Investigation of copy number alterations in patients with minor anomalies and epilepsy

DOCTORAL (Ph.D.) THESIS

ANDRÁS SZABÓ

University of Pécs
Faculty of Medicine
Interdisciplinary Medical Sciences Doctoral School
Clinical Center, Department of Medical Genetics

Pécs, 2016
Introduction

Epilepsy background

The emergence of the epilepsy in BC 1600-1700 attracted the interest of people engaged in medicine. In the earliest Egyptian and Chinese written evidence, based on the typical clinical picture of epilepsy it was mentioned as a supernatural phenomenon. From the XVII-XVIII century the investigation of the epilepsy background had an explosive development. Several physiological experiments and interpretations were written off during this time associated with the disease. Based on the results obtained by the analysis of patients with epilepsy the relationship between epilepsy and CNS became obvious. Through the experience gained by the renewable diagnostic tools, the definition of the epilepsy extended to the functional and / or morphological abnormalities of the brain.

According to the latest available definition (2014) of the International League Against Epilepsy, people with epilepsy have to meet at least one of the following conditions:

1. At least two unprovoked (or reflex) seizures occurring >24 h apart;
2. One unprovoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures, occurring over the next 10 years;
3. Diagnosis of an epilepsy syndrome.

Demographic distribution of epilepsy

Worldwide, approximately 1% of the people suffer from epileptic disorder. Approximately 80% of epilepsy cases are from the developing countries. The likelihood of the development of the disease increases with age.

The classification of the epilepsies

Through the wide spectrum of different seizures, the classification could be done by several ways. Based on the brain involvement we can distinguish focal and generalized seizures. Focal seizures start from the epileptic nodules of the cortex, and lead to motor, sensory, cognitive, emotional and complex phenomena. Seizures which are associated with bilateral motor movement are called generalized seizures. Absence seizures are part of the
generalized seizures. The most common focal and generalized seizures which show motor activity are tonic, atonic, clonic, tonic-clonic, clonic-tonic-clonic, myoclonic, myoclonic-atonic and hypermotor seizures, and the epileptic spasm. Seizures, which do not show motor activity creates the non-motor group of the seizures, which includes the sensory, cognitive, emotional and autonomic features as well. In the classification of the epileptic seizures the presence or absence of the relevant state of the mind plays an important role. Classification of the epileptic seizures could be done based on the pathogenesis. In the development of the different types of seizures congenital brain malformations, lesions and genetic origin plays a huge role. In daily practice a previous ILAE definition is still used, which defines the seizures with genetic origin as idiopathic epilepsies. Cryptogen epilepsies include those epilepsies, which presumably do not have genetic origin, but the exact origin is unknown. In those cases, when the development of the clinical signs are origin from a single gene defect, mostly ion channel defects, defect in the GABA enzymes and in the G-protein coupled receptors occur.

**Genomic alterations associated with the development of the epilepsies**

Array CGH investigation of the different types of epilepsies lead to the detection of recurrent and non-recurrent copy number variations. The function and the copy number changes of the genes of the concerned genomic regions determine the development of the mostly complex clinical picture. Most common recurrent microdeletions associated with the different types of epilepsies affect the 1q21.1, 15q11.2, 15q13.3, 16p11.2, 16p13.11 and 22q11.2 genomic regions. Despite the regular detection of the recurrent microdeletions in patients with epilepsy, the incidence of the recurrent microdeletions in these people does not exceed separately the 0.5-1% and overall the 2.5%. The increased number of the cases with recurrent microdeletions made it obvious that, not only the copy number loss of the dosage sensitive genomic regions could lead to the clinical signs, but copy number gain also. The development and spread of the high-resolution approaches highlighted the importance of the different microduplication syndromes. Besides the above mentioned genomic alterations affecting the 1q21.1, 15q11.2, 15q13.3, 16p11.2, 16p13.11, 22q11.2, other dosage sensitive gene containing recurrent copy number changes could lead to the disease.

The widespread usage of the whole-genome analysis tools also proved the association of non-recurrent copy number variations with the development of different types of epilepsies.
The therapeutic aspects of the epilepsies

Among the patients with epilepsy the most commonly used therapy is the anticonvulsant therapy. The type of the seizure, the classification of the epilepsy syndrome, the existence of health problems and other medications as well as the person’s age and lifestyle could determine the applicable anticonvulsant. Despite the advanced epileptic therapy in nearly 30% of cases the anticonvulsant therapy does not solve the problems.

