Genetic investigations on rare monogenic diseases

Summary of the Ph.D. Thesis

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LIST OF PUBLICATIONS

Publications providing the basis of the dissertation

I. **Sulák A**, Tóth L, Farkas K, Tripolszki K, Fábos B, Kemény L, Vályi P, Nagy K, Nagy N, Széll M. One mutation, two phenotypes: a single nonsense mutation of the *CTSC* gene causes two clinically distinct phenotypes. *Clin Exp Dermatol* 2016; 41(2):190-195. **IF: 1.315**

II. Tripolszki K, Knox R, Parker V, Semple R, Farkas K, **Sulák A**, Horváth E, Széll M, Nagy N. Somatic mosaicism of the *PIK3CA* gene identified in a Hungarian girl with macrodactyly and syndactyly. *Eur J Med Genet* 2016; 59(4):223-226. **IF:1.81**

III. Tripolszki K, Farkas K, **Sulák A**, Szolnoky G, Duga B, Melegh B, Knox RG, Parker VER, Semple RK, Kemény L, Széll M, Nagy N. Atypical neurofibromatosis type 1 with unilateral limb hypertrophy mimicking overgrowth syndrome. *Clin Exp Dermatol* 2017; doi: 10.1111/ced.13154. [Epub ahead of print] **IF: 1.315**

Publications directly related to the subject of the dissertation

I. Nagy N, Valyi P, Csoma Z, **Sulak A**, Tripolszki K, Farkas K, Paschali E, Papp F, Toth L, Fabos B, Kemeny L, Nagy K, Szell M. *CTSC* and Papillon–Lefèvre syndrome: detection of recurrent mutations in Hungarian patients, a review of published variants and database update. *Molecular Genetics & Genomic Medicine* 2014; 2:(3) 217-228.

II. Vályi P, Farkas K, Tripolszki K, **Sulák A**, Széll M, Nagy N, Nagy K. Rekurrens európai misszensz mutáció egy magyar Papillon-Lefévre szindrómában szenvedő családban. *Fogorvosi Szemle* 2014; 107:(3) 87-92.

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Publications indirectly related to the subject of the dissertation

I. Nagy N, Farkas K, Tripolszki K, **Sulák A**, Kemény L, Széll M. A cylindromatosis gén mutációi által okozott genodermatosisok. *Bőr Vener Szemle* 2014; 90:(5) 185-193.

II. Nemes E, Farkas K, Kocsis-Deák B, Drubi A, **Sulák A**, Tripolszki K, Dósa P, Ferenc L, Nagy N, Széll M. Phenotypical diversity of patients with LEOPARD syndrome carrying the worldwide recurrent p.Tyr279Cys *PTPN11* mutation. *Arch Dermatol Res* 2015; 307(10):891-895. **IF: 2.327**

III. Tóth L, Fábos B, Farkas K, **Sulák A**, Tripolszki K, Széll M, Nagy N. Identification of two novel mutations in the *SLC45A2* gene in a Hungarian pedigree affected by unusual OCA type 4. *BMC Med Genet* 2017; 18(1):27. **IF: 2.198**

IV. Tripolszki K, Török D, Goudenège D, Farkas K, **Sulák A**, Török N, Engelhardt JI, Klivényi P, Procaccio V, Nagy N, Széll M. High-throughput sequencing revealed a novel *SETX* mutation in a Hungarian patient with amyotrophic lateral sclerosis. *Brain Behav* 2017; 7(4):e00669. **IF: 2.157**

V. Fábos B, Farkas K, Tóth L, **Sulák A**, Tripolszki K, Tihanyi M, Németh R, Vas K, Csoma Z, Kemény L, Széll M, Nagy N. Delineating the genetic heterogeneity of *OCA* in Hungarian patients. *Eur J Med Res* 2017; 19;22(1):20. **IF: 1.414**

1. INTRODUCTION

Rare diseases (RDs) are defined by the European Union as life-threatening or chronically debilitating conditions whose prevalence is less than 5 in 10 000 of the general population. Nowadays, there are more than 8000 distinct RDs and 6-8% of the European population is affected by a rare disease at some point in their lives. RDs are usually monogenic disorders and they are mostly determined by the presence or the absence of any causative genetic alteration which can cause the consequential failure of the certain protein and can lead to the development of the disease.

