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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Supervisor: Prof. Dr. Éva Oláh

UNIVERSITY OF DEBRECEN

DOCTORAL SCHOOL OF CLINICAL MEDICINE

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The examination takes place at the Centre of Life Sciences, III/201-202. Discussion Room, University of Debrecen, Medical and Health Science Center, on September 23. 2013. 10:30.

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The Ph.D. defense takes place at the Lecture Hall of the Inst. of Internal Medicine, building "A", University of Debrecen, Medical and Health Science Center, on September 23. 2013., 13:00.

Introduction

Diseases that affect fewer than 1/2000 individuals are referred to as rare; those with a prevalence lower than 1/50 000 are referred to as ultra-rare. Increasing attention is devoted to this group of patients for several reasons: 1. The recognition of a rare disease and confirmatory molecular/biochemical tests 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. 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.

To date, approximately 7000 rare diseases are known – the number of patients in Hungary is estimated to 50 000 citizens.

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 Pediatrics, 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.

Review of the literature

Prevalence of rare diseases and their significance in health care

80% of rare diseases are genetic of origin, and 80% of genetic disorders are rare. The remaining 20% are caused by infections, environmental damage, or are immunological, degenerative and proliferative by nature. Increasing evidence supports the major role of genetic predisposition in this group of diseases, too. Rare diseases are characterized 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.

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. 20-30% of all neonatal deaths and 30-50% of post-neonatal deaths are due to genetic disorders, and up to 71% of inpatient hospital admissions are for children with a genetic defect - representing an 81% share of the total health care charges.

The majority of genetic disorders display mental retardation as a primary feature, thus further increasing the burden of these conditions. The prevalence of mental retardation in the population is 1-3%, that of multiple congenital anomalies is 2-3%.

Due to steady improvements in general health care, many rare disease patients now survive into adulthood and require medical help for chronic, age-related and associating symptoms in addition to the primary genetic defect.

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. 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 (uniparental disomy) and imprinting defects in the genetically ill population. In an estimated 38-40% of cases the genetic cause remains unknown.

In Hungary, the total birth prevalence of all isolated major congenital anomalies is about $600/10^4$.

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. 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. Choosing the right method with

the highest possible diagnostic yield and the lowest possible cost in a given case demands precise phenotyping, accurate evaluation of morphological and functional symptoms, identification and characterization of dysmorphic 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 syndromologists, Prof. Raoul Hennekam. For this, a profound knowledge in several fields of medicine (embryology, anatomy, endocrinology, neurology, psychiatry) is needed.

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*".

Structure and strategy of the laboratory diagnosis of rare diseases

Given the rarity of most conditions, confirmatory laboratory tests are centralized all over the world to ensure expertise and cost-effectiveness in testing. 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.

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 of the Inst. of Pediatrics, University of Debrecen.
- 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.
- Based on the experiences gained from the diagnostic utility of genetic tests, it seemed necessary to develop an algorithm for the diagnostic management of rare genetic diseases, based on international standards but adapted to the limited resources of the Hungarian health care system.
- 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.

Materials and methods

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** (common microdeletion syndromes, cases with small supernumerary marker chromosomes, certain cases of mosaicism), **3. Chromosomal submicroscopic** imbalances detected with **comparative genomic hybridization**, **4. Mendelian disorders**, **5. Uniparental disomies and methylation defects**, **6. Mitochondrial diseases**, **7. Polygenic/Multifactorial**, **8. Phenotypically diagnosed but not molecularly proved** patients, **9**. Patients with **infertility**, **10. Unclarified** conditions with presumably genetic origin.

Patient data are displayed in corresponding tables, two unique cases from each group are described in details. 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 (evaluated by Dr. Erzsébet Balogh, Clinical Genetics Center, University of Debrecen), region-specific, multicolor and subtelomeric FISH (performed by Dr. Anikó Ujfalusi

and Dr. Gabriella P. Szabó, Clinical Genetics Center, University of Debrecen), DNA sequencing (performed by Beáta Bessenyei - Clinical Genetics Center, University of Debrecen, Dr. István Balogh - Inst. of Laboratory Medicine, Dr. Noémi Polgár, Dr. Judit Bene and Dr. Katalin Komlósi – Inst. of Genetics, University of Pécs, and foreign laboratories), CGH (performed by Dr. Alida C. Knegt, Academisch Medisch Centrum Amsterdam), Southern-blot (performed by Beáta Bessenyei, Clinical Genetics Center, University of Debrecen) , methylation sensitive PCR (performed by Beáta Bessenyei- Clinical Genetics Center, University of Debrecen and Dr. Petra Zeitlhofer, Medgen At. Wienna on postnatal and Dr. Veronika Karcagi - Inst. of Environmental Health, Budapest on prenatal samples), DNA microsatellite marker analysis (performed by Dr. Márta R. Czakó- Inst. of Genetics, University of Pécs) and biochemical assays in metabolic disorders (performed by Dr. Eszter Karg and Dr. Ferenc Papp – University of Szeged, and foreign laboratories). Given the large heterogeneity of the conditions and of their genetic cause included in the present work, the description of the testing of each and every disorder is not provided here.