Genome analysis techniques – aCGH

The development of the NGS and aCGH techniques made it possible to confirm the results of the traditional cytogenetic techniques, and to detect new genomic alterations. aCGH is a type of the traditional comparative genomic hybridization, in which the metaphase chromosomes are replaced with 25-60 bp oligonucleotides covering the whole genome. The oligos are printed to a special slide, and the genomic positions are determined by the manufacturer. This method, with its 5-10 Kb resolution offers a specific, fast and sensitive technique facilitating the detection of the copy number changes both in clinical and research field. The combination of the traditional (Karyotyping, FISH) and next-generation (NGS, aCGH) molecular biological approaches confirms each other and allows the clinical usage. It is very important to emphasize, that next-generation techniques do not replace, but supplement the traditional techniques. The limitations of the different techniques could be eliminated by a simultaneous usage (e.g. detection of balanced translocations). The development of the science and the improvement of the different techniques influenced the investigation of the background of the epilepsy. The combination of the aCGH and FISH made it possible to detect the different sized copy number variations and the examination of the connection between the clinical picture and the genotype. In addition to the epilepsies based on one gene defect, epilepsies based on larger chromosomal changes due to a deletion or duplication became highly investigated.
**Aims of the study**

During the investigation my goal was:

- to detect the expected genomic alterations of patients having epilepsy and minor anomalies;
- to detect recurrent and non-recurrent copy number changes of patients having epilepsy and minor anomalies;
- to investigate the relationship between the epilepsy, the minor anomalies and the detected copy number changes;
- to analyze the function of the genes of the genomic regions affected by the detected alterations, using literature data and public databases;
- to evaluate the literature data of the dosage sensitivity of the concerned genes;
- to determinate the genotype-phenotype correlation of the detected genomic alterations, by comparing our results to the published literature data similar to our genotypic and phenotypic features;
- to narrow the huge genomic regions to smaller regions, genes based on our results and published literature data;
- and to reinforce the applicability of aCGH in patients with epilepsy and minor malformations, based on the detected genomic alterations and phenotype.
Materials and methods

Clinical examination of the patients and their families was carried out during a genetic counseling. After providing information about the examinations and signing the written consent, 5-7 ml EDTA anticoagulated blood was taken from each person for the laboratory tests. The isolation of DNA was performed using Omega E.Z.N.A. Blood Maxiprep Kit.

In the first step of the G-banded karyotyping we cultured the lymphocytes of the investigated persons and then the preparation was stained by Giemsa. After drying we evaluated the seen results in a microscope. We counted 15 metaphase chromosomes and ordered them into groups. After Giemsa staining and evaluation the G-banding was performed. The preparation was treated with Leishmann’s stain and then after the microscopic analysis we created 5 karyotype from each sample.

In the case of UPD examination the polymorph STR markers of the parental and investigated person samples were amplified by PCR. During the analysis we compared the alleles of the investigated persons with the parental alleles and evaluated the pattern of the results.

For the FISH investigation of the selected persons we used peripheral blood. After the pretreatment of the preparations we performed denaturation and hybridization steps. After the posthybridization and staining step we analyzed the results.

aCGH was performed using Agilent SurePrint G3 Human Gene Expression V2 8X60K Microarray Kit. After the fragmentation of the patient and reference samples we amplified the fragmented DNA samples and then we labelled the patient and reference samples with different fluorescent dyes (Cy5 and Cy3). After purification and concentration of the labelled samples we measured the incorporation of the fluorescent dye to the samples and then we pipetted the reference-patient pairs into one tube. After the hybridization of the reference-patient sample pairs onto the slide we performed washing steps and we put the slides to a scanner which can detect the fluorescence intensity of the hybridized samples. The last step was the evaluation of the data.
 Patients

1. patient

Patient 1. is a 27 month old boy showing epilepsy and psychomotor delay. The family history and the pregnancy were unremarkable. He was delivered at the 40th week of gestation with a birth weight of 3200 g (25-50 pc) and an Apgar score 9/1 and 10/5, respectively. In the perinatal period cardiological examination was performed because of extrasystole. The Holter monitoring revealed bigeminic extrasystoles, but it regressed spontaneous shortly. His first epileptic seizure developed at 5.5 months of age, and he was never seizure free in spite of numerous different antiepileptic drug combinations. His seizure pattern is very variable, and at four years of age hyperactivity developed. His developmental milestones were delayed, he crept and stood at 18 months, but he could not walk. Speech development was delayed as well, only bubbling was present at 28 months.