In my thesis, I have summarized the results of my genetic investigations in very stigmatizing, rare monogenic diseases: the clinical variants of the *cathepsin C* gene (*CTSC*) mutation-caused disease spectrum such as Papillon-Lefévre syndrome and Haim-Munk syndrome, atypical neurofibromatosis type 1 mimicking overgrowth syndrome and unilateral overgrowth of two fingers (macrodactyly).

1.1. The *CTSC* mutation-caused disease spectrum

1.1.1. Papillon-Lefévre syndrome

Papillon-Lefévre (PLS; OMIM 245000) syndrome is a rare, autosomal recessive disorder, characterized by symmetrical palmoplantar hyperkeratosis and severe periodontitis leading to the loss of both the primary and permanent teeth. Patients with PLS can also develop mild mental retardation, calcification of the dura mater, hyperhidrosis and increased susceptibility to infections. The prevalence of PLS is estimated four cases per million and to date, approximately 300 cases have been reported worldwide.

1.1.2. Haim-Munk syndrome

Haim-Munk syndrome (HMS; OMIM 245010) and PLS are characterized by overlapping dermatological and dental symptoms such as hyperkeratosis of the palms and soles as well as severe periodontitis. Besides these symptoms, specific features of HMS include pes planus, arachnodactyly, acroosteolysis and onychogryphosis. The prevalence of HMS is approximately one case per million. Fewer than 100 HMS cases have been reported in the literature to date.

1.1.3. Aggressive periodontitis type 1

Aggressive periodontitis type 1 (AP1; OMIM 170650) is characterized by severe periodontal inflammation leading to tooth loss. The prevalence of the disease is approximately less than one case per million individuals and so far only a few cases were described in the literature.

1.1.4. Genetic background

PLS, HMS and AP1 are both inherited in an autosomal recessive manner and develop as a consequence of mutations of the *cathepsin C* (*CTSC*) gene. To date, 79 distinct mutations have been reported for the *CTSC* gene and the majority of these mutations were detected in PLS cases, while only a few mutations were reported in HMS or AP1 cases. Due to the overlapping clinical symptoms of the diseases and their manifestation within the same families; PLS, HMS, and AP1 are not different entities: they represent the phenotypic spectrum of a single disease.

1.2. *PIK3CA*-related overgrowth spectrum

Somatic mutations in the *phosphatidylinositol 3-kinase catalytic alpha* (*PIK3CA*) gene cause segmental overgrowth disorders. These *PIK3CA*-related overgrowth diseases include fibroadipose hyperplasia and non-classifiable conditions characterized by muscular, boney and fatty tissue overgrowth, congenital lipomatous overgrowth, vascular malformations, epidermal nevi and skeletal abnormalities (CLOVES) syndrome, macrodactyly, hemihyperplasia multiple lipomatosis, and the brain overgrowth conditions megalencephaly capillary malformation and megalencephaly-polymicrogyria-hydrocephalus syndrome (MPPH). These previously described disease entities have overlapping clinical symptoms and represent a phenotypic spectrum.

1.2.1. Macrodactyly

Macrodactyly (OMIM 155500) refers to a rare congenital malformation occurring in approximately 1 in 100,000 live births and is characterized by an increase in the size of all the structures of the limbs, including soft tissues, bones, vessels, nerves and skin. It typically affects the terminal portions of the limb within a “nerve territory” and the individual peripheral nerve is both enlarged and elongated. Recently macrodactyly has been added to the growing list of overgrowth syndromes.