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. 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.

Hidden aberrations in patients were sought with the involvement of professionals from other subspecialties. Particularly important was brain MRI in cases with unexplained mental retardation, performed by Dr. Ervin Berényi, Dept. of Biomedical Laboratory and Imaging Science. Consultation with foreign experts were performed in the most challenging cases.

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 in male patients if the phenotype and/or the pedigree suggested so. 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 molecular 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. 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 performed in a foreign laboratory often as last step. This protocol was consistent with the guideline of Shevell et al. 2003, but contradicted some most recent others that suggest CGH to be the first-tier test. Nevertheless, to a certain extent we had to compromise with national possibilities.

Results

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**.** 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. Down-syndrome is the only chromosomal abnormality that does not count as a rare disease, given its 1/1000 prevalence. Trisomy 13 was diagnosed in one patient, full trisomy 18 was diagnosed in one patient, 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, 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) were 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 detected in one patient. 46,XY gonadal dysgenesis with female external genitalia was proved in two cases. Combined autosomal and gonosomal aneuploidy was present in one patient.

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).

Using the probes our laboratory possesses, the following microdeletion syndromes were detected: 1p36 deletion syndrome (2 cases), 3q29 (3q subtelomeric deletion syndrome, 1 case) 4p16.3 (Wolf-Hirschhorn syndrome – 3 cases), 5p15.3 (Cri-du-chat syndrome – 1 case, and 2 further cases visible by G-banding), 5q35 (Sotos syndrome – 0 case, 1 case proved with MLPA), 7p11.2 (Williams-Beuren syndrome – 11 cases), 9q34 (Kleefstra syndrome – 0 case, 1 case detected by CGH), 15p11.2 (Prader-Willi/Angelman syndrome 6/3 cases), 17p11.2 (Smith-Magenis syndrome – 1 case), 21q22.1 microduplication (Down-syndrome – 1 case), 22q11.2 (DiGeorge/Velocardiofacial syndrome – 6 cases), 22q13.3 (Phelan-McDermid syndrome – 1 case), and Subtelomeric rearrangements (4 cases).

Chromosomal submicroscopic copy number changes detected with comparative genomic hybridization (CGH)

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 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, the country's present financial situation does not allow to replace first-tier karyotyping with first-tier CGH.

To date, six patients requesting medical genetic help were proved to be affected by a microdeletion, none had a microduplication. Regions and sizes of deletions were: 1p36.21p36.12 (10.3 Mb), 3p25.3p25.2 (3.4 Mb), 3q29 (not reported), 9q34.3 (958 kb), 12p12.1 (254 kb), 21q22.1 (308kb). In one case, an unexpected side finding was the deletion of the Von Hippel Lindau (VHL) gene within the deleted region (3p25.3), causing Von Hippel Lindau disease.

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**.** In overview, mutations of the fibroblast growth factor receptors FGFR2, FGFR3 and TWIST genes were found in 7 cases of craniosynostoses; FMR1 mutations causing Fragile X syndrome were identified in 12 individuals including female carriers, neuromuscular diseases resulting from dystrophin, POMT1 (Protein O Mannosyltransferase), SMN1 (Survival of motoneurons 1), CHRNE (Choline Receptor, nuclear, ε subunit) were found in 15 cases including carriers; NF1 mutations were detected in 9 neurofibromatosis patients; osteochondrodysplasias associated with FGFR2 and FGFR3 mutations were found in 8 patients. Marfan syndrome causing FBN1 (fibrillin 1) mutations were detected in 7 patients. MECP 2 ((Metly CpG-binding protein) mutations in Rett-syndromic children were found in 2 cases; metabolic/neurodegenerative diseases arising from NPC1,

(Niemann-Pick C), IDUA (α-L iduronidase), GAA (α-glucosidase), GAMT (Guanidino-acetate methlytransferase) deficiencies, ATM (Ataxia teleangiectasia were identified in 18 patients including carriers. Three cases out of these were proved by other institutes. Mutations responsible for single cases of dysmorphic syndromes were identified in 13 cases - NSD1 (SET-Domain Protein 1), KAT6B (Lysine-acetyltransferase 6B), TCOF1 (Treacher-Collins-Franceschetti 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), HPS1 (Hermansky-Pudlak syndrome) -, others, e.g. mutations causing familiar cancer syndromes (WT1, BRCA2) were found in 19 cases.

