University of Ulm, Germany. It remains a question of the molecular cytogenetic and molecular tests ahead whether the underlying genetic mechanism can be clarified.

Fig.20. *Patient with multiple congenital anomalies and moderate intellectual deficit. Onychodystrophy is a strong diagnostic clue for DOOR syndrome, yet, pathological copy number changes are to be excluded prior to biochemical or molecular genetic investigations.*

Case report – Patient 16. Unclarified genetic disorder, no further diagnostic suspicion

Patient was born to term with normal somatic parameters, perinatal period was uneventful, early development was reported to be normal. Seizures started at 2 years of age, since then, mental and motor development reached a plateau, but definitive regression did not occur. The seizures react partially to combined antiepileptic treatment. Severe mental retardation, mouthing, sleep disturbance, no major congenital anomalies characterize the clinical picture. Facial appearance is best described by round face, rounded chin, mandibular prognathism.

(Fig. 21.) Karyotyping, 17p11.2 region specific FISH (Smith-Magenis syndrome), MECP2, CDKL5, FOXG1 molecular genetic tests for Rett- and atypical Rett syndrome, brain MRI, metabolic screening for 26 inborn errors of metabolism, array CGH are negative. No further diagnostic test can be offered at present.
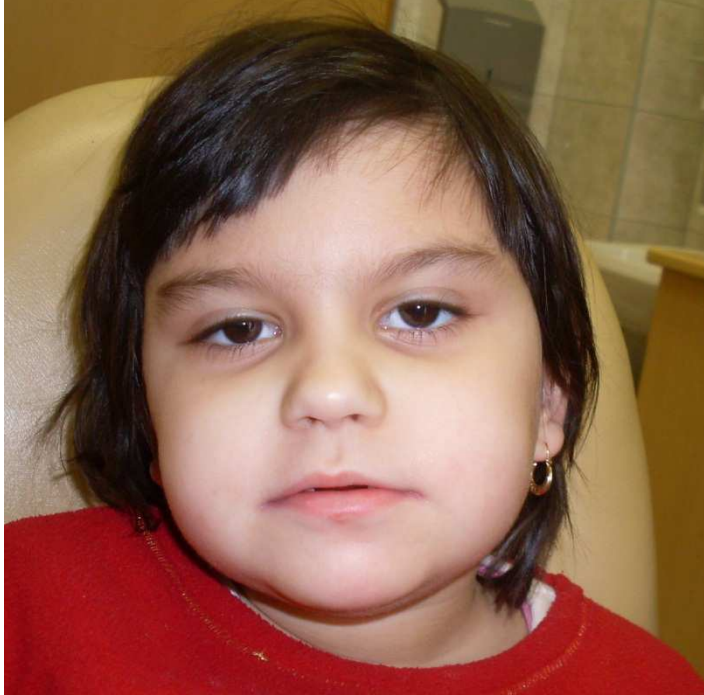
Fig. 21. Patient with unknown origin of severe intellectual deficit, partially therapy-resistant seizures, sleep disturbance.

V. Discussion

The diagnosis and management of rare diseases is a constantly expanding field of medicine and an increasingly important public health issue. The unique combination of low prevalence, yet severe, devastating or chronically debilitating nature of orphan diseases, lack of sufficient knowledge on their cause, symptoms and treatment call for a global act to improve the quality of life of the affected patients and reduce recurrence risk in families. Scientists, medical professionals and civil organisations together have already achieved enormous advances in the field.

Approximately 80% of rare diseases have genetic origin, and 50% effect children, manifesting as congenital malformations and/or intellectual disability *(Eurordis- Rare diseases Europe 2012).* Addressing them requires special combined efforts with making the proper diagnosis in the first place, reducing recurrence risk and providing access to therapy in the second. Given the rarity of most conditions, the diagnostic procedure alone may last for a very long time, often for years.

In the past 5 ½ years of work as a clinical geneticist the author's aim was to provide definitive diagnoses to patients seeking help at the outpatient clinic of the Clinical Genetics Center operating in the Inst. of Pediatrics, University of Debrecen, Medical and Health Science Center: recognize if there was a suspicion for an underlying genetic abnormality, confirm the presumed diagnosis with properly chosen genetic tests, assess recurrence risk, provide a basis for future prenatal diagnosis, inform families about the expected outcome, and initiate therapy where possible. The results of this diagnostic work is summarized here. Another important goal was to define in what proportion of patients referred to our clinical genetics center a genetic abnormality/rare disease could be proved; which group of genetic origin they represented and in what ratio; whether the distribution of the diagnoses reflect the international prevalence data; whether the strict policy and limited resources of the Hungarian health care system facilitate or hinder the diagnosis of rare and extremely rare diseases; and what means would be urgently necessary to step forward in the critical issue of rare diseases.

Data of overall 2049 patients were assessed and categorized by the genetic origin of symptoms. Of them 741 patients (36.2%) did not have genetic abnormalities – this group of patients were tested for carrier status of a genetic illness, were referred for isolated minor anomalies or behavioral problems without true mental retardation, or were healthy relatives of patients providing blood samples for testing the propositus (DNA microsatellite marker

analysis for instance). An additional 285 patients were referred because of infertility and tested negative for chromosome abnormalities. Thus, the diagnosis of 1023 patients remained to be solved. Of them, 21 were carriers of an autosomal recessive or X-linked recessive disease but symptom-free themselves – as their genetic defects have importance in future prenatal tests they are counted as diagnosed patients. The number of phenotypically abnormal patients was 2049-(741+285-21) = 1002. The number of mentally retarded patients were 573, the remaining 429 patients had normal cognitive functions or corrigible, mild developmental delay.

