Genetic, haplotype and functional 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. Nemes E*****, **Farkas K***, Kocsis-Deák B, *et al.* Phenotypical diversity of patients with LEOPARD syndrome carrying the worldwide recurrent p.Tyr279Cys PTPN11 mutation. *Arch Derm Res* 2015; 307(10):891-895. **IF: 1,902**
*****E. Nemes and K. Farkas contributed equally to this work.

II. **Farkas K**, Kocsis-Deák B, Sánchez LC, *et al.* The CYLD p.R758X worldwide recurrent nonsense mutation detected in patients with multiple familial trichoepithelioma type 1, Brooke-Spiegler syndrome and familial cylindromatosis represents a mutational hotspot in the gene. *BMC Genet* 2016; 17(1):36. **IF: 2,397**

Publications directly related to the subject of the dissertation

I. Nagy N, **Farkas K**, Kinyo A, *et al.* A novel missense mutation of the CYLD gene identified in a Hungarian family with Brooke-Spiegler syndrome. *Exp Dermatol* 2012; 21(12):967-969.

II. Nagy N, Rajan N, **Farkas K**, *et al*. A Mutational Hotspot in CYLD Causing Cylindromas: A Comparison of Phenotypes Arising in Different Genetic Backgrounds. *Acta Derm-Venereol* 2013; 93(6):743-745.

III. Nagy N, **Farkas K**, Tripolszki K, *et al.* A cylindromatosis gén mutációi által okozott genodermatosisok. *Bőr Vener Szemle* 2014; 90:(5) 185-193.

IV. Nagy N, **Farkas K**, Kemény L, Széll M. Phenotype-genotype correlations for clinical variants caused by CYLD mutations. *Eur J Med Genet* 2015; 58(5):271-278. **IF: 1,466**

V. Nagy N, **Farkas K**, Kemeny L, Szell M. Knowledge explosion for monogenic skin diseases. *World J Dermatol* 2015; 4(1):44-49.

Publications indirectly related to the subject of the dissertation

I. **Farkas K**, Nagy N, Kinyo A, *et al.* A newly identified missense mutation of the HR gene is associated with a novel, unusual phenotype of Marie Unna Hereditary Hypotrichosis 1 including limb deformities. *Arch Derm Res* 2012; 304(8):679-681. **IF: 2,708**

II. **Farkas K**, Paschali E, Papp F, *et al.* A novel seven-base deletion of the CTSC gene identified in a Hungarian family with Papillon-Lefévre syndrome. *Arch Derm Res* 2013; 305(5):453-455. **IF:2,270**

III. Nagy N, **Farkas K**, Bacsa S, *et al.* NRP1 Activates NF-κB Signaling Pathway and Initiates Proliferation in Keratinocytes. *Int J Genomic Med* 2013; 1:102.

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V. Horvath E, **Farkas K**, Herczegfalvi A, *et al.* Identification of a novel missense GLRA1 gene mutation in hyperekplexia: a case report. *J Med Case Rep* 2014; 8(1):233.

VI. Kinyo A, Valyi P, **Farkas K**, *et al.* A newly identified missense mutation of the EDA1 gene in a Hungarian patient with Christ–Siemens–Touraine syndrome. *Arch Derm Res* 2014; 306(1):97-100. **IF:2,270**

VII. Nagy N, **Farkas K**, Kinyó Á, *et al.* A synonimous polymorphism of APCDD1 affects translation efficacy and is associated with androgenic alopecia. *J Life Sci (Libertyville)* 2014; 8(2):106-114.

VIII. Nagy N, Valyi P, Csoma Z, Sulak A, Tripolszki K, **Farkas K**, *et al*. 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.

IX. Vályi P, **Farkas K**, Tripolszki K, *et al*. Rekurrens európai misszensz mutáció egy magyar Papillon-Lefévre szindrómában szenvedő családban. *Fogorvosi Szemle* 2014; 107(3):87-92.