An exceptionally rare mendelian disorder was identified in a patient presenting with features of blepharophimosis-ptosis-mental retardation Say-Barber/Biesecker/Young-Simpson type, where a de novo heterozygous nonsense mutation c.5389C>T (p.Arg1797*) in the KAT6B gene (Lysine-acetyltransferase 6B; OMIM 605880) was detected, as the first confirmatory study following the discovery that mutations of the KAT6B gene are responsible for the SBBYS phenotype. In addition, our work-group was the first to identify neuroanatomical malformations in a SBBYS patient, the significance of which is supported by the knowledge that KAT6B plays a major role in the structural and functional development of the brain.

In two cases of Niemann-Pick disease type C, disease-causing mutations of the NPC1 gene were identified as first in Hungary. The establishment of the molecular genetic test in Debrecen was motivated by the two Hungarian patients recognized by the author and has been offered to other centers, performed by Dr. István Balogh, Inst. of Laboratory Medicine.

Uniparental disomies, methylation defects

Uniparental disomies and methylation defects were identified in four patients (0.2% each), causing Angelman, Prader-Willi, Beckwith-Wiedemann and Russel-Silver syndromes.

Mitochondrial diseases

Mitochondrial diseases were molecularly proved in three patients, and by brain MRI spectroscopy in an additional one.

Multifactorial disorders

22/2049 (1.1% of all patients) in the reported time interval showed phenotypic features – isolated abnormalities - that were considered to have arisen as multifactorial traits.

Phenotypically diagnosed but not molecularly proved syndromes

In the reported time interval 249/2049 (12.2%) patients received a diagnosis based on phenotypic features and not molecular or molecular cytogenetic tests**.** Latter (array-comparative genomic hybridization) 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 have been very recently identified), 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 centers 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 pregnancies. 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).

A patient with Solitary Median Maxillary Central Incisor Syndrome is highlighted from this etiological group, where the multiplex midline defect involved the hypophysis region and caused panhypopituitarism, making the case unique in the literature. Patient data and photos were requested to be included in the London Dysmorphology Database. This patient was followed-up by endocrinologists and received multiple hormone substitution for 15 years prior to the recognition of the seemingly isolated anomaly – the solitary maxillary central incisor -

Patients with infertility with or without detectable genetic anomalies accounted for 13.9% of all cases (285/2049 patients). This group of patients constitute the research field of a colleague in the cytogenetics laboratory and are therefore not analysed in details.

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.

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.*

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 organizations together have already achieved enormous advances in the field.

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 and of this diagnostic work is summarized, and conclusion is drawn based on the experiences obtained to improve our further diagnostic work. 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 (21.5%) were chromosomal (G-banded numerical and structural), 40 (3.9%) were microdeletions detectable by FISH including 4 cases with subtelomeric rearrangements, 6 had pathological CNVs (0.6%, irrelevant ratio, due to limited access to the method), 110 patients (10.7%) were affected by a mendelian disorder, 4 (0.4%) had methylation defects, 4 (0.4%) had UPD-s, 3 (0.3%) were mitochondrial and 2,2% multifactorial of origin. 249 patients (24.3%) 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 (35.7%) remained undiagnosed.

Considering that literature data relevant for comparison are available only on the distribution of genetic causes in the mentally retarded patient population, our data were reevaluated using only

the data of the 573 patients who were intellectually disabled. In this patient population, using the report of Rauch et al. 2006. as a basis for comparison, the following etiological distribution was found: Numerical chromosome aberrations were detected in 19.5% in our center vs. 11.3% of cases reported by Rauch et al.; data derived from the array technique are irrelevant (0.6% vs.6.6% by Rauch et al.) due to our very limited access to the technique; the proportion of microdeletion syndromes with 5.6% and 5.3% are quite similar; monogenic/mendelian disorders with 5.4 % vs. 7.5% differ - other than failure in recognition, differences in the percentage 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.

Detected subtelomeric rearrangements represent almost the same proportions with 1.2 and 1.3%. The ratio of genetic or suspected conditions of unknown origin are also similar, 53.9% vs.59.3%. Comparison of the data reflects that the diagnosis of rare genetic diseases as much as it is a matter of human knowledge and experience can compete with international standards, however, the spectrum and availability of certain genetic tests must be expanded urgently.

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 (64.3%), in 365 (35.7%) the underlying genetic defect remained unknown. This is in accordance with international standards. 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 supported by the clinical picture and anamnestic data. 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.
- 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 abnormaltities, due to our limited access to CGH.
- 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. Four scientific publications were produced from this international work and another is in progress. The value of these connections lies in their utility in

improving the genetic diagnosis of rare diseases in the future and in helping our professional obtain sufficient knowledge in even rare entities.

