Genetic investigations in rare genodermatoses with pigmentaional abnormalities

Summary of the Ph.D. Thesis

Beáta Fábos MD

Graduate School of Clinical Medicine

University of Szeged

Supervisor:

Nikoletta Nagy MD, Ph.D.

Department of Medical Genetics

University of Szeged

Szeged

2017.

TABLE OF CONTENTS

LIST OF PUBLICATIONS

Publications providing the basis of the dissertation

I. 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 Mar 15;18(1):27. **IF: 2.198**

II. **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 Jun 19;22(1):20. **IF: 1.414**

Impact factors of these publications: 3.612

Publications indirectly related to the subject of the dissertation

I. Szabó K, Gáspár K, Dajnoki Z, Papp G, **Fábos B**, Szegedi A, Zeher M.: Expansion of circulating follicular T helper cells associates with disease severity in childhood atopic dermatitis. *Immunol Lett.* 2017 Apr 18. pii: S0165-2478(17)30085-8. **IF: 2.860**

II. 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 Mar;41(2):190-5. **IF: 1.315**

III. Nagy N, Vályi P, Csoma Z, Sulák A, Tripolszki K, Farkas K, Paschali E, Papp F, Tóth L, **Fábos B**, Kemény L, Nagy K, Széll M.: CTSC and Papillon-Lefèvre syndrome: detection of recurrent mutations in Hungarian patients, a review of published variants and database update. *Mol Genet Genomic Med.* 2014 May;2(3):217-28.

Impact factors of all publications: 7.787

1. INTRODUCTION

1.1. Rare monogenic skin diseases

From ancient times to the present, the basic approach for diagnosing skin diseases has been to classify the diseases according to their visible signs and symptoms. This approach highlights that dermatology is still a highly morphology-orientated specialty (Shelley *et al.,* 1976; Nagy *et al.,* 2015). Since that time, the desire to understand the nature of observed skin lesions constantly drives the development of dermatology and the incorporation of novel investigative methods into its everyday practice (Shelley *et al.,* 1976; Nagy *et al.,* 2015).

Breeding agricultural plants and animals characterized the pre-Mendel era of genetics (Stern *et al.,* 1950; Hansen *et al.,* 2014). After Gregor Mendel established the basic rules of heredity in the nineteenth century (Mendel *et al.,* 1993), several major discoveries, such as the identification of DNA as the material encoding inheritable information, of the genetic code and of the mechanisms of gene expression, have initiated the era of molecular genetics (Watson *et al.,* 1953; Min Jou *et al.,* 1972). Very recently, the enormous technical development of sequencing methods and platforms has resulted in large-scale genomic projects, which produce amounts of data that were unimaginable a few decades ago (Sanger *et al.,* 1977; Stoneking *et al.,* 2011).

These discoveries and techniques have been used to identify several normal genetic variations, as well as candidate genes and their disease-causing mutations, accelerating the elucidation of the genetic background of several monogenic skin diseases (genodermatoses).

Genodermatoses are defined as life-threatening or chronically debilitating skin conditions whose prevalence is less than 5 in 10 000 of the general population (Baldovino *et al.*, 2016). In opposite with common diseases, which are considered as the consequence of multifactorial – life style, environmental and genetic – etiological factors; genodermatoses 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 (Aronson *et al.*, 2006).

Although dermatology and genetics are considered separate disciplines, the combination of these two fields has already resulted in enormous improvement in the understanding of monogenic skin diseases (Nagy *et al.,* 2015). Investigations on genodermatoses are essential for the development of the knowledge about the genetic background on these diseases and the research can help family planning in the affected families and can also provide a novel therapeutic modality for the affected patients in the future (Aymé *et al.*, 2015). This latter one has also great importance, since currently, int he majority of the cases, only symptomatic treatment is available for patients with genodermatosis (Nagy *et al.*, 2015).

The following clinical entities are classified as genodermatoses: epidermolysis bullosa, keratotic disorders, disorders of skin color, ectodermal genodermatoses, genodermatoses associated with connective tissue, vascular genodermatoses and genodermatoses with skin manifestation and elevated cancer risk (Aymé *et al.*, 2015).

1.2. Genodermatoses with abnormal pigmentation

One of the most clinically heterogenous group of genodermatoses, is the one with pigmentational abnormalities.