1.3. Neurofibromatosis type 1

Neurofibromatosis type 1 (NF1; OMIM 162200) is a rare monogenic disease with autosomal dominant inheritance due to mutations in the *neurofibromin* gene (*NF1*). The clinical features of NF1 involve pigmentary changes such as café-au-lait macules and axillary freckling, the development of cutaneous fibromatous tumors and the development of hamartomas of the iris known as Lisch nodules. Besides these symptoms, central nervous system and skeletal abnormalities (mainly scoliosis, pseudoarthrosis of the tibia, macrocephaly and short stature) can occur. Frequently reported vascular symptoms are the vascular dysplasia with cerebral, gastro intestinal and/or renal involvement and the renovascular hypertension. NF1 is one of the most common monogenic disorders worldwide, the prevalence of the disease is approximately 1 in 3000 live births.

2. AIMS

RDs represent a major challenge for health care organizations due to the small number of patients and the lack of the relevant knowledge and expertise of the specific rare disease. Therefore, my thesis focuses on the elucidation of the disease-causing mechanisms in RDs. The investigated RDs belong to the groups of the *CTSC*, *PIK3CA* and *NF1* mutations-caused disease spectrum. With the performed genetic and haplotype studies, my aim was to identify the underlying molecular mechanisms in patients affected by Papillon-Lefévre syndrome, Haim-Munk syndrome, atypical neurofibromatosis type 1, and unilateral macrodactyly. Although the investigated diseases involve rare phenotypic variants, their symptoms result in a huge life-long burden physically and mentally. Revealing the mechanism of the observed atypical phenotypes and phenotypic diversity in the investigated RDs contribute to the understanding of these diseases. The proposed genetic, molecular biology investigations might also lead to the identification of novel therapeutic target molecules and, eventually, to the development of novel therapeutic modalities for patients with RDs. These investigations have been performed in accordance with the current trends of biomedical research of the European Union, which supports the investigation of rare, so-called “neglected” diseases, since the mechanisms revealed in RDs would also lead to the further understanding of the mechanisms of common diseases. As my investigation on Papillon-Lefévre syndrome, Haim-Munk syndrome, atypical neurofibromatosis type 1, and unilateral macrodactyly might provide further insights into common mechanisms of palmoplantar hyperkeratosis and overgrowth of certain body parts.

3. PATIENTS AND METHODS

3.1. Hungarian patient affected by Haim-Munk syndrome

A 39-year-old Hungarian woman (Patient I) presented with a typical HMS phenotype. Mild hyperkeratotic plaques were observed symmetrically on her palms and soles. Onychogryphosis and arachnodactyly were noted on her fingers and pes planus on her soles. The patient lost all permanent teeth and uses a permanent dental prosthesis. She was brought up in state care without knowing her parents and has no husband or child. She was not aware of any known relatives.

3.2. Hungarian patient affected by Papillon-Lefévre syndrome

A 25-year-old Hungarian man (Patient II) presented with the classical PLS phenotype. The hyperkeratosis on his palms and soles was more severe than the symptoms of Patient I. Onychogryphosis, arachnodactyly and pes planus were not present. He was also missing all permanent teeth and using a permanent dental prosthesis. His parents and his wife were clinically unaffected. He had no siblings or children. He was not aware of any family members that are clinically affected.

Both patients were referred to the out-patient clinic of the Mór Kaposi Teaching Hospital (Kaposvár; Hungary).

3.3. Hungarian girl affected by isolated macrodactyly and syndactyly

A 4-year-old Hungarian girl was referred to the University of Szeged, Department of Medical Genetics with isolated macrodactyly on the third and fourth fingers of the left hand. X-ray imaging proved that the disease is characterized not only by the overgrowth of the soft tissues but also by the overgrowth of the bones of the affected fingers. In addition to macrodactyly, syndactyly was associated with concrescence of the fingers and was constrained to the soft tissues of the affected fingers and not to the bones. The macrodactyly and syndactyly of the left hand was present at birth and slowly progressed with the growth of the child. On examination, no vascular abnormality was present. There was no associated abnormality of the internal organs. Other body parts were symmetric and equally developed. The patient's parents were clinically unaffected, and they were not aware of any other family members with either macrodactyly or syndactyly.