X. Gajda A, Horvath E, Hortobagyi T, Gergev G, Szabo H, **Farkas K**, *et al*. Nemaline Myopathy Type 2 (NEM2): Two Novel Mutations in the Nebulin (NEB) Gene. *J Child Neurol* 2015; 30(5):627-630. **IF:1,666**

XI. Hamon Y, Legowska M, Fergelot, Nagy N, **Farkas K**, *et al*. Analysis of urinary cathepsin C for diagnosing Papillon-Lefèvre syndrome. *FEBS J* 2016; 283(3):498-509. **IF: 4,001**

XII. Sulak A, Toth L, **Farkas K**, *et al*. 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: 1,092**

XIII. Tripolszki K, Knox R, Parker V, Semple R, **Farkas K**, *et al*. Somatic mosaicism of the PIK3CA gene identified in a Hungarian girl with macrodactyly and syndactyly. *Eur J Med Genet* 2016; [Epub ahead of print] **IF: 1,466**

1. INTRODUCTION

Incidence of rare diseases is 1:2000 or less as defined by the European Union. They can impair the life quality of the patient significantly and they can also result in stigmatization and difficulties in socialization. Rare diseases are usually monogenic, meaning that one defined genetic alteration, one gene defect and consequently failure of one protein can be critical and can lead to the development of the disease. In my thesis, I have summarized the results of my genetic investigations in rare, very stigmatizing diseases: LEOPARD syndrome (LS), multiple familial trichoepithelioma type 1 (MFT1), familial cylindromatosis (FC) and Brooke-Spiegler syndrome (BSS).

1.1. LEOPARD syndrome

LEOPARD syndrome (MIM 151100) is an autosomal dominant condition belonging to the family of neuro-cardiofacio-cutaneous syndromes. The name is an acronym of its major features such as multiple **L**entigines, **E**lectrocardiographic conduction abnormalities, **O**cular hypertelorism, **P**ulmonary stenosis, **A**bnormal genitalia, **R**etardation of growth and sensorineural **D**eafness. LS is the consequence of mutations located in the protein-tyrosine phosphatase nonreceptor-type 11 (*PTPN11)* gene

1.2. Multiple familial trichoepithelioma type 1

Multiple familial trichoepithelioma type 1 (MIM 601606) is an autosomal dominant condition characterized by numerous firm skin-colored papules that are trichoepitheliomas. Trichoepitheliomas are small benign skin-colored tumors and are typically present at the center of the face, mostly around the nose, periorbitally and in the nasolabial.

1.3. Familial cylindromatosis

Familial cylindromatosis (MIM132700) is also an autosomal dominantly inherited disorder. Patients with FC have cylindromas, which are slowly growing benign tumors that are usually located on the scalp and face. Typically, they appear as multiple turban-like protrusions on the scalp, which are also referred as turban tumors.

1.4. Brooke-Spiegler syndrome

Brooke-Spiegler syndrome (MIM 605041) is also a rare monogenic skin disease characterized by the development of a wide variety of benign skin appendage tumors, such as trichoepitheliomas, cylindromas and/or spiradenomas. Spiradenomas are purple benign nodular tumors, which are usually located on the trunk or limbs.

MFT1, FC and BSS have been independently mapped to chromosome 16q12-q13. In the mapped region, the *cylindromatosis* (*CYLD*) gene was identified as the causative gene responsible for the development of these three diseases. MFT1, FC and BSS were originally described as distinct clinical entities, but due to their overlapping clinical symptoms and their manifestation within the same families, they are now considered as clinical variants that represent a phenotypic spectrum of a single entity.

2. AIMS

In my thesis, the primary aim was to summarize the results of the genetic and functional investigations in stigmatizing rare monogenic disorders, as LS caused by *PTPN11* gene mutations and the clinical variants of the *CYLD* mutation-caused disease spectrum such as BSS, FC and MFT1.

Concerning LS, the aim was to identify the underlying causative genetic abnormality in a 51-year-old Hungarian male patient. Besides, it was also among my goals to compare this variant with the reported ones in the literature in order to define genotype-phenotype correlations and Hungarian population specific mutations.