The pigmentation of the skin is, except in rare pathological instances, the result of three pigments or chromophores: melanin, a brown/black (eumelanin) or red/yellow polymer (pheomelanin) produced by melanocytes; hemoglobin in red blood cells in the superficial vasculature; and dietary carotenoids (Rees *et al.,* 2003; Chatzinasiou *et al.,* 2015). Melanin, the most important, is formed from tyrosine, via the action of tyrosinase in the lysosome-related organelles of melanocytes, called melanosomes. Melanocytes are dendritic cells, arising from the neural crest during embryonic development and located in the basal layer of the epidermis. The melanosomes are transferred from a melanocyte to a group of 36 keratinocytes called the epidermal melanin unit, to which they provide melanin (Chatzinasiou *et al.,* 2015).

Interest in the genetics of human pigmentation is longstanding. Variation in human pigmentary form - of skin, hair, and eyes - is one of the most striking polymorphic human traits (Rees *et al.,* 2011; Chatzinasiou *et al.,* 2015). More than 150 genes have now been identified that affect pigmentation of the skin, the hairs and the eyes (Chatzinasiou *et al.,* 2015).

The availability of largescale DNA analysis and genome-wide scans, together with our existing knowledge of the genes involved in pigmentation, have contributed to the interpretation of the mechanism of skin pigmentation (Yamaguchi *et al.,* 2009; Rees *et al.,* 2011).

Disorders of pigmentation can result from migration abnormalities of melanocytes from the neural crest to the skin during embryogenesis (Plensdorf *et al.,* 2009; Fistarol *et al.,* 2010). In addition, impairment of melanosome transfer to the surrounding keratinocytes, an alteration in melanin synthesis and a defective degradation or removal of melanin may lead to abnormal skin pigmentation (Plensdorf *et al.,* 2009; Fistarol *et al.,* 2010). Immunologic or toxic mediated destructions of melanocytes can also cause pigmentation abnormalities (Plensdorf *et al.,* 2009; Fistarol *et al.,* 2010).

Disorders of pigmentation are can occur as a genetic or acquired disease (Plensdorf *et al.,* 2009; Fistarol *et al.,* 2010). In my thesis, I have focused on the ones with genetic background, which considered as genodermatoses with abnormal pigmentation.

These pigmentational abnormalities can affect the whole body of the patient and therefore can be generalized, but can manifest only in one body part and considered as localized abnormality (Chatzinasiou *et al.,* 2015). The previous one is usually the consequence of germline mutations of genes playing key role in the regulation of pigmentation, the latter ones can be the consequence of somatic mosaicizm. In these latter cases, the mutation is present in a mosaic form and only affect the body part with altered pigmentation. In my thesis, I have investigated the putative underlying germline mutations of the genes involved in the development of the pigmentation of the skin, the hairs and the eyes.

Pigmentational abnormalities either can be associated with increased amount of pigments resulting in hyperpigmented lesions or with decreased or complete lack of pigments leading to the development of hypopigmented lesions (Chatzinasiou *et al.,* 2015). In my thesis, I have summarized the results of my genetic investigations in very stigmatizing, rare monogenic skin disease characterized with decreased or

complete lack of pigments, the oculocutaneous albinism. The complexity of this genodermatoses is increased by the fact that the abnormalities of the color might not only affect the skin of the patients, but also the hairs and the eyes as well.

1.3. The oculocutaneous albinism

Oculocutaneous albinism (OCA) is a clinically and genetically heterogenic group of rare monogenic diseases characterized by diffuse reduced melanin production in the skin, hair and/or eyes (Mártinez-García *et al.,* 2013). OCA affects one in 20,000 individuals worldwide; however, the prevalence of its subtypes varies among different populations (Gargiulo *et al.,* 2011). To date, six genes have been implicated in the development of the isolated OCA forms, and an additional 24 genes have been associated with syndromic OCA variants (Simeonov *et al.,* 2013). I have focused my scientific work to the investigation of the isolated (non-syndromic) OCA variants, in which besides the pigmentational abnormalities of the hairs, skin and eyes there is no further affected organ.