At 28 months of age as he was first examined in our institution, his weight was 10 kg, height was 90 cm and OFC was 46 cm. Flat occipital region, epicanthal folds, hyperteloristic eyes, crease of earlobes, broad nasal bridge, micrognathia and severe generalized hypotonia were present.

2. patient

Patient 2. is a 17 months old girl showing epilepsy and minor anomalies. The girl was delivered at 41st weeks of gestation with a birth weight of 3520 g (75-90 pc) and Apgar score 1/10 and 5/10. Because of developmental delay she received neurohabilitation from the fourth month of age and West syndrome developed at the sixth month. Vigabatrin treatment caused significant seizure frequency reduction. Congenital brain malformation was supposed in the background but the performed brain MRI out of dilated frontal and temporal liquor spaces gave negative results. Her developmental milestones were delayed.

She was referred to our institution because of epilepsy, dysmorphic features and developmental delay at the age of 17 months. At the examination her weight was 13 kg, height 85 cm, OFC 46 cm, and only mild craniofacial dysmorphic signs (prominent forehead, flat occiput, broad face, turned-up nose), broad thorax and small feet could be observed. In her
neurological status severe generalized hypotonia, tiptoe walking and stereotype hand movements were seen. Therefore valproic acid was administered instead of vigabatrin.

3. patient

Patient 3. was born as a first child to non-consanguineous healthy parents at 40th week of gestation by caesarean section, her birth weight was 2600 g, Apgar scores were 9-10 respectively. On the second day of life convulsion occurred, barbiturate administration was introduced. The performed skull US examination confirmed intraventricular hemorrhage (IVH) grade III. Skull MRI was performed, which raised corpus callosum dysgenesis. At two years of age she had no more seizures and the EEG showed normal brain activity, the barbiturate administration was stopped, but three weeks later the seizures recurred. The skull MRI performed at that age excluded the corpus callosum anomaly and found a dilatation of right ventricle, as a consequence of the perinatal intraventricular hemorrhage.

She was under cardiological control because of ASD/VSD and under gastroenterological control because of GERD. Her mental development was severely delayed, so neurohabilitation was introduced at the age of two years.

We examined her for the first time at the age of 28 months, at that time her weight was 10 kg, her length was 80cm and her head circumference was 44 cm, microcephaly, micrognathia, flat nasal bridge, epicanthic folds, hypertelorism, prominent philtrum, high arched palate, syndactyly of 2-3 toes and multiple café-au-lait spots of the skin could be observed in her dysmorphology. Bruxism, hyperactivity and autistic-like features characterized her behavior.

At the age of 39 months, dystrophy and microcephaly were present, the most striking dysmorphic features were the same as at the previous examinations. She had seizures at valproate and clobazam therapy. Beside intensive neurohabilitation her gross motor skills are appropriate to her age, but her speech development was delayed. Bruxism, hyperactivity and autistic-like features were constant at that age.

At 6 of age according to the ring chromosome her somatic development was slow and the microcephaly was expressed. The EEG showed normal brain activity, but she required the same AED therapy at low dose. Speech developmental delay was observed. She required methylphenidate administration because of severe ADHD and stereotypic hand movement.
could be observed. In her dysmorphology the characteristic facial features and the skin pigment anomaly were present.

4. **patient**

Patient 4. is a 22 months old girl showing minor anomalies, hearing loss and psychomotor delay. Following the diagnosis of a hypoplastic aortic arch at the 26th week of pregnancy the girl was delivered at 40 weeks of gestation with a birth weight of 2740 g. An aortic stenosis and coarctation of the aortae was confirmed by cardiological examination on the first day of life. Her developmental milestones were delayed. The objective audiometry showed bilateral hearing impairment. After adjustment of a hearing aid, considerable advance was not detected in her auditive attention. The first epileptic seizure developed at 22 months, which had an adequate therapeutic response to valproate treatment. Brain MRI detected symmetrical dilated liquor space with a consequent gracile hippocampus and subcortical ischemic lesions. She was referred to our institution because of dysmorphic features at the age of 8 months. At that examination her weight was 7850 g, height 68 cm and OFC 42 cm, she had brachycephaly, flat face, midface hypoplasia, downslanting palpebral fissure, convergent strabismus, short nose, high palate, tented lip and severe generalized hypotonia.