Regarding CYLD mutation-caused disease spectrum, my aim was to investigate families and sporadic cases affected by MFT1, FC or BSS in order to identify the underlying genetic abnormalities. I have also aimed to perform further genetic investigations including haplotype analysis to demonstrate whether different cases and families affected by different clinical variants of the CYLD mutation-caused disease spectrum carrying the same *CYLD* mutation are the consequence of the same founder event or independent mutational events. In case of novel mutations, I have also aimed to perform functional investigations to prove their ability to impair the function of the encoded CYLD enzyme. Besides these investigations, my further goals were to describe genotype-phenotype correlations and population specific mutation database.

3. PATIENTS AND METHODS

3.1. Hungarian pedigree affected by LEOPARD syndrome

The investigated 51-year-old Hungarian male patient has the typical symptoms of LS including facial anomalies, pigmentation abnormalities, cardiovascular and urological symptoms. The patient is deaf and dumb and mild growth as well as mental retardations were also present. The patient was born out of wedlock. There are no any other clinically affected relatives either on his father, or on his mother side.

3.2. Spanish pedigree affected by multiple familial trichoepithelioma type 1

The 62-year-old Spanish male has multiple skin-colored papules in both nasolabial folds, on the forehead, above the eyebrows and, to a lesser extent, on the ears, on the back of the head and on the back. The patient's only child, a 33-year-old daughter, has lesions similar to those of her father but are fewer in number. The lesions first appeared in both nasolabial folds and, over time, began to appear on her forehead, temples, ears and scalp. No other clinically affected member has been identified in this pedigree.

3.3. Dutch patients affected by familial cylindromatosis

The investigated Dutch patients were previously reported by Van den Ouweland *et al*.

3.4. Austrian patient affected by Brooke-Spiegler syndrome

The investigated Austrian patient was previously reported by Grossmann *et al*.

3.5. Hungarian pedigree from Szekszárd affected by Brooke-Spiegler syndrome

21 affected family members were identified in the seven-generation BSS family in Hungary. The affected individuals have serious skin appendage tumors. Some of them have cylindromas on the scalp and trichoepitheliomas on the face. The tumors appeared on the back and on the extremities of the patients.

3.6. Anglo-Saxon pedigree affected by Brooke-Spiegler syndrome

The Anglo-Saxon pedigree contained 8 affected family members spanning five generations. The affected individuals had a comparatively milder phenotype, with cylindromas and spiradenomas on the scalp and trichoepitheliomas on the face.

3.7. Hungarian pedigree from Szeged affected by Brooke-Spiegler syndrome

A Hungarian pedigree suffering from BSS has 2 affected and 5 unaffected individuals spanning two generations. One of the affected individuals, a 60-year-old male has numerous soft, hairless, skin-colored papules around his nose, in his ears, on his scalp and on his shoulders. His daughter, a 35-year-old female presented with milder symptoms.

3.8. Methods

Peripheral blood samples were taken from the affected and unaffected family members as well as from healthy controls for genetic analysis. Genomic DNA was isolated and after the amplification of the coding regions and the flanking introns of the investigated gene, DNA sequencing was performed. For the haplotype analysis, common polymorphisms located in the 3' and 5' prime region of the identified mutation were genotyped using direct sequencing. For functional analysis after immunoprecipitation Western blot was performed.

4. RESULTS

4.1. Genetic investigation of the *PTPN11* gene

In case of LS patient, direct sequencing of the coding regions and the flanking introns of the *PTPN11* gene revealed a missense mutation (c.836A/G; p.Tyr279Cys) in the seventh exon. The clinically affected LS patient carried one of the most common missense mutation of the *PTPN11* gene in heterozygous form, while the unrelated healthy controls carried the wild type sequence.