Four genes have been implicated in the etiology of the most common isolated OCA forms: the tyrosinase gene (*TYR*; OMIM 606933), which is responsible for the development of OCA type 1 (OCA1) (King *et al.,* 2003; Ghodsinejad Kalahroudi *et al.,* 2014), mutations of the oculocutaneous albinism two gene (*OCA2*; OMIM 611409), which are associated with OCA type 2 (OCA2) (Durham-Pierre *et al.,* 1994), pathogenic variants of the tyrosinase-related protein gene (*TYRP*; OMIM 115501), which are linked with OCA type 3 (OCA3) (Rooryck *et al.,* 2008), and mutations in a membrane-associated transporter gene (*SLC45A2*; OMIM 606202), which are implicated in OCA type 4 (OCA4) (Inagaki *et al.,* 2004). Although OCA2 and OCA4 are present in Caucasian populations, OCA1 is the most common form and OCA3 is very rare (Rooryck *et al.,* 2008).

Rare isolated OCA variants include OCA type 5 (OCA5), OCA type 6 (OCA6) and OCA type 7 (OCA7). In OCA5 the causative gene has not yet been elucidated, there is only one reported Pakistanian family affected with OCA5 (Kausar *et al.,* 2013). OCA6 (OMIM 606574) results as the consequence of mutations in the solute carrier family 24 member 5 *(SLC24A5)* gene. OCA6 patients have been reported from the Faroe Islands (Gronskov *et al.,* 2013). OCA7 (OMIM 615179) develops as the results of mutations in the *C10ORF11* gene encoding the leucine-rich melanocyte differentiation-associated protein (LRMDA), OCA7 has only been reported in one Faroese family so far (Gronskov *et al.,* 2013).

Since OCA3 common in South Africans and OCA5, OCA6 and OCA7 are extremely rare variants reported only in a few patients, I have focused my scientific work for the investigations of the OCA1, OCA2 and OCA4 non-syndromic variants.

The *TYR* gene in OCA1 encodes the tyrosinase enzyme, which catalyzes the first and second steps in melanin synthesis: the hydroxylation of tyrosine to L-DOPA and the oxidation of L-DOPA to DOPA-quinone (King *et al.,* 2003). The *OCA2* and *SLC45A2* genes in OCA2 and OCA4 encode transporter proteins, which are implicated in the trafficking of tyrosinase to melanosomes.

1.4. Aims

Genodermatoses 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. Among genodermatoses, one of the most complex disease group is the genodematoses with pigmentational abnormalities. Therefore, my thesis focuses on the elucidation of the genetic background of genodermatoses with pigmentational abnormalities.

Within the group of genodermatoses with pigmentational abnormalities, I have focused my scientific work for the investigation of OCA, which is characterized by variable hair, skin and ocular hypopigmentation. In order to identify the causative mutations in the investigated Hungarian OCA patients (n=12), I have performed the genetic screening of the *TYR*, *OCA2* and *SLC45A2* genes, which have been implicated in the etiology of the most common isolated OCA forms (OCA1, OCA2 and OCA4).

With the performed clinical workup and mutation screening of the *TYR*, *OCA2* and *SLC45A2* genes, the main aims of my scientific work were the followings:

1. to promote the understanding of the heterogeneity of OCA,
2. to assess the independent and cumulative contributions of the *TYR*, *OCA2* and *SLC45A2* genes to the development of OCA,
3. to compare relative and cumulative frequencies of the clinical variants of OCA in a representative Hungarian OCA population.

My results revealed rare (mutation) and common (polymorphism), novel and recurrent genetic variants of the investigated genes and contributed to the understanding of the genotype-phenotype correlations in this clinically and genetically heterogenic group of genodermatoses. My results also demonstrated that the concomitant analysis of OCA genes is critical, providing new insights to the phenotypic diversity of OCA and expanding the mutation spectrum of OCA genes in Hungarian patients.

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 OCA.

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 rare monogenic skin diseases would also lead to the further understanding of the mechanisms of common diseases. As my investigation on OCA might provide further insights into mechanisms of common skin diseases with pigmentational abnormalities such as vitiligo or melasma.

2. PATIENTS AND METHODS

2.1. Patients

The individuals (n=12) participating in this study were recruited at the Mór Kaposi Teaching Hospital of the Somogy County (Kaposvár, Hungary), at the Hospital of Zala County (Zalaegerszeg, Hungary) and at the Department of Dermatology and Allergology, University of Szeged (Szeged, Hungary). The enrolled patients fulfilled the clinical criteria for OCA.