Altogether 658/1023 patients (carriers included) received a reliable diagnosis: 220 were chromosomal (G-banded numerical and structural), 40 were microdeletions detectable by FISH (including 7 patients with subtelomeric rearrangements), 6 had pathological CNVs, 110 patients were affected by a monogenic disorder, 4 had methylation defects, 4 had UPD-s, 3 were mitochondrial and 22 multifactorial of origin. 249 patients had a clear diagnosis based on phenotypic features, but molecular tests could not be offered for them because of no estimated recurrence risk in the family in the near future, financial obstacles or no known gene causing the syndrome. 365 patients remained undiagnosed. *(Fig.22.)*

ıg) -21.5%

.9%

·elevant) - 0.6%

;) - 10.7%

⁄

:d - 24.3%

ɟnosed in 2007-
ıogenic disorders.

Chromosomal abnormalities

220 patients (10.7% of all registered 2049 patients but 21.5% of phenotypically abnormal individuals) were proved to have chromosomal abnormalities visible by G-banding (>10Mb size) – this ratio is higher than suggested by other authors *(Miller et al 2010; Turnpenny and Ellard 2005).* 40 patients (1.9% of all and 3.9% of patients with abnormal phenotype) had microdeletions detected by FISH.

Among karyotypically abnormal individuals (260 patients, microdeletions detected by FISH included to serve as a basis for comparison with other reports), Down-syndrome was found in free-trisomic form in 95 patients (36.5%). Other autosomal aneuploidies were found in 1.2%, sex chromosome aneuploidies in 11.9%, deletions altogether in 6.2% + 15.4% (detected by G-banding and FISH, respectively), duplications in 5.4%, ring chromosome in 0.8%, small markers in 3.5%, balanced rearrangements in 10.8%, unbalanced translocations in 2.7% of all karyotypically abnormal individuals *(Fig. 23A),* each ratio higher than described by Phelan et al. *(Fig. 23B) (Phelan et al 1996).* Technical standards and quality of evaluation are best reflected by the number of structural aberrations identified – numerical anomalies can be detected at lower professional levels, too.

Considering that neither our team in 2007-2013 nor that of Phelan in 1996 could include CGH-derived results in the evaluations, comparison seems to be acceptable.
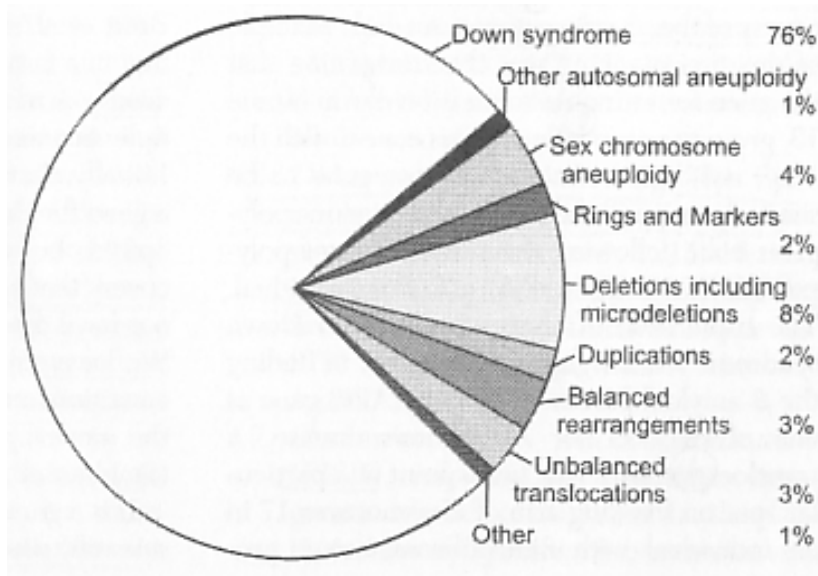
ly) - 36.5%

uploidy - 1.1%

uploidy - 11.9%

.3%

nicrodeletions - 21.6%

ents - 10.8%

tions - 2.7%

Down syndrome	76%
Other autosomal aneuploidy	1%
Sex chromosome aneuploidy	4%
Rings and Markers	2%
Deletions including microdeletions	8%
Duplications	2%
Balanced rearrangements	3%
Unbalanced translocations	3%
Other	1%

Fig. 23. The relative proportions of different cytogenetic categories in karyotypically abnormal individuals in A) our clinical genetics center, B) as reported by Phelan et al. (Phelan et al 1996). Possible explanations to differences are listed above.

The reason for a seemingly better recognition of chromosomal anomalies with karyotyping in our center when compared to several others may be attributed to the following factors: 1. The knowledge of the cytogeneticists and assistants, their technical skills, and the over 40 years of practice in the method by dedicated, extremely experienced professionals – Dr. Erzsébet Balogh and Dr. Anikó Ujfalusi. 2. Our genetics center is responsible for the care

of over 2.5 million inhabitants in the Eastern part of the country – postnatal genetic care of pediatric and adult patients from this population including those with malignant diseases are covered by three clinical geneticists and two cytogeneticsist only. Thus, experience with a constantly high number of patients and samples is more likely to allow expertize to develop. However, the same factor may lead to a less efficient diagnosis of non-chromosomal abnormalities – patients with attenuated phenotypes or those with extremely rare conditions may slip through our overloaded health care system or wait unreasonably long for a correct diagnosis. 3. Another important factor contributing to the efficient detection of chromosomal anomalies can be the proper choice and indication of genetic tests, adequate referral of patients to genetic counseling and filtering them prior to referral based on the severity of their condition by primary care physicians and practicioners of other subspecialties. This "prescreening" certainly leads to a faster diagnosis and an increased number of new patients from the moderate/ severe MCA/MR group, but again, it may have an adverse effect in that patients with milder degree of malformations and/or intellectual deficit may not reach the genetic outpatient clinic. 4. And finally, the "must" to continue karyotyping without regular access to CGH, noticing even small unbalances and trying to clarify their origins from parental karyotyping or FISH largely contributes to the advanced knowledge and maintenance of high quality karyotyping.