4.2. Genetic, haplotype and functional investigations of the *CYLD* gen

4.2.1. Genetic investigation of the Spanish pedigree

Direct sequencing of the coding regions (exon 9-20) and the flanking introns of the *CYLD* gene revealed a previously described nonsense mutation (c.2272C/T, p.Arg758X) in exon 17. Both investigated patients carried the mutation in heterozygous form, whereas the unaffected family members and the unrelated controls carried the wild-type sequence.

4.2.2. Haplotype analysis of the Spanish pedigree, the Dutch and the Austrian patients

Previously reported Dutch and Austrian cases carry the same mutation that was identified in the Spanish pedigree (c.2272C/T, p.Arg758X). Haplotype analysis of the Spanish

patients with MFT1, as well as Dutch patients with FC and an Austrian patient with BSS was performed to investigate whether the same or different mutational events are responsible for the development of these cases. My results demonstrated that the Spanish and the Dutch pedigrees carry the same haplotype, whereas the Austrian patient carries a different haplotype. Thus, it can be assumed that different mutational events are responsible for the development of the Austrian case and the Spanish and Dutch cases.

4.2.3. Genetic investigation of the Hungarian pedigree from Szekszárd

Direct sequencing of the coding regions and the flanking introns of the *CYLD* gene revealed a previously described nonsense mutation (c.2806C>T, p.Arg936X) in exon 20. The investigated affected family members carried the mutation in heterozygous form, while the clinically unaffected family members and the unrelated healthy controls carried the wild type sequence.

4.2.4. Haplotype analysis of the Hungarian pedigree from Szekszárd and the Anglo-Saxon pedigree

An Anglo-Saxon BSS pedigree also carried the same nonsense mutation (c.2806C>T, p.Arg936X), that was identified the Hungarian pedigree from Szekszárd. Haplotype analysis was performed to reveal whether the mutation they carry is the result of two independent mutational events or they are carrying the same founding mutation. As the result of the haplotype analysis, in case of the Hungarian pedigree, two polymorphisms were found, which were inherited linked to the mutation in the *CYLD* gene. The haplotype of the Anglo-Saxon pedigree was different, which means that the same nonsense mutation was the result of two independent mutational events in the two pedigrees. Thus this part of the *CYLD* gene could be a mutational hotspot.

4.2.5. Genetic investigation of the Hungarian pedigree from Szeged

Mutation analysis of the *CYLD* gene revealed a novel missense mutation (c.2613C>G p.His871Gln**)** located in exon 19, in heterozygous form in the investigated affected patients. This mutation could not be identified in any of the clinically unaffected family members or in the healthy controls.

4.2.6. Functional investigation of the Hungarian pedigree from Szeged

To reveal the function of this novel missense mutation (c.2613C>G, p.His871Gln), the known CYLD-regulated pathways were studied. CYLD protein is known to directly interact with the NF-κB signaling NEMO protein. Functional analysis was performed on fibroblasts isolated from biopsy samples of the patients affected by BSS and from healthy individuals. NEMO protein was immunoprecipitated from fibroblasts. After loading equal amounts of the NEMO, Western blot was performed to detect the ubiquitination of the loaded samples. The data suggest that decreased NEMO expression is associated with its altered deubiquitination. NEMO immunoprecipitated from fibroblasts carrying the CYLD mutation demonstrated significantly higher ubiquitination than NEMO immunoprecipitated from control fibroblasts.