2.2. Methods

The performed genetic investigations got ethical approved by the Hungarian National Public Health and Medical Officer Service. After written informed consent was obtained from all investigated individuals, peripheral blood samples were collected from the investigated patients (n=12) and from unrelated controls for genetic analysis (n=100). Genomic DNA was extracted from the whole blood samples by a BioRobot EZ1 DSP Workstation (QIAGEN, Hilden, Germany). Genomic DNA was dissolved in 100 µl distilled water.

The coding regions and flanking introns of the investigated *TYR*, *OCA2* and *SLC45A2* genes were amplified by polymerase chain reaction (PCR) with specific primers. Amplifications were carried out in 20 µl volumes containing 4 µl sample DNA, 9 µl Dream Taq Green PCR Master Mix (Fermentas), 4 µl distilled water and 1,5 - 1,5 µl of each primers. The using primer sequences were obtained from the UCSC Genome Browser (www.genome.ucsc.edu) and Primer3 (http://bioinfo.ut.ee/primer3-0.4.0/). The PCR conditions were the following: after an initial denaturation step at 95 °C for 10 min, 40 cycles of amplification was performed consisting of 30 sec at 95 °C (denaturation), 30 sec at the optimal annealing temperatures of the primers (~59°C) and 45 sec at 72°C (synthesis). The synthesis reaction time was determined according to the length of the reaction product. Finally a 10 min terminal elongation step was followed at 72°C in a MyCycler PCR machine (BioRad).

The PCR products were checked on 2% agarose gel (SeaKem LE agarose, Lonza) using TBE buffer (Lonza) and visualized by 2,5 µl GelRed (Biotium) staining. The gel was analyzed by BioRad Molecular Imager® GelDoc™ XR gel documentation system with QuantityOne software.

After amplifying the coding regions and flanking introns of the investigated *TYR*, *OCA2* and *SLC45A2* genes, DNA sequencing was performed on the purified amplification products. The suitable PCR products were sequenced by a traditional capillary sequencer in an ABI Prism 3100 (Applied Biosystems) sequencing machine with Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Bio systems). The service of the sequencing was provided in scientific cooperation by the Delta Bio 2000 company (Szeged, Hungary).

3. RESULTS

In order to identify the causative mutations in the investigated Hungarian OCA patients (n=12), I have performed the genetic screening of the *TYR, OCA2* and *SLC45A2* genes, which have been implicated in the etiology of the most common isolated OCA forms (OCA1, OCA2 and OCA4). Molecular genetic investigation of Hungarian OCA patients identified pathogenic mutation in all of the patients: five patients carried a combination of two heterozygous pathogenic mutations, whereas only one heterozygous pathogenic mutation was identified in seven patients. In six of these patients, non-pathogenic variants of the *TYR, OCA2* and *SLC45A2* genes were also detected. None of these variants alone is expected to result in the development of OCA; however, these variants were also included in the subsequent analysis, as they might provide further insight into modifying mechanisms of disease development and might lead to the establishment of genotype–phenotype correlations in OCA.

3.1. Recurrent pathogenic and recurrent non-pathogenic variants were identified on the *TYR* gene

Direct sequencing of the *TYR* gene revealed pathogenic mutations in 75% (n=9) of the investigated patients. The most frequently detected mutation was the p.Pro406Leu missense mutation, which was present in heterozygous form in 66% (n=8) of the patients. Two patients carried the p.Pro406Leu mutation in combination with the p.Arg217Gln missense mutation, and one patient carried it with the p.Arg402X nonsense mutation. In five patients carrying the p.Pro406Leu mutation in heterozygous form, no further pathogenic mutation could be identified, and this was confirmed by screening the *TYR, OCA2* and *SLC45A2* genes. However, all five patients carrying the p.Pro406Leu pathogenic mutation also carried common polymorphisms, such as the p.Arg402Gln polymorphism of the *TYR* gene, the p.Arg305Trp variant of the *OCA2* gene, and the p.Leu374Phe polymorphism of the *SLC45A2* gene. These polymorphisms are considered as benign variants, which are unable to cause OCA by themselves, but might contribute to the development of OCA in combination with other pathogenic mutations (Simeonov *et al.*, 2013). In one patient, only the p.Arg217Gln heterozygous pathogenic missense mutation was identified. The p.Ser192Tyr polymorphism of the *TYR* gene affects a copper-binding domain of the protein; all the other pathogenic and non-pathogenic variants are located outside of the known functional domains of the enzyme.