Although the ratio of Down-syndromic patients among all chromosomal imbalances is significantly lower in our center than reported by Phelan et al., (36.5% vs 76%) a constant attention surrounds its non-decreasing incidence. 105 patients (47.7%) of the detected chromosomal anomalies in our survey were trisomies 21 – free trisomies were found in 95 of them (36.7%), representing the largest group of chromosomal disorders. Reasons for this are very likely the following: 1. Extremes in child-bearing age: an increasing number of women opt (or have the possibility) for getting pregnant in their late thirties or early forties, while

other young and typically uneducated women engage in pregnancy in their teenage years. Both age-extremes are known to be error-prone in meiotic cell division. A considerable ratio of individuals with Down-syndrome belong to the roma ethnic group. Roma families are known to have a large number of children, of whom the youngest ones are often born at an advanced maternal age. Many of these mothers do not attend to the scheduled genetic screenings of pregnancies due to their low-education and social circumstances, or accept the genetically ill child as it is when informed about the increased risk. 2. No access to non-invasive screening for trisomies (free fetal DNA in maternal blood); many pregnant women decline invasive amniocentesis even if biochemical markers raise suspicion of Down syndrome. 3. Overemphasized, exaggerated positive effects of a Down-syndromic child on a family, often seen in booklets and handouts of civil foundations distributed in gynecology units suggesting mothers-to-be to choose to continue a screened Down-syndromic pregnancy. 4. Miscounting gestational age, performing the 11-13th week biochemical tests and ultrasonography earlier or later than targeted. 5. Lack of experience and FMF certification of ultrasonography specialists in certain hospitals. 6. Chromosome 21 is a relatively small chromosome and its trisomic state is better tolerated by the living organism than even smaller imbalances of larger chromosomes.

According to our practice without regular access to CGH, in patients whose karyotype proves to be normal, but whose symptoms suggest chromosomal origin at a submicroscopic level possibly matching into any of the well-known microdeletion syndromes – region-specific fluorescent in situ hybridization can be used as an alternative for molecular karyotyping. Important it is to note that while karyotyping and array CGH provide a genom-wide screening for chromosomal aberrations, FISH can detect the gain or loss of a certain chromosomal region only, smaller than 3-5 Mb. In other words, while karyotyping and CGH may provide a solution to the „What is wrong with this patient" question, FISH gives a „Yes

or No" answer to the „Does this patient have this syndrome?" question. Thus, the diagnostic yield of FISH is very much a matter of the proper choice of the probes applied, and as such, highly dependent on the quality of the clinical evaluation prior to testing.

In addition to the 40 patients whose diagnosis was derived solely from FISH, 22 patients had chromosomal abnormality that could be recognized but could not be precisely characterized by G-banding. In this group, too, the final diagnosis was obtained using FISH.

In six patients, CGH proved submicroscopic losses of the genome – latter is not relevant in drawing conclusion about its true diagnostic yield as it was used in very few, strictly selected cases and only in the past two years. Based on recent papers, molecular karyotyping is able to detect up to 20%-29% of genetic abnormalities in patients with multiple congenital anomalies and mental retardation *(Miller et al 2010; Rauch et al 2006)*. Its diagnostic yield is especially high if congenital heart defect is, intrauterine and/or postnatal growth retardation and moderate mental retardation are also part of the clinical picture, for the following reasons:

1. An incorrect amount of genetic material carried by the conceptus disturbs and distorts its normal growth pattern.*(Gardner and Sutherland 2004)* 2. Major genes and/or gene cluster responsible for the normal formation of the heart are present on every chromosome, it is therefore very likely that any microdeletion or duplication will affect the structural development of the heart. *(Fig.24.)* 3. Severe mental retardation has a greater likelihood to have monogenic origin if not a large, microscopically visible gain or loss, while mild mental retardation has a greater likelihood to have polygenic or multifactorial origin.
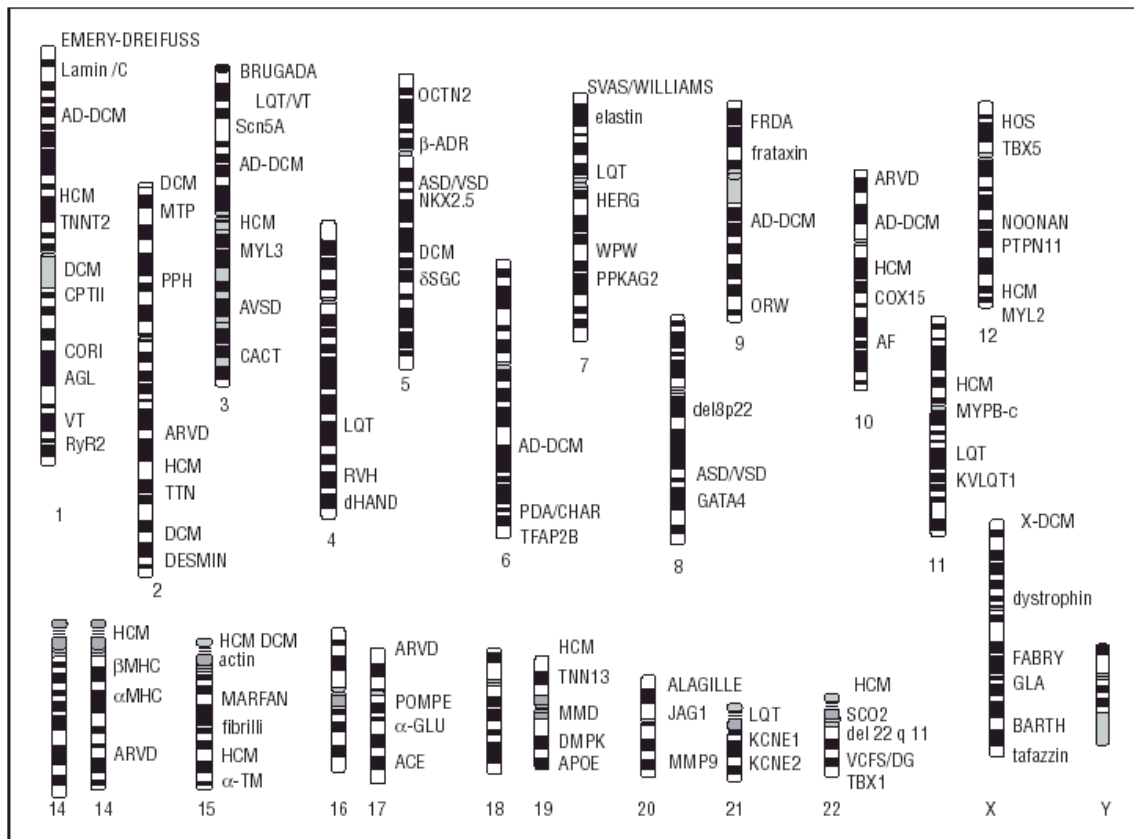
Fig. 24. *Genes responsible for congenital cardiac disorders. Figure from Richards et al., 2010. (Richards and Garg 2010).*

Multifactorial disorders

Multifactorial disorders consist of congenital malformations of a heterogenous nature arising from the interaction of a large number of small impact genes and provoking environmental factors. Each parent transmits a set of pathological alleles into the offspring that – added up with the alleles of the other parent and exposed to triggering environmental factors– cross the treshold of presenting as a pathological phenotype. Thus, disease phenotypes develop only after a certain critical liability threshold is reached –the further the liability threshold is surpassed, the more severe the disease phenotype is.*(Mossey 1999)* Traits that fall into discrete categories are discontinuous (cleft lip/palate, hip dislocation, scoliosis), while those that display a gradient of phenotypes (hypertonia, diabetes) are classified as continuous*(Lobo 2008)*.

Examples to multifactorial disorders are spina bifida, cheilo-gnatho-and palatoschizis, congenital hip dislocation, idiopathic scoliosis, pyloric stenosis (latter is now more often attributed to the NOS1 gene located on chromosome 12q with contradictory reports). (*Soderhall and Nordenskjold 1998*).

Mendelian disorders

Mendelian diseases were found in a total of 110 patient including carriers (21 patients) (5.4% of all patients and 10.7% of phenotypically abnormal patients). Diagnoses varied greatly and ranged from syndromic mental retardation to neuromuscular and neurodegenerative diseases. Prenatal diagnosis based on postnatal findings of an affected offspring was performed in five cases: in two (Treacher-Collins and Neurofibromatosis 2) the fetus was proved to be positive for the mutation and the pregnancy was terminated, in three (Fragile-X, Guanidino-acetate methyltransferase deficiency, ataxia teleangiectasia) the fetus tested negative and healthy children were born. In the majority of cases, the genetic defect is inherited in an autosomal dominant fashion and appeared as new mutation in the affected offspring, thus recurrence risk is low. In others cases where the inheritance is autosomal or X-linked recessive or the affected individuals of an autosomal dominantly inherited disorder have a high risk to produce offspring themselves, prenatal diagnosis can be offered if needed.

An extremely rare (<1/1 000 000) autosomal dominant congenital malformation syndrome whose diagnosis was made in the past 5 ½ years is Say-Barber/Biesecker/Young-Simpson syndrome, a variant of the Ohdo-blepharophimosis syndrome group *(Ohdo et al 1986; Verloes et al 2006)*. Affected patient share a characteristic facial phenotype and a combination of additional anomalies including heart defects, optic atrophy, deafness, hypoplastic teeth, cleft palate, joint limitations, and hypothyroidism. Mental retardation is ususaly severe, and although it is a constant feature of the syndrome, its cause remained long

unclear. No imaging studies have been published on SBBYS patients before - our working grop was the first to perform functional brain MRI on an affected individual and find neuroanatomical defects of which the signifinace was not clear at the time of description. The case was first published in the American Journal of Medical Genetics Part A in March 2011. *(Szakszon et al 2011).*

Mutations of the gene encoding the histone-acetyltransferase KAT6B were identified as a cause of SBBYS syndrome by Clayton-Smith et al. in November 2011. using whole exome sequencing *(Clayton-Smith et al 2011).* Our work-group detected KAT6B mutations in the previously described and in one additional patient, and ours was the first independent confirmatory finding after the initial description of Clayton-Smith. KAT6B encodes the K(lysine) acetyltransferase 6B, a component of the MOZ/MORF complex which has a histone H3 acetyltransferase activity; it is highly expressed in adult neural stem cells residing in the subventricular zone and is essential for the normal number, multipotency and renewal of the stem cells *(Merson et al 2006).* Recent studies demonstrate that KAT6B deficiency leads to developmental brain defects in mice *(Thomas et al 2000; Thomas and Voss 2004)*and disruption of the human KAT6B by a translocation breakpoint causes intellectual disability *(Kraft et al 2011).* These findings may be relevant to the understanding of the severe cognitive deficits observed in individuals with SBBYS syndrome and may provide an explanation for the disturbed myelination and axon disorganisation seen on the MRI of our patient.