3.4. Hungarian patient affected by neurofibromatosis type 1 and overgrowth of the left leg

A 52-year-old Hungarian woman was referred to our out-patient clinic with an unusual phenotype exhibiting the clinical features of NF1. The patient was presented with the typical skin symptoms of NF1, including neurofibromas and cafe-au-lait macules on her body and axillary freckling. Ophthalmological examination determined the presence of Lisch nodules. Imaging studies did not find any indication of central nervous system malignancies. The skeletal abnormalities scoliosis, tibial pseudoarthrosis, short stature and macrocephaly were not present. Based on the clinical symptoms, the diagnosis of NF1 was established. In addition to the above described clinical features, the patient was also noted to have hypertrophy of the left leg, resulting in significant differences in the circumference and length of the legs. This abnormality of the left leg was already present at birth. Imaging studies verified unilateral osteohypertrophy affecting the left leg.

When questioned about her family history, the patient was not aware of any relevant chronic diseases or other family members with NF1 or with overgrowth syndromes.

3.5. Methods

Peripheral blood samples, tissue biopsy and deep surgical excision of the affected tissues were collected from the investigated patients and from unrelated healthy controls for genetic analysis. DNA was extracted and after the amplification of the coding regions and flanking introns of the investigated genes, DNA sequencing was performed on the purified amplification products.

The mutational hotspots of the *PIK3CA* gene were screened using an in-house PCR-based restriction fragment assay which has been previously described by Keppler-Noreuil *et al*. in 2014 (Cambridge, UK).

For the haplotype analysis, common polymorphisms located in the 3’ and 5’ prime region of the identified mutation were genotyped using direct sequencing of the flanking coding and non-coding regions of the *CTSC* gene.

4. RESULTS

4.1. Genetic and haplotype investigations of the *CTSC* gene

In cases of the PLS and HMS patients, direct sequencing of the coding regions and the flanking introns of the *CTSC* gene revealed a nonsense mutation in the fifth exon (c.748C/T,

p.Arg250X). The patients carried the mutation in homozygous form, while the unrelated controls (n=100) carried the wild type sequence.

The presence of the same homozygous nonsense mutation in both patients raised the possibility of familial relationship between them. To address this, the polymorphisms located in the 3' and 5' regions of the identified mutation were genotyped and the haplotypes were determined. The patients were homozygous for all the genotyped polymorphisms, and all genotypes were the same for both patients, indicating they carried exactly the same haplotype.

4.2. Genetic investigations of the *PIK3CA* gene

In case of patients with overgrowth, neither in the genomic DNA of the peripheral blood samples nor in the genomic DNA isolated from affected tissue samples was *PIK3CA* gene mutation present using conventional capillary sequencing. In case of the macrodactyly patient, on the genomic DNA of the affected tissue sample a somatic heterozygous missense mutation at codon 542 (c.1624 G/A, p.Glu542Lys) was identified with 4% mutation burden using in-house PCR-based restriction fragment assay. This genetic investigation confirmed that the development of macrodactyly and syndactyly of the third and fourth fingers of the left hand are the consequence of the mosaicism of the p.Glu542Lys heterozygous missense mutation in the *PIK3CA* gene. In the other patient with NF1 clinical symptoms and the overgrowth of the left leg, somatic mutation responsible for the detected overgrowth was not identified with the targeted new generation sequencing of the *PIK3CA* gene and further 50 oncogenes and tumor suppressor genes.