3.2. Only one recurrent non-pathogenic genetic variant was identified on the *OCA2* gene

No pathogenic *OCA2* mutation was identified in the investigated OCA individuals. However, one patient with the pathogenic p.Pro406Leu *TYR* mutation also carried the common p.Arg305Trp polymorphism of the *OCA2* gene in heterozygous form. This variant does not affect any known functional domains of the OCA2 protein.

3.3. Two novel pathogenic mutations and recurrent non-pathogenic variants were identified on the *SLC45A2* gene

Based on our results, six of 12 patients carried SLC45A2 variations: two patients carried the combination of the newly identified p.Gly411Asp pathogenic missense mutation and also novel the p.Gln487X nonsense mutation. These two mutations are novel mutations, which have not been reported in the literature previously, therefore here I gave detailed phenotypic descriptions of the patients and detailed genetic results.

The two affected OCA patients have complete absence of pigment in their hair, pale skin, pink nevi and blue eyes with nystagmus. One of them has been suffering from Crohn's disease for 9 years and hypothyreosis for 4 years. The other one is not aware of any known concomitant diseases. The parents of the affected siblings are clinically unaffected by OCA4.

Both two OCA patients carry both novel mutations: the c.1226G/A p.Gly411Asp missense and the c.1459C/T p.Gln487X nonsense ones. Regarding their location on the SLC45A2 protein (Uniprot: Q9UMX9), they are situated within transmembrane domains. The c.1226G/A p.Gly411Asp missense mutation is located within the ninth and the c.1459C/T p.Gln437X nonsense mutation within the tenth transmembrane domains of the SLC45A2 protein. Concerning the location of the identified mutations, it can be hypothesized that they impair the transport function of the SLC45A2 protein. The dysfunctional SLC45A2 might cause acidic melanosomal lumen, which leads to improper incorporation of copper into typrosinase and thus to reduced tyrosinase activity and the development of the OCA phenotype (Bin *et al.*, 2015). The p.Gly411Asp missense mutation affects an evolutionary conserved region of the MATP protein further emphasizing the putative pathogenic role of this mutation in the development of the observed pigmentation abnormalities of the affected patients.

In four patients, only the non-pathogenic p.Leu374Phe missense polymorphism was detected. All the detected pathogenic and non-pathogenic variants are located within trans-membrane domains of the encoded protein.

4. DISCUSSION

With the performed clinical and genetic workup, I have investgated clinical and genetic heterogenity of OCA1, OCA2 and OCA4 in Hungarian OCA patients (n=12) in order to promote the understanding of the heterogeneity of OCA, to assess the independent and cumulative contributions of the *TYR*, *OCA2* and *SLC45A2* genes to the development of OCA and to compare relative and cumulative frequencies of the clinical variants of OCA in a representative Hungarian OCA population.

4.1. *TYR* mutations accounts for approximately 75% of the isolated OCA cases in the investigated Hungarian OCA population

Pathogenic *TYR* mutations were present in 75% (n=9) of the patients. This result correlates well with previous findings that OCA1 is the most common isolated OCA subtype and *TYR* mutations account for approximately 50% of the isolated OCA cases worldwide (Rooryck *et al.,* 2008; Simeonov *et al.,* 2013). Among the approximately 320 *TYR* mutations identified to date, missense mutations are the most common (Ghodsinejad Kalahroudi *et al.,* 2014). In our study, four missense and one nonsense variants were detected for the TYR gene. Two of these missense mutations are considered pathogenic and two are considered benign polymorphisms. In contrast, the protein carrying the p.Arg402Gln polymorphism exhibits reduced tyrosinase activity at physiological temperature and is considered a temperature-sensitive variant (Berson *et al.,* 2000; Halaban *et al.,* 2000; Toyofuku *et al.,* 2001; Tripanthi *et al.,* 1992). The contribution of the p.Arg402Gln TYR polymorphism to the OCA phenotype is still unknown. By itself, this variant is unable to cause OCA; however, its increased frequency in OCA patients with one heterozygous pathogenic TYR mutation suggests that it can contribute to the development of OCA in combination with a pathogenic mutation (Hutton and Spritz, 2008; Chiang *et al.,* 2009). One Hungarian patient carried the p.Arg402Gln polymorphism in combination with the p.Pro406Leu pathogenic variant. Previous reports (Hutton and Spritz, 2008; Chiang *et al.,* 2009) suggest this combination might contribute to the development of the OCA symptoms of the patient. It is important to emphasize that certain variants might not cause a disease phenotype in isolation, but contribute to the development of the disease in combination with other pathogenic variants. The difficulty of assessing the impact of these variants highlights the importance of the databases providing information about OCA gene variants, detailed phenotypic descriptions and delineation of OCA genetic heterogeneity.