With the identification of the causative role of KAT6B in SBBYS and knowing the biological role of the protein encoded by the gene, we can assume a relationship between the functional deficiency of the protein and the neuroanatomical malformations/cognitive impairment of our patient. It is not yet clear whether neuroanatomical anomalies were unique

findings in this single case or they are frequent features of SBBYS patients, but future neuroradiological studies will clarify their nature and prevalence in the syndrome.

For the affected family, consultation could provide the information of a de novo mutation in the child and no risk of recurrence in the parents' future pregnancies. Based on her intellectual status, the affected child is also very unlikely to pass on the mutation to further generations.

A special group of patients where single gene defects cause severely disabling, most often fatal illness is metabolic diseases. In most centers this group of patients are tested and consulted separately from dysmorphic syndromes, because they cannot be recognized by morphological features and the need for urgent treatment to avoid irreversible neurological damage where possible demands the proper choice of biochemical/molecular tests by experienced experts. In Hungary the care of patients with metabolic disorders are integrated in the work of genetic or rare disease centers, excepted the metabolic units of the Semmelweis University, Budapest and the University of Szeged.

In the reported period of 2007-2013 nine patients were proved to suffer from a metabolic disease – three of them were diagnosed in other national centers and referred to us as the regional health facility for further management and treatment. Five patients had treatable lysosomal storage disease, three of them died in spite of treatment, the condition of two patients improved and could be stabilized. Two patients with confirmed Niemann-Pick C disease were the first ones diagnosed in Hungary. Miglustat treatment was introduced as soon as the drug was approved as the only specific therapy of the disease, but by this time the condition of one patient worsened to a great extent. In this patient, the advantegous effects of miglustat could not be experienced, neurological decline was irreversible and side effects caused serious complications. In another juvenile NPC patient, miglustat proved to be highly

effective in restoring cognitive functions and ensuring a non-symptom-free but acceptable quality of life. Its typical side effect – diarrhea – resolved spontaneously after 6 months of therapy, as it happens in most cases. Although we have a very low number of cases with own experience, we postulated that the early onset and rapidly progressive disease in one patient and the the late-juvenile onset, slowly advancing if not stagnating disease with good response to treatment of the other patient reflect clear genotype-phenotype correlations, and the explanation to the clinical differences lies in the differences of the underlying genetic mutations. The mutation of the NPC1 gene resulting in premature stop codon and nonsense-mediated mRNA decay in the rapidly progressive form vs. two common mutations in the more benign form code for different residual protein functions and hence determine the phenotype.

An important differencial diagnostic issue regarding neurometabolic diseases is that they often mimic other neurodegenerative disorders such as leukodystrophies or mitochondrial encephalopathies – latter may de facto manifest as multiple lacunar damage in the white matter on neuroimaging studies, as seen in our Leigh-disease patient.

Mitochondrial diseases are a group of disorders caused by the defective function of mitochondria and subsequently disturbed energy supply of cells. Except for red blood cells, all eukaryotic cells contain mitochondria – structures responsible for converting energy derived from nutrients into ATP. Being so critical in cell function, especially in tissues with the highest energy consumption (muscle, heart, brain), mitochondrial diseases may cause a large variety of symptoms with similarly varying severity, presenting at „any age, any time, in any gender". Three major categories of mitochondrial diseases exist according to the primarily affected organs and predominant symtoms: 1. encephalopathy-myopathy, including Leigh syndrome, Myoneurogenic gastrointestinal encephalopathy (MNGIE), Mitochondrial myopathy- encephalopathy- lactic acidosis-stroke-like symptoms (MELAS), Myoclonic

epilepsy with Ragged Red Fibers (MERRF), Neuropathy-ataxia-retinitis pigmentosa-ptosis (NARP); 2. sensory organ deficits such as Leber's hereditary optic neuropathy, and 3., mitochondrial DNA depletion syndrome where the quantity of mitochondria is insufficient (Mitochondrial DNA depletion Syndrome)

Mitochondrial disorders may be caused by mutations in the mitochondrial DNA (encoding proteins of the respiratory chain) or in nuclear genes that code for mitochondrial components (numts). Given that mitochondria can be inherited maternally, and that each egg cell might have a different constitution of normal or defective mitochondria (heteroplasmia), inheritance cannot be fitted into mendelian rules and recurrence risk is unpredictable. Affected couples may opt for using enucleated donor oocytes to reduce risk in further pregnancies. In addition, the random distribution of mitochondria into daughter cells during mitotic divisions result in similarly randomly affected organs making the diagnosis difficult and peripheral blood often useless in genetic testing.