5. **patient**

Patient 5. is a 30 month old boy presenting minor anomalies. In the fetus dysmaturity was observed from the 34th week of gestation. He was delivered at 38 weeks of gestation with a birth weight of 2600 g. His developmental milestones were delayed. Speech development was delayed as well. The objective audiometry showed bilateral hearing impairment and in the first year of life he went through multiple pneumonias. The pulmonological examination revealed tracheomalacia in the background. His first epileptic seizure developed at 20 months of age, since then the seizures are therapy resistant focal seizures. An abnormal AED metabolism was observed in the boy, namely minimal AED doses already cause a toxic drug blood level. At 18 months of age as he was first examined in our institution his weight was 10 kg, height was 81 cm and OFC was 45 cm. Brachymicrocephaly, flat face, midface hypoplasia, hypertelorism, short nose, tented lip, thick lower lip, pointed chin, malformed ears and mild hypotonia were present.
Results

1. patient

G-banded chromosome painting of the 1. patient resulted an E-group acrocentric supernumerary marker chromosome 15. Uniparental disomy of normal chromosomes 15 has been excluded using polymorphic STR markers.


2. patient

GTG-banded chromosomes at the (550)-band level showed an additional bisatellited chromosome in 2. patient. Uniparental disomy of normal chromosomes 15 has been excluded using polymorphic STR markers.

Metaphase FISH analysis of the 2. patient resulted a 47,XX,+idic(15)(pter→q14::q14→pter) karyotype. aCGH investigation of the 2. patient showed an abnormal female profile with additional dosage of the regions 15q11.2q13.2 (22,765,628-31,183,907) and 15q13.3 (31,261,835-32,861,626). 2. patient had 4 copies of 15q11.2q13.2 (8.42 Mb, chr15:22,765,628-31,183,907) and of 15q13.3 (1.6 Mb, chr15:31,261,835-32,861,626) chromosomal regions, demonstrating the +idic(15) contained two 15q11.2q13.2 segments with 8.42 Mb (chr15:22,765,628-31,183,907) and 1.6 Mb of 15q13.3 (chr15:31,261,835-32,861,626) regions in 2. patient.

3. patient

G-banded karyotype analysis of the patient at the 450-band level revealed abnormal female karyotype with a r(15) and a sSMC(15). FISH analysis of the 3. patient resulted a 47,XX,r(15),+mar[82]/46,XX,r(15)[18] karyotype in lymphocytes while the fibroblast cell culture analysis resulted in a 47,XX,r(15),+mar[52] karyotype. The cells with
47,XX,r(15),+mar chromosomal constitution can be detected dominantly, however, cells without the supernumerary marker chromosome are also present in lymphocytes. Uniparental disomy of chromosome 15 has been excluded using polymorphic STR markers.

Array CGH analysis of our patient revealed a copy number gain in the 15q11.2 chromosomal region and a copy number loss in the 15q26.3 chromosomal region. The investigated patient has additional copy of the 2.6 Mb 15q11.2 (22,765,628-25,383,882) region and a missing copy of the 1 Mb 15q26.3 (101,373,740-102,383,473) region, illustrating that the sSMC(15) contains a 2.6 Mb 15q11.2 segment (22,765,628-25,383,882) and the r(15) has a 1 Mb 15q26.3 deletion (101,373,740-102,383,473).

4. patient

GTG-banded chromosomes at the 450 (550)-band level showed 46, XX normal female karyotype and subtelomeric FISH resulted a 9q34.3 terminal deletion. aCGH investigation of the 4. patient revealed a 2.188 Mb deletion of the ch9:138,831,145–141,018,984 chromosomal region.

5. patient

GTG-banded chromosomes at the 450 (550)-band level were normal and subtelomeric FISH resulted a 9q34.3 terminal deletion. aCGH investigation of the 5. patient revealed a 1.211 Mb deletion of the 9q subtelomeric region with the breakpoints ch9:139,641,471–140,852,911.
Discussion

1. and 2. patient

Comparison of the aCGH investigated patients having 15q duplication syndrome of the literature and our 1. patient resulted a postnatal growth retardation in or patient. The analysis of the concerned genes confirmed that not only the copy number loss of the *CHRNA7* gene could stand in the background of the development of the postnatal growth retardation, but the copy number gain also. Besides the *CHRNA7*, the copy number gain of the *NDN, MAGEL2, NIPA1* and *NIPA2* genes also could be associated with the postnatal growth retardation and the epilepsy.