5. DISCUSSION

5.1. Comparison of the patient suffering from LEOPARD syndrome with the literature

A 51-year-old male patient suffering from LS was investigated. Genetic screening of the *PTPN11* gene revealed one of the most common missense mutation (c.836A/G; p.Tyr279Cys) in heterozygous form of the *PTPN11* gene. The p.Tyr279Cys mutation has previously been reported in 47 different LS patients with Italian, French, Spanish, German, Estonian, Bosnian, Chinese Han, South-Korean, Japanese and Australian origin. The most common symptom is multiple lentigines, which was present in 46 (96%) out of 48. The development of café-au-lait spots were observed in only 22 (46%) patients. Ocular hypertelorism was detected in 40 (83%), palpebral ptosis in 32 (67%) and dysmorphic ears in 31 (65%) patients. Besides, cardiovascular anomalies can develop: hypertrophic cardiomyopathy was diagnosed in 25 (52%) patients. Short stature was present only in 19 (40%) patients. The p.Tyr279Cys mutation is rarely associated with deafness, which was reported in 12 (25%) patients. This analysis identified that certain symptoms - such as cryptorchidism, macrocephaly, horse kidney, hydrothorax, myelodysplasia and umbilical hernia - are rarely associated with the p.Tyr279Cys phenotype. The observed differences in the clinical symptoms of the 48 LS patients carrying the same missense mutation clearly demonstrate the wide phenotypic diversity and the variable expressivity of the disease.

5.2. Haplotype analysis of the patients carrying the recurrent nonsense p.Arg758X *CYLD* mutation represents a mutational hotspot in the gene

In case of a Spanish MFT1 pedigree with two affected family members (father and daughter), direct sequencing of the *CYLD* gene revealed a recurrent nonsense mutation (c.2272C/T, p.Arg758X) in exon 17. The p.Arg758X nonsense mutation has also been detected in patients with BSS, FC and MFT1. Furthermore, this mutation has been detected in different ethnic groups including Dutch and Austrian. Previously reported Dutch and Austrian cases carrying the same mutation were also investigated. To determine whether the worldwide recurrent p.Arg758X mutation of the *CYLD* gene is the result of one or more independent mutational events, haplotype analysis was performed. The haplotype analysis of the Spanish, Dutch and Austrian patients demonstrated that, although the Spanish and the Dutch patients carry the same haplotype, the clinical appearance, MFT1 and FC, respectively, is different. These results suggest the importance of modifying genetic and/or environmental factors. In contrast with these, the Austrian patient carried a different haplotype than the Spanish and Dutch families. Thus, presumably the presence of the same mutation is the consequence of different mutational events. These data suggest that the p.Arg758X nonsense mutation is located at a mutational hotspot in the *CYLD* gene.

5.3. Haplotype analysis of the patients carrying the recurrent nonsense p.Arg936X *CYLD* mutation represents a mutational hotspot in the gene

A large Hungarian BSS pedigree carrying a nonsense mutation (c.2806C>T, p.Arg936X) of the *CYLD* gene was investigated. This nonsense p.Arg936X mutation was also present in an Anglo-Saxon BSS pedigree, therefore haplotype analysis was performed to elucidate whether the mutation they carry is the result of the same or two independent mutational events. Haplotype analysis of the Hungarian and the Anglo-Saxon BSS pedigrees demonstrated that the same mutation carried by the two geographically distant pedigrees was the result of two independent mutational events. I hypothesize that these positions may be mutational hotspots on the *CYLD* gene.

5.4. The functional analysis of the newly identified missense mutation represents a disease causing mutation in the *CYLD* gene

A novel missense mutation (c**.**2613C>G, p.His871Gln) of *CYLD* gene was identified in a Hungarian BSS pedigree. CYLD protein has a role in the regulation of many signaling pathways, such as NF-κB signaling pathway throuhg the deubiquitination of NEMO protein

as its interaction partner. Ubiquitination of NEMO was investigated and based on the results presumably this novel mutation through the increased ubiquitination of NEMO leads to decreased NEMO expression and as a consequence may influence the NF-κB pathway. Further studies are needed to elucidate the exact mechanism of the development of BSS symptoms.

5.5. Mutations on the *CYLD* gene

To date, a total of 95 mutations have been published for the *CYLD* gene. The majority of the *CYLD* mutations (98%) were reported in coding regions. Distribution of the mutations within exons is unequal: 99% of the mutations are located within exons 9–20. The majority of the sequence changes are frameshift (48%), nonsense (27%), missense (12%) or splice-site (11%) mutations, however, two in-frame deletions have also been reported.