4.3. Genetic investigations of the *NF1* gene

In the patient with NF1 clinical symptoms and the overgrowth of the left leg, direct sequencing of genomic DNA isolated from the peripheral blood of the patient revealed a novel frameshift mutation (c.5727insT, p.V1909fsX1912) in exon 39 of the *NF1* gene. The patient carried the mutation in heterozygous form, while the unrelated controls (n=50) carried the wild type sequence. In tis patient, mutation responsible for the detected overgrowth was not identified with the targeted new generation sequencing of the *PIK3CA* gene and further 50 oncogenes and tumor suppressor genes. In the case of the patient, instead of the diagnosis of overgrowth syndrome, the diagnosis of atypical NF1 disease was established and the altered size of the bone and soft tissues of the left leg was attributed not to overgrowth syndrome, but to a rare phenotypic variant of the NF1 disease.

5. DISCUSSION

5.1. A single nonsense mutation of the *CTSC* gene causes two clinically distinct phenotypes

Two Hungarian patients affected by different phenotypic variants, one with PLS and one with HMS, who nonetheless carry the same homozygous nonsense mutation (c.748C/T; p.Arg250X) of the *CTSC* gene were investigated. Polymorphisms surrounding the mutation were investigated to determine whether these patients are relatives and to possibly identify a genetic modifier factor within the *CTSC* gene, which could be responsible for the development of the different phenotypes.

The p.Arg250X homozygous nonsense mutation detected in the investigated patients is located in the exon 5 of the *CTSC* gene, which encodes the heavy chain region of the cathepsin C protein. The p.Arg250X mutation leads to the formation of a truncated protein and may significantly impair enzyme activity. This hypothesis correlates well with previous studies demonstrating that pathological changes in the *CTSC* gene are loss-of-function mutations resulting in the inactivation of enzymatic activity and altered regulation of the immune response, which increase the susceptibility to periodontal inflammation and skin infections.

The p.Arg250X nonsense mutation has already been previously reported in patients with PLS; however, this is the first study of its association with HMS. A previous investigation detected this mutation in homozygous form in Turkish patients. Based on the common history of the two ethnic groups, we can not exclude that the identified mutation is the consequence of a single founder effect.

The two investigated Hungarian patients were affected by different variants (PLS and HMS) of the phenotypic spectrum caused by *CTSC* mutations. Clinical differences between the PLS and HMS symptoms of the patients were striking, although, surprisingly, genetic screening identified the presence of the same nonsense mutation (p.Arg250X) in homozygous form in both patients. Haplotype analysis revealed that the two patients exhibit the same haplotype, indicating a strong likelihood of relatedness. The patients were not aware of any such relationship. Patient I was brought up in state care and did not know any of her relatives. Patient II was not aware of consanguinity within his family. However, our results and the fact that they share a common family name strongly suggest familial relationship between the two investigated patients.

As the patients had the same homozygous disease-causing mutation as well as the same haplotype, it was possible to examine genetic variations in the *CTSC* gene to identify

any differences that could account for the development of the phenotypic differences. Our investigation could not identify any such genetic variant with the *CTSC* gene and flanking regions. Therefore, we hypothesize that the putative modifier factor, which results in the development of different phenotypic variants for this *CTSC* mutation, is not located in the region of *CTSC*, but in another region. Moreover, we cannot exclude the possibility that the reason for the phenotypic variation is a non-genetic influence, such as environmental or life style factors.

Our results further support the accepted viewpoint that PLS and HMS are not different disease entities, but that they are phenotypic variants of the same disease and their development is influenced by other factors. These observations highlight the importance of genetic investigation and the establishment of genotype–phenotype associations.