Another fascinating non-mendelian pathogenetic mechanism, even though the basis of only a small number of well-defined clinical conditions, is uniparental disomy and disorders of imprinting. The occurrence of uniparental disomy could be explained by models postulating postfertilization error, gamete complementation, monosomic conception with subsequent chromosome gain, or trisomic conception followed by chromosome loss *(Spence et al 1988)*. UPD may occur for the entire chromosomal complement, resulting in hydatiform mole (paternal), ovarian teratomas (maternal); for a complete chromosome or part of the chromosome (segmental UPD). Complete chromosomal UPD of chromosome 15 is the mechanism whereby Prader/Willi and Angelman syndrome arise, segmental UPDs are seen in cases of Russel-Silver, Beckwith-Wiedemann syndrome and transient neonatal diabetes.

The latter three conditions may arise from a different mechanism causing silencing or unsilencing originally genetically active or inactive segments of DNA via imprinting. Through imprinting, a chromosomal segment receives an epigenetic mark that is called methylation – methyl groups attached to cytosine bases. A segment of chromosome, or just a single locus is genetically active or not active according to whether it was transmitted from the mother or the father – thus, a parent of origin effect. Imprintable segments function monoallelically *(Gardner and Sutherland 2004)*. Imprinting errors (methylation defects) may cause an incorrect, biallelic or nullallelic expression of genes located in the regions subjects to imprinting.

In a number of patients none of the above genetic mechanisms could be confirmed or could be suspected as a cause of their conditions. In this group the diagnosis relies on phenotypic features and on a normal karyogram with the underlying genetic defect being unknown or with no financial resources to molecularly clarify them if the suspected recurrence risk in the family is negligible until the affected patient enters his/her fertile years. According to present professional standards, a normal array CGH profile should support their diagnosis, but it is very unlikely that all these patients would gain access to such test in the near future.

In several patients with MR/MCA, CGH was performed and failed to prove a chromosomal etiology – this was the case in our Solitary Median Maxillary Central Incisor Syndrome patient (SMMCI), OMIM 147250. (Detailed description see on page 61.) This rare malformation syndrome consists of multiple, mainly midline defects of development resulting from unknown factors operating in utero about the 35th-38th day(s) from conception *(Hall 2006),* hence the normal array profile was not surprising. Novum was the panhypopituitarism associating with the symptoms, not described previously in the literature. The case was

published in the European Journal of Medical Genetics. *(Szakszon et al 2012; Turnpenny and Ellard 2005)*

The need for CGH in the routine diagnostic work and the increasing need for whole exome sequencing in syndromes of unknown etiology reaches into our last group of patients – those with mental retardation and/or multiple congenital anomalies whose diagnosis is unclear. It is not unlikely that in a number of them monogenic syndromes could be identified, had they been seen by other, more experienced syndromologists, and the author did make efforts to present the toughest cases to professionals of other expert centers, but again, the large number of patients and the time-consuming preparation of files for presentation does not allow to do this in every single case. Those with apparently severe dysmorphic features and young parents wanting to have more children are chosen for consultation with foreign experts, whose valuable advice is deeply appreciated. All the more so, because their expert help cannot be offered payment or any other help in return – our center does not possess knowledge or methods other institutes do not have.

The most important foreign cooperations in molecular genetic, molecular cytogenetic tests and case-consultations are shown in *Fig. 25*.

Fig. 25. *Foreign cooperations for performing molecular genetic tests that are not available in Hungary and for consultations of the most difficult cases.*

- Prof. Raoul C.M. Hennekam, Dr. Alida C. Knegt, Academisch Medisch Centrum Amsterdam, The Netherlands
- Dr. Guntram Borck, University of Ulm, Germany
- Dr. Corinne Collet, Service de biochimie et biologie moléculaire, Hôpital Lariboisière, Paris, France
- Dr. Andrew Jackson, Inst. of Genetics and Molecular Medicine, University of Edinburgh, UK
- Dr. Petra Zeitlhofer, Medgen.at, Genetische Diagnostik&Beratung, Wien, Austria
- Prof. Franziska Joncourt, Hospital Universitaire De Berne, Bern, Switzerland
- Prof. Jan-Eric Mansson, Dr. Niklas Mattsson, Sahlgren's University Hospital, Molndal, Sweden
- Prof. Katharina Wimmer, Medizinische Universität, Innsbruck, Austria
- Regional Genetics Laboratory, Salisbury, UK
- Prof. Raymonda Varon-Mateeva, Charité Universitätsmedizin, Berlin, Germany
- Prof. Thomas Liehr, Institute of Human Genetics and Anthropology, Jena University Hospital, Germany
- Dr. Erik-Jan Kamsteeg, University Medical Centre St. Radboud, Nijmegen
- Prof. Arndt Rolfs, CeGaT GmbH, Tübingen, Germany
- Prof. Andreas Gal, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany
- Dr. Saskia Biskup, Albrecht-Kossel-Institute for Neuroregeneration, Medical Faculty, University of Rostock, Germany
- Dr. María Garcia Hoyos, Science Park. University of Valencia, Paterna, Spain