Approximately half of the identified *CYLD* mutations (48%) are frameshift mutations arising from the insertion or deletion of a small number of nucleotides. The majority of these changes lead to the formation of premature stop codons causing truncation and, thus, the dysfunction of the CYLD protein. Frameshift mutations are unequally distributed in the *CYLD* gene. Exon 17 is a mutational hotspot, containing 20% of all identified frameshift. Only a quarter of the frameshift mutations (27%) are recurrent

Nonsense mutations causing truncation and, thus, CYLD protein dysfunction are also common, accounting for one quarter (27%) of the *CYLD* mutations identified so far. The distribution of nonsense mutations is also unequal in the *CYLD* gene. Nearly half of the nonsense mutations (40%) are recurrent.

Missense mutations account for 12% of all mutations identified on the *CYLD* gene. The distribution of missense mutations is also unequal on the *CYLD* gene: all are located within the region spanning exons 12–20. Only a quarter (27%) of these are recurrent mutations.

Splice-site mutations account for 11% of all mutations. The distribution of the splice-site mutations is also unequal: all of them occur within the region spanning exons 10–18. Less, than a quarter (18%) are recurrent.

In addition, two in-frame deletions have also been reported: these mutations are located within exons 19 and 20. Both of them lead to the development of the FC clinical variant.

5.6. Distribution of the mutations in the CYLD protein

The protein encoded by the *CYLD* gene exhibits deubiquitinase activity. The N-terminal of the CYLD protein contains three cytoskeleton-associated glycine rich domains (CAP-GLY), at which the CYLD protein connects to microtubules. The N-terminal of the CYLD protein can be divided into two regions based on the occurrence of the mutations: no mutations have been detected in the region encoded by exons 4 and 5, whereas the region encoded by exons 5–11 contains approximately one fifth (18%) of the mutations identified to date. Mostly frameshift and nonsense mutations occur in this region, as well as the two known splice-site mutations. Most frameshift mutations occur in the first and the third CAP-GLY domains, whereas most of the nonsense and splice-site mutations occur in the region of the third CAP-GLY domain. Missense mutation has not been detected at the N-terminal of the CYLD protein.

The C-terminal of the CYLD protein, encoded by exons 12–20, contains the ubiquitin-specific protease domain that is responsible for the deubiquitinase activity of the protein. This region contains the majority (82%) of identified *CYLD* mutations, including frameshift (72%), nonsense (72%) and splice-site (81%) mutations as well as all known missense mutations. Functional studies of the identified mutations suggest that mutations of this region may decrease the deubiquitinase activity of the CYLD protein.

5.7. Genotype and phenotype correlations in case of patients carrying *CYLD* mutation

Genotype–phenotype correlations are difficult to establish, as all types of known *CYLD* mutation – frameshift, nonsense, missense and splice-site – lead to the development of each clinical variant of the *CYLD* mutation-caused spectrum.

Frameshift mutations of the *CYLD* gene have been identified for all clinical variants of the *CYLD* mutation-caused spectrum. Interestingly, most frameshift mutations occur in the region of exon 17.

Nonsense mutations of the *CYLD* gene exhibit the largest phenotypic diversity. Some nonsense mutations have been detected in patients diagnosed with FC, BSS or MFT1. As many recurrent nonsense mutations are due to de novo events, their frequency and location indicates mutational hotspots on the *CYLD* gene. Patients carrying the same nonsense mutation from different mutational events often exhibit extreme differences in their clinical manifestations. These differences might be the consequences of yet unknown genetic factors that modify the development of the phenotype. Nonsense mutations are also responsible for

the most variable expression within families. These differences might be explained by environmental and/or lifestyle factors.

Missense mutations of the *CYLD* gene are more frequently associated only with MFT1 (73%) than the other types of mutations. Missense mutations also lead to the development of milder phenotype. This observation might be explained by the fact that missense mutations are distributed differently than the other types of mutations: they are located only between exons 12–20 and not at all in the 5' end. In general, missense mutations are associated with low phenotypic diversity, as the majority of missense mutations result in the MFT1 phenotype.