5.2. The somatic p.Glu542Lys *PIK3CA* mutation causes high phenotypic diversity in patients suffering from segmental overgrowth syndromes

Here we investigated a 4-year-old Hungarian patient with isolated macrodactyly and syndactyly of the third and fourth fingers of the left hand. Genetic investigation identified a somatic missense mutation (p.Glu542Lys) of the *PIK3CA* gene. This mutation affects the helical domain of the p110α catalytic subunit of the PI3K protein. Functional studies have previously shown that this p.Glu542Lys variant caused hyperactivation of AKT, a down-stream target of PI3K in the nerve cells of a patient with macrodactyly. This particular mutation has been reported in eight patients with different forms of segmental overgrowth. Patients with somatic p.Glu542Lys mutation of the *PIK3CA* gene show high phenotypic diversity, Kurek *et al.* (2012) for example, described a female and a male patient affected by CLOVES syndrome. In addition to macrodactyly, both patients developed lipomatous overgrowth of the trunk and the limbs and vascular anomalies including lymphatic, capillary and venous malformations. The affected female patient also had a hypoplastic right kidney. A subsequent study reported the prenatal diagnosis of CLOVES syndrome in a 27-week-old fetus carrying the same somatic mutation of the *PIK3CA* gene. The observed clinical symptoms at birth were asymmetric chest and abdomen, bilateral multicystic malformations and asymmetric growth of the left leg with macrodactyly of the left foot and a sandal gap between the first and second toes. Rios *et al.* (2013) reported two patients with the same somatic mutation; both were affected by macrodactyly. However, one of the patients was also affected by true muscular hemihypertrophy, which was also attributed to the presence of the somatic p.Glu542Lys *PIK3CA* mutation. A subsequent study has also reported three further

patients with facial infiltrating lipomatosis, which was attributed to the presence of the somatic p.Glu542Lys mutation. These patients did not exhibit macrodactyly.

The high phenotypic diversity associated with the somatic p.Glu542Lys mutation might be explained by the different time points during embryogenesis of the mutational events. Patients with CLOVES syndrome might have developed the same somatic p.Glu542Lys mutation earlier during the embryogenesis than the ones with regional overgrowth (macrodactyly or facial infiltrating lipomatosis).

Genetic analysis has a huge significance for these patients, as once the genetic cause is determined, pharmacological intervention could be considered as a therapeutic option. In the interim, there are no clinically approved therapies for this condition; however there is a theoretical possibility that small molecule inhibitors of the PI3K-AKT-mTOR signaling pathway could be effective therapies for these patients. Rapamycin (sirolimus) indirectly targets PI3K and may also be useful in treating macrodactyly. In addition, rapamycin has been reported to be effective in isolated cases of allied conditions and may promote a breakthrough in the treatment of macrodactyly and overgrowth syndromes. However, long-term safety data of this treatment in *PIK3CA*-related overgrowth is currently lacking and, thus, indicates the need for formal clinical trials to evaluate safety and efficacy. In light of the current situation, detailed genetic investigation and publication of these isolated cases is essential.

5.3. A novel mutation of the *NF1* gene associated with atypical NF1 phenotype

Recently we investigated a Hungarian woman presenting with the clinical features of both NF1 and left leg overgrowth. Such an atypical clinical form of NF1 has not been reported previously. However, the phenomenon of ‘vascular neurofibromatosis phenotype’ and the relatively frequent association of NF1 with vascular dysplasia have been frequently reported in the literature. NF1 can also be accompanied by skeletal abnormalities, such as sphenoid wing dysplasia, macrocephaly, scoliosis, vertebral disc dysplasia, pseudoarthrosis of tibia and short stature. Therefore, it was important to distinguish whether the patient was affected by two independent rare diseases or whether the symptoms of the left leg were the results of the atypical vascular and skeletal manifestations of NF1.

Our results demonstrated a novel heterozygous single-nucleotide insertion in the *NF1* gene, leading to frameshift and the formation of a premature termination codon (c.5727insT, p.V1909fsX1912). Because this mutation was present in the genomic DNA isolated from the peripheral blood of the patient, we suggest that this sequence change is a germline mutation. Further *NF1* gene mutation was not present in the DNA sample of the patient isolated from

the affected left leg. Regarding the DNA sample of the affected left leg, mutation responsible for the detected overgrowth was not identified with targeted new generation sequencing of the *PIK3CA* gene and further 50 oncogenes and tumor suppressor genes.