The copy number changes of the *KLF13* gene could be associated with heart morphological changes based on literature data. Clinical investigation of the 1. patient resulted a bigemin extrasystole, which is not a heart morphological alteration. Based on these findings we could not confirm the literature data, which describes that copy number gain of the *KLF13* is always associated with heart morphological changes.

Investigation of our 1. and 2. patient showed the copy number gain of the *GABRB3, GABRA5* and *GABRG3* genes, which are subunits of the GABA receptors. The increase of the mentioned genes could lead to the increase of the GABA receptor protein level. Vigabatrin and valproate treatment of epilepsy are inhibiting the gamma aminobutirate transaminase, which increases the level of the GABA proteins. Thus, the increased GABA receptor level due to the copy number gain of the concerned genes and the increased level of GABA proteins due to the antiepileptic treatment could explain the reduction of the seizure frequency.

Based on the overlap of the 22,765,628-31,183,907 and 31,261,835-32,861,626 genomic regions with the affected genomic region of the 1. patient and the clinical features of the 1. and 2. patient, the observations of the 1. patient are also applicable at the 2. patient. Copy number gain of the *CHRNA7, OTUD7A, NDN2, MAGEL2, MKRN3, NIPA1* and *NIPA2* genes could lead to the detected epileptic features and neuropsychological signs of the 2. investigated patient.

Similar to the 1. patient we did not detect heart malformation. Based on these findings we could confirm our previous results, that copy number gain of the *KLF13* gene does not always lead to heart malformations.
3. **patient**

By comparing the clinical data and molecular findings of our 3. patient with the literature data, we could suppose that the copy number gain of the genes of the PWSCR could lead to the facial dysmorphism and the delayed mental development. Based on literature data the copy number loss of the TUBGCP5, CYFIP1, NIPA2 and NIPA1 protein coding genes of the 15q11.2 BP1-BP2 region could be associated with the motor and speech development delay and the development of epileptic features. We could support, that the copy number gain of the TUBGCP5, CYFIP1, NIPA2 and NIPA1 protein coding genes also could lead to motor and speech development delay and epilepsy.

In the case of our 3. patient the detected deletion affected the SELS, SNRPA1 and PCSK6 genes, thus based on the detected CHD and the concerned genes, we could confirm the previous published results by Alenne et.al. and Flaquer et.al. about the association of the copy number loss of the SELS, SNRPA1 and PCSK6 genes with the development of the ASD/VSD.

4. **and 5. patient**

Molecular diagnostic results and the clinical findings of the 4. and 5. patients resulted the diagnosis of Kleefstra syndrome. Based on the current scientific knowledge the deletion or the loss of function of the EHMT1 stand in the background of the Kleefstra syndrome.

aCGH investigation of the 4. patient revealed the deletion of the 9q34.3 (2,188 Mb, 138,831,145–141,018,984) chromosomal region. The concerned 2.188 Mb genomic region contains 78 genes, including the mentioned EHMT1 gene. The clinical findings of the patient confirmed the diagnosis of the Kleefstra syndrome.

By comparing the phenotypic signs of the 5. patients of the literature data, and our 4. patient, we could detect AED resistance, which is not a typical symptom of the Kleefstra syndrome. aCGH investigation of the 5. patient resulted a smaller, but same region containing deletion like the 4. patient. The difference in the number of the genes of the deleted chromosomal region of the 4. and 5. patient is 21. Thus, we could assume that these genes could not play a role in the development of the AED resistance of the 5. patient.

Based on the above mentioned results we could suppose that not the copy number loss of the affected chromosomal region stands in the background of the modified AED response.
Copy number changes of other chromosomal regions, or changes in the DNA sequences could lead to the non-typical AED resistance in patients with Kleefstra syndrome.
Summary

- In our study we could detect the previous published recurrent duplications of the 15q11.2 and 15q13.3 genomic regions, the non-recurrent deletion of the 15q26.3 genomic region and the deletion of the 9q34 genomic region of the Kleefstra syndrome in patients with epilepsy and minor anomalies using aCGH.

- By comparing the genomic alterations and phenotypic signs of our patients with the published literature data, we could define the genes of the affected genomic regions, which could play a role in the development of the clinical symptoms.

- By studying the association between the copy number changes of the 15q11.2q13.3 chromosomal region and the epileptic features, we could suppose that the copy number gain of the concerned *CHRNA7, OTUD7A, NDN, MAGEL2, MKRN3, TUBGCP5, CYFIP1, NIPA1* and *NIPA2* genes could lead to postnatal growth delay and epilepsy.