- 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. Some of these syndromes are exceptionally rare worldwide, one with such association of symptoms has not been described before. 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. Our positive results called the attention of decision-makers to the importance of the method and to the need of its introduction in Hungary.
- Based on the results of molecular genetic tests, **prenatal diagnosis** was successfully performed on 6 occasions. In 2 cases a healthy child was born, in 4 the pregnancy was terminated. Prenatal diagnosis can be offered in the future for all diagnosed patients at risk.
- Several cases, including the one with the solitary median maxillary central incisor underpin the importance of physical and morphological examination in the diagnosis of rare syndromes.

- 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, and by personal consultations as well. My two most valued consultants are Prof. Raoul Hennekam and Dr. Alida C. Knegt, in Amsterdam.

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 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.

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 „backstage" 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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Lectures related to the topic of the thesis:

- **Diagnostic and therapeutic approaches in Tangier-disease**. Debrecen, Evenings of Great-Forest's Pediatrics: 23. February, 2007.
- **Clinical and molecular biological aspects of Niemann-Pick C disease.** National Mucopolysaccharisis Conference, Eger, 20. November, 2007.
- **Experiences with the enzyme replacement therapy of type I. Mucopolysaccharidosis.** Conference of Young Pediatricians, Pécs, 29. March, 2008
- **Rare genetic syndromes.** Scientific meeting of the Regional Association of Pediatricians of North-Eastern Hungary. Miskolc, 7. June, 2008.
- **Rare genetic syndromes – case presentations.** VII. Congress of the Hungarian Association of Human Genetics. Pécs, 12. July, 2008.
- **Rare genetic syndromes – case presentations.** Conference of Young Pediatricians, Pécs, 29. March, 2008.
- **Rare genetic syndromes – case presentations.** Conference of Young Pediatricians, Kőszeg, 03-05. April, 2009.
- **Niemann-Pick C disease**. 8th Congress of the Hungarian Clinical Neurogenetic Association, Visegrád, 04-05. December, 2009.
- **Say-Barber/Biesecker/Young-Simpson syndrome- case of blepharosphimosis-mentál retardation syndrome.** VII. Congress of the Hungarian Association of Human Genetics, Debrecen, 04. Sept. 2008.

- **If there are no dysmorphic signs.** Scientific meeting of the Committee of the Hungarian Academy of Sciences, Debrecen, 16. May, 2011.
- **Pompe-disease – Experiences with enzyme replacement therapy.** Scientific meeting of the Regional Association of Pediatricians of North-Eastern Hungary. 04. June, 2011.
- **Hurler disease – Experiences with enzyme replacement therapy.** Scientific meeting of the Regional Association of Pediatricians of North-Eastern Hungary. 04. June, 2011.
- **Endocrine and neuroanatomical findings in a case of Solitary Median Maxillary Central Incisor Syndrome resembling Turner phenotype.** Porto, 8th European Cytogenetics Conference, 02-05. July, 2011. Poster.
- **Niemann-Pick disease type C – characteristics of the juvenile and adult onset forms.** Grand symposium of the Hungarian Pediatrics Association. Pécs, 02. Sept., 2011.
- **Case report on the first Hungarian pediatric case of Pompe disease.** Pompe Disease Expert Day, Rotterdam, 28. October, **2011.**
- **About Niemann-Pick C.** XXXVI. Congress of the Hungarian Association of Pediatric Neurology, Neurosurgery, and Youth Psychiatry, Budapest, 04.19-21. 2012.
- **Adult onset Niemann-Pick C**. XXXV. Annual meeting of the Hungarian Association of Neurologists and Psychiatrists. Debrecen, 22-24. November, 2012.
- **GM1 gangliosidosis – a rare lysosomal strorage disease. .** Grand symposium of the Hungarian Pediatrics Association. Tapolca, 07. Sept. 2012.
- **Unexplained mental retardation with or without multiple congenital anomalies -** Scientific meeting of the Committee of the Hungarian Academy of Sciences, Debrecen, 13. October, 2012.

- **Recommendations for the improvement of the diagnosis of rare diseases in Hungary** – Audition at the Hungarian Ministry of Health, Budapest, 16. November, 2012.
- **Complete recovery from psychosis upon miglustat treatment in a juvenile Niemann-Pick C patient** - 5th Scientific Symposium on Niemann-Pick C, Wien, 15-17. March, 2013. Poster.
- **Clinical utility of array CGH – case presentations**. HealthTIES Symposium, Debrecen, 16. February, 2013.

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List of publications related to the dissertation

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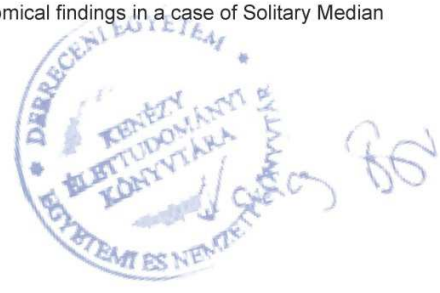

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