Distribution of etiological causes

In their recent paper Rauch et al. present the distribution of various etiological causes found in patients with mental retardation. *(Fig.26.A.)*
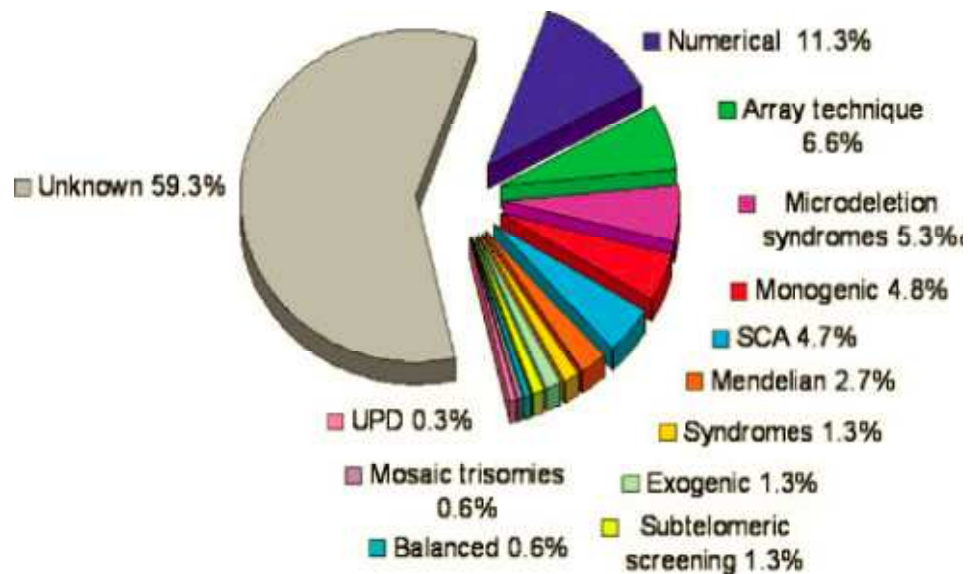
Fig. 26 A.
Distribution of etiological causes identified in patients with mental retardation by Rauch et al. (Rauch et al 2006)

As the last step in the evaluation, I attempted to compare the above ratios with those found in our patients *(Fig.26 B)*
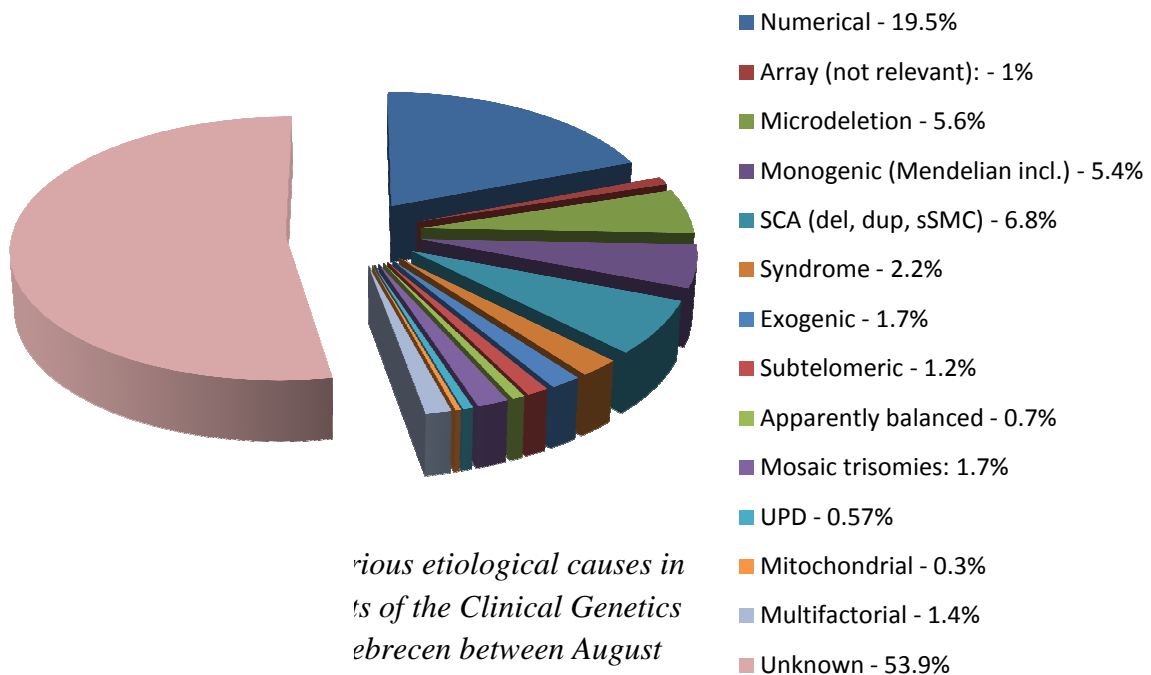
...rious etiological causes in
...ts of the Clinical Genetics
...ebrecen between August

Data of individuals with mental retardation are used here only (573 patients) – e.g. patients with monogenic syndromes without mental retardation are not included in the calculations.

As visible on the two diagrams, slight differences can be seen between almost all etiological groups excepted numerical chromosomal anomalies, but the ratios within each diagram are strikingly similar, and it is obvious that more than half of the patients with unexplained mental retardation remained "unexplained". Data derived from array are not relevant in our diagram for reasons discussed earlier, causing loss of 10-14% of possible diagnoses. Similarly, other than failure in recognition, differences in the percentage ratio of confirmed monogenic syndromes are likely to be related to the very long procedure, paperwork and limited approval of molecular genetic tests prior to testing in each syndrome whose genetic diagnosis is not available in Hungary.

VI. Original observations

- A survey to assess the results and efficiency of the diagnosis of rare genetic diseases in the Clinical Genetics Center of the University of Debrecen was performed in a 5 ½ year long period between August 01. 2007. – March 31. 2013. To my knowledge, this is the first comprehensive report in Hungary that relies on such a large number of patients of all major etiological groups.