- By studying the association between the copy number changes of the 15q11.2q13.3 genomic region and other clinical symptoms, we could suppose that the copy number gain of the *KLF13* gene of the 15q13.3 genomic region, not always responsible for the development of the heart morphological changes published by Derwinska et.al.

- By studying the copy number changes of the 15q11.2q13.3 regions of our patients, we could assume an association between the copy number gain of the gamma aminobutirrate receptor subunit coding *GABRB3, GABRA5* and *GABRG3* genes and the effect of the vigabatrin and valproate antiepileptic drugs, which decrease the GABA transaminase activity.

- By comparing the 15q26.3 terminal deletion detected on the ring 15 chromosome and the observed clinical symptoms with the published literature data, we could confirm that the copy number loss of the *SELS, SNRPA1* and *PCSK6* genes could associate with the detected heart developmental problems.

- Studying the association between the copy number loss of the 15q26.3 region of the ring 15 chromosome and the dystrophy of the 3. patients did not confirmed the published literature data, which described that in the patients with terminal deletions including the *IGF1R* gene, always the copy number loss of the *IGF1R* gene lead to the development of short stature. We suppose that the ring 15 chromosome itself or the haploinsufficiency of
the genomic region distal from the \textit{IGF1R} also could be responsible for the development of the short stature.

- Copy number loss of the 9q34 genomic region which is associated with the development of the Kleefstra syndrome resulted typical symptoms of the Kleefstra syndrome in our 4. and 5. patient, but in the 5. patient a non-typical AED resistance occurred. Based on our results we could suppose that not the copy number loss of the affected chromosomal region stands in the background of the modified AED response. Copy number changes of other chromosomal regions, or changes in the DNA sequences could lead to the non-typical AED resistance in patients with Kleefstra syndrome.

- In case of patients with epilepsy and minor malformations we demonstrated the importance of the usage of the aCGH for the determination of the genetic background of the non-specific symptoms.
Publications

Publications supporting the dissertation

1. Partial tetrasomy of the proximal long arm of chromosome 15 in two patients: the significance of the gene dosage in terms of phenotype
   Szabó A, Czakó M, Hadzsiev K, Duga B, Komlósi K, Melegh B
   Impact Factor: 2,14

2. Kleefstra syndrome in Hungarian patients: additional symptoms besides the classic phenotype
   Impact Factor: 2,14

Other publications

1. Marked differences of haplotype tagging SNP distribution, linkage, and haplotype profile of IL23 receptor gene in Roma and Hungarian population samples
   *Cytokine* 65:(2) pp. 148-152. (2014)
   Impact Factor: 2,664

2. Genetic update on inflammatory factors in ulcerative colitis: Review of the current literature
   Sarlós P, Kövesdi E, Magyari L, Bánfai Zs, Szabó A, Jávorházy A, Melegh B
   Impact Factor: 0
3. Genetic polymorphisms in promoter and intronic regions of CYP1A2 gene in Roma and Hungarian population samples


ENVIRONMENTAL TOXICOLOGY AND PHARMACOLOGY 38:(3) pp. 814-820. (2014)

Impact Factor: 2,084

Impact factor of publications which have served as a base for the thesis: 4,28

Impact factor of other published articles: 4,748

Cumulative impact factor: 9,028
Acknowledgements

First of all, I would like to thank especially to my supervisor, Professor Béla Melegh, who supported me throughout the research and introduced me to the human molecular genetics and diagnostics. I also thank him for his useful advices during my work, for his leadership and help.

I would like to express my gratitude to Dr. Márta Czakó for the transfer of the essential knowledge associated with aCGH and for her friendly support during my work.

I am very grateful to Dr. Kinga Hadzsiev, to Dr. Anett Lőcsei-Fekete and to Dr. Katalin Komlósi who supported me with a very thorough clinical knowledge.

I want to say a specific thank to the current and former employees and Ph.D. students of the Department of Medical Genetics, Dr. Judit Dr. Berenténé-Bene, Dr. Erzsébet Kövesdi, Balázs Duga, Zsolt Bánfai, Renáta Szalai, Katalin Sümegi, Petra Mátyás, Etelka Pöstyéni and Titanilla Szabó.

I am also thankful to all the technicians and specialists in our department for their excellent assisting.

Last but not least my warmest thanks to my family for supporting me with their patients, love and encouragement.