Splice-site mutations of the *CYLD* gene can lead to the development of any clinical variant of the *CYLD* mutation-caused spectrum, but little is known about their phenotypic significance.

5.8. Geographical occurrence of *CYLD*-mutation caused disease spectrum

Mutations of the *CYLD* gene have been reported among patients with Irish, Japanese, Spanish, German, Algerian, Turkish, Hungarian, Slovakian, Italian, Scandinavian, Taiwanese, Turkish, Canadian and African backgrounds. However, the majority of the published mutations are reported from the UK, USA and China. Comparing the ethnicity and the reported allelic variants, geographical differences can be observed. The clinical phenotype of MFT1 was observed in African, African American, Taiwanese, Algerian, Turkish, Italian and Spanish patients though the most of it has been reported from China. The FC clinical variant was present in Dutch, Italian and Irish patients, however, the majority have been reported from the UK and from the USA. The clinical phenotype of BSS was detected in patients from Hungary, Slovakia, Austrian, Italy, Scandinavia, Spain, Canada and the majority – similarly to FC – is coming from the UK and the USA.

6. SUMMARY

In my thesis, I have summarized the genetic and functional investigations in stigmatizing rare diseases: LS and the clinical variants of the CYLD mutation-caused disease spectrum namely MFT1, FC and BSS.

A 51-year old Hungarian male patient has been identified with LS. LS develops due to mutations in the *PTPN11* gene. Direct sequencing of the *PTPN11* gene revealed a worldwide recurrent missense mutation (c.836A/G; p.Tyr279Cys), which has been previously

identified in 47 LS patients. Comparison of the clinical phenotypes of our patient and the ones reported in the literature demonstrates great phenotypic diversity despite of the same genotype.

In case of a Spanish pedigree suffering from MFT1, direct sequencing of the *CYLD* gene revealed a worldwide recurrent heterozygous nonsense mutation (c.2272C/T, p.Arg758X). This mutation has already been detected in patients with all three clinical variants – BSS, FC and MFT1 – of the *CYLD*-mutation spectrum. Haplotype analysis was performed for the Spanish patients with MFT1, Dutch patients with FC and an Austrian patient with BSS, all of who carry the same heterozygous nonsense p.Arg758X mutation. The results demonstrated that different mutational events are responsible for the development of the Austrian case and the Spanish and Dutch cases. My results indicate that this position is a mutational hotspot in the gene and that patients carrying the mutation exhibit high phenotypic diversity.

A Hungarian BSS pedigree with Bukovinian (Romanian) origin has also been investigated. Direct sequencing of the coding regions of the *CYLD* gene revealed a nonsense mutation (c.2806C>T, p.Arg936X) in exon 20. Since this nonsense mutation is present in an Anglo-Saxon pedigree, I performed the haplotype analysis of the two pedigrees and revealed that the mutation they carry is the result of two independent mutational events. The results suggest that this may be a mutational hotspot in the *CYLD* gene. Regarding the phenotypic features of the investigated pedigrees carrying the same nonsense mutation, I observed huge differences in the severity of the symptoms suggesting the presence of a yet unidentified modulatory factor.

Another Hungarian BSS pedigree has been investigated. Direct sequencing of the *CYLD* gene demonstrated a novel missense mutation (c.2613C>G; p.His871Gln) in exon 19 of the *CYLD* gene within the ubiquitin-specific protease domain of the encoded protein. Analyzes have been performed to reveal the functional role of this novel mutation. Data suggest that this novel *CYLD* mutation leads to increased ubiquitination of NEMO through influencing deubiquitinating activity of the CYLD protein and thus may result in enhanced NF-κB signaling.

These investigations have great importance because they could be the basis of future genetic studies for the development of novel causative therapies that will be more specific and effective than the symptomatic treatments. Genetic screening and the identification of the disease-causing mutation have great significance for family planning in prenatal and preimplantation diagnosis.

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