- An overall 658/1023 patients received a diagnosis (64%), in 365 (36%) the underlying genetic defect remained unknown. In 387 patients (37.8%), the diagnosis was based on positive cytogenetic or molecular genetic results, in 271 (25.5%) the diagnosis relied on phenotypic features but is considered to be correct because the clinical picture and the anamnestic data support it. In 32 patients, the underlying genetic defect is unknown and as such, cannot be proved at the present status of genetic knowledge; in 11 patients clear evidence of a teratogenic cause exist, and in 22 the symptoms suggest

multifactorial origin. In the remaining 204 patients further genetic tests would be needed to prove the suspected syndrome (already in progress in 30 cases). In the 365 patients whose diagnosis is unclarified, array CGH would be absolutely necessary, and depending on its results, further genome-wide tests should be considered. In some of them, dysmorphic syndromes could probably be recognized by more experienced syndromologists, but a one-by-one consultation is obviously not executable in so many patients.

- In the past 5 ½ years the author has established regular cooperation with 17 European expert centers, and an additional 15 centers were involved in occasional genetic testings of rare disease patients. 4 scientific publications were produced from this international work and another is in progress.

- Patients with Niemann-Pick C disease, GAMT deficiency, Mowat-Wilson, SMMCI, Say-Barber/Biesecker/Young-Simpson, Phelan-McDermid, Sotos, 3q29 deletion, Kleefstra, Hermansky-Pudlak syndromes were recognized by the author and were proved molecularly as first in the country, with the help of foreign and home laboratories. Upon the author's initiation, the molecular diagnosis of Niemann-Pick C and Hermansky-Pudlak syndromes were established at the University of Debrecen as the only site in Hungary.

- The number of proved microdeletion syndromes (proved by FISH) has increased by 9 fold in the past $5^{1/2}$ years.

- Array CGH was first performed in 2011. for our patients in a foreign laboratory upon the authors request, and a fruitful collaboration has been maintained ever since, providing a diagnosis for 6 patients and excluding submicroscopic copy number changes in many others to support the suspected diagnosis of a monogenic disease or a syndrome of unknown origin.

- Based on the results of molecular genetic tests, prenatal diagnosis was successfully performed on 6 occasions, and can be offered in the future for all diagnosed patients at risk.

- A fibroblast bank to ensure DNA for postmortem diagnoses was established and is handled by the author.

- Case-consultations with expert centers were introduced on a regular basis mostly through the Internet, but a huge progress was made when Prof. Raoul Hennekam accepted my invitation and provided consultation in person on the most difficult 14 patients. His exceptional knowledge lead to prompt diagnosis in several cases, and helped to establish further cooperations in others. In a number of patients diagnostic procedure is still in progress. A few months later, Dr. Alida C. Knegt agreed to educate our laboratory specialists on CGH.

- On occasion of personal consultations, consultants were asked to deliver lectures in syndromology or the array-technique attracting other geneticists and professionals.

- First in Hungary, the diagnostic success of the author, the number and ratio of different etiological groups of genetic disorders were compared to that of foreign experts/expert centres. Results are similar, except for the ratio of submicroscopic chromosomal abnormalties, due to our limited access to CGH.

VII. Summary

The diagnosis and management of rare genetic syndromes are often extremely difficult, time consuming and pricy. Recognition itself – not to mention biochemical or molecular confirmation – may take years due to lack of knowledge of physicians, limited or no access to certain diagnostic tests, and confusing patient routes. Numerous rare diseases are rapidly fatal or devastating, a considerable ratio of affected individuals die shortly after the onset of

symptoms, therefore a fast and reliable diagnosis would be necessary to predict outcomes, reduce recurrence risk in families and decide on the availability of curative treatment for the patient. This discrepancy between expectations and limitations largely determine today's situation in the health care of rare disease patients.

In the present report the author aimed to assess the results of her (and many others' in the field of laboratory diagnostics) work as a clinician dealing with patients with mental retardation and/or congenital anomalies. Apart from defining in what proportion of them a genetic abnormality/rare disease could be proved and which group of genetic origin they represented, an important goal was to compare the succes rate and distribution of our diagnoses with international prevalence data. It was challenging to find out whether the clinical recognition and laboratory confirmation of rare diseases is as good as or worse than in Western-European countries and to what factors success and failure can be attributed to.

Some very rare syndromes diagnosed in the reported period are presented.

Our data reflect that the detection of chromosomal abnormalities visible by G-banding, that of microdeletions detected by FISH, UPD and methylation defects can compete with international figures, and that the diagnosis of extremely rare syndromes with or without known etiology also meet high standards – this hypothesis is supported by figures and accepted publications. Emphasizing the significance and irreplacable utility of syndromologic knowledge, Van Karnebeek et al. reported that dysmorphological evaluation was essential for the proper diagnosis of 62% of cases with a rare condition, and contributory in 79% of cases *(van Karnebeek et al 2005)*. We do, however, have much to improve in the diagnosis of monogenic disorders (including metabolic diseases), especially X-linked mental retardation, submicroscopic pathological copy number changes and nonsyndromic mental retardation. It is obvious that the introduction of CGH into routine diagnostics can no longer be postponed, and promising steps are made in this issue at a nation-wide level. Likewise, it is evident and

acceptable that centralization of laboratory tests of rare diseases serves cost-efficiency and maintenance of expertize regarding entities that are included in the diagnostic spectrum of a given lab. I am convinced, however, that an easier route for the use of certain diagnostic tests offered in foreign countries, inclusion of frequently ordered genetic tests of foreign laboratories into routine financing that proved to be beyond reproach on several occasions; acknowledging the work of a syndromologist or rare disease expert by making their background work „visible" in the now existing code-system of the national health care would significantly improve the time and costs the diagnosis of a rare disease demands.

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