Considering that there is no other clinically affected member in the patient's family and that NF1exhibits autosomal dominant inheritance, we hypothesize that this is a *de novo* mutation of the *NF1* gene. The genotype–phenotype correlation is generally poor in NF1, with the exception that patients with large deletions in the *NF1* gene tend to have severe phenotypes. *NF1* mutations usually result in loss of tumor suppressor function by disrupting the neurofibromin protein's ability to maintain the proto-oncogene RAS in an inactive form. We suggest that this novel *NF1* mutation is the causative mutation for the development of NF1, and the presence of the unilateral limb hypertrophy is highly possible also the consequence of this mutation.

In conclusion, we investigated a patient affected with both the clinical features of NF1 and overgrowth of the left leg. Our results demonstrate that the patient is not suffering from two independent rare diseases, but the unusual clinical phenotype of NF1. To our knowledge, our study is the first to clearly elucidate the genetic background of such a complex case, and further confirms the causative role of the somatic mutations of the *NF1* gene in the development of overgrowth.

6. SUMMARY

In my PhD dissertation, my aim was to summarize the genetic and haplotype investigations in Hungarian patients affected by different rare diseases, such as PLS, HMS, unilateral macrodactyly and atypical NF1.

Mutation screening of the *CTSC* gene from two Hungarian patients affected by PLS and HMS revealed the presence of the same homozygous nonsense mutation (c.748C/T; p.Arg250X). The performed haplotype analysis revealed that they carry the same haplotype, and the possibility that they are related cannot be excluded. Our results further support the hypothesis that PLS and HMS are the phenotypic variants of the same disease and, additionally, exclude the presence of a putative genetic modifier factor within the *CTSC* gene that is responsible for the development of the two phenotypes. We hypothesize that this putative genetic modifier factors are located outside the *CTSC* gene or, alternatively, that the development of the different phenotypes is the consequence of different environmental or life style factors.

Here I investigated a Hungarian girl with macrodactyly and syndactyly. Genetic screening at hotspots in the *PIK3CA* gene identified a somatic mutation (c.1624 G/A, p.Glu542Lys) in the DNA sample of the affected tissue, but not on the DNA sample of the peripheral blood. To date, this somatic mutation has been reported in eight patients affected by different forms of overgrowth syndromes. Detailed analysis of the Hungarian child and previously reported cases suggests high phenotypic diversity associated with the p.Glu542Lys somatic mutation, which might be explained by the different time points during embryogenesis of the mutational events. The identification of the *PIK3CA* causative mutations might provide novel therapeutic modalities for the affected patients with the administration of the inhibitors of the PI3K-AKT-mTOR signaling pathway.

Recently I investigated a Hungarian woman with the clinical phenotype of NF1 over her whole body and the clinical features of unilateral overgrowth involving her entire left leg. This unusual phenotype suggested either the atypical form of NF1 or the coexistence of NF1 and overgrowth syndrome. Direct sequencing of the genomic DNA isolated from peripheral blood revealed a novel frameshift mutation (c.5727insT, p.V1909fsX1912) in the *NF1* gene. Further NF1 gene mutation was not present in the DNA sample of the patient isolated from the affected left leg. Regarding the DNA sample of the affected left leg, mutation responsible for the detected overgrowth was not identified with targeted new generation sequencing of the PIK3CA gene and further 50 oncogenes and tumor suppressor genes. Based on these results, we concluded that the patient is not surrefing from two independent rare diseases (NF1 and orvergrowth syndrome), but affected by an unusual phenotype of NF1, and the observed unilateral overgrowth of the left leg might be the consequence of the identified c.5727insT mutation.

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