Three of the nine Hungarian OCA patients with *TYR* mutations carry a combination of two pathogenic mutations. In six of the nine, only one heterozygous pathogenic mutation was identified. These results correlate well with the recently reported investigation of an Iranian OCA population: pathogenic *TYR* variants were identified in 19 of 30 patients (Ghodsinejad Kalahroudi *et al.,* 2014). In this study, six patients carried only one pathogenic TYR mutation, and no pathogenic mutation was identified in five patients (Ghodsinejad Kalahroudi *et al.,* 2014).

All of the pathogenic *TYR* mutations detected in the Hungarian OCA patients have previously been identified in OCA patients of different ethnicity. The p.Pro406Leu mutation was detected in Caucasians from Iran, the p.Arg217Gln mutation in Caucasians from USA, Canada and Northern-Europe, and the p.Arg402X in Caucasians from Lebanon (Hutton and Spritz, 2008; Simeonov *et al.,* 2013). The frequency of pathogenic mutations differs in different populations, and, therefore,

these populations might vary in their genetic susceptibility to certain diseases. Based on our results and the results of previous studies, the identified pathogenic TYR mutations are not specific to the Hungarian population, as they have been detected worldwide in OCA patients (Hutton and Spritz, 2008; Simeonov *et al.*, 2013).

4.2. *OCA2* mutation was not detected in the investigated Hungarian OCA population

No pathogenic OCA2 mutation was identified in the investigated Hungarian individuals, although one patient carried the benign p.Arg305Trp polymorphism in heterozygous form. This variant has been associated with human eye color and might be an inherited biomarker of cutaneous cancer risk (Rebbeck et al., 2002; Jannot et al., 2005). This also suggest that the genetic screening of the TYR and SLC45A2 genes should preceed the genetic investigation of the OCA2 gene, since based on my results, the mutations of this gene is probably less frequent than the mutations of the TYR and SLC45A2 genes among Hungarian OCA patients.

4.3. Two novel mutations in the *SLC45A2* gene identified in two Hungarian OCA patients are associated with unusual OCA4 phenotype

Among the investigated OCA patients, there were two patients who carry two novel heterozygous pathogenic variants of the *SLC45A2* gene: the p.Gly411Asp missense and the p.Gln487X nonsense mutations. The OCA4 symptoms of the affected patients is highly possibly the consequence of the identified mutations of the *SLC45A2* gene. However, it is still uncovered, whether the concomitant diseases of Patient 2 (Crohn's disease for 9 years and hypothyreosis for 4 years) are related to the identified *SLC45A2* mutations. Regarding Crohn's disease, there is one previous study in the literature, which reports a Canadian patient affected by both OCA4 and Crohn's diseases (Fernandez *et al.*, 2012). High throughput genetic investigations of this Canadian patient identified two pathogenic homozygous mutations, one in the *SLC45A2* gene and another in the *G6PC3* gene encoding the third subunit of the glucose-6-phosphatase enzyme (Fernandez *et al.*, 2012). The authors concluded that the patient suffers from two distinct diseases: OCA4 and severe congenital neutropenia type 4 (SCN4). The Crohn's disease of the patient was concerned as a manifestation of the SCN4 (Fernandez *et al.*, 2012). In case of the investigated Hungarian OCA patients the mutation screening of the *SCN4* gene was not performed, since their clinical symptoms do not support this diagnosis. However, we hypothesize that the concomitant diseases (Crohn's disease and hypothyreosis) of Patient 2 are not related to the identified *SLC45A2* mutations.

Mutations of the SLC45A2 gene can either cause complete or partial loss of pigmentation and thus contribute to the development of several different OCA phenotypes (Simeonov *et al.*, 2013). However, with the comparison of the *SLC45A2* mutations and the patients' clinical symptoms, genotype–phenotype correlations have not yet been established in OCA4 (Simeonov *et al.*, 2013). Mutations of the *SLC45A2* gene typically associated with the so called "brown OCA" phenotype referring to partial loss of pigmentation in the affected patients (Kamaraj *et al.*, 2014). In contrast with this, here we report two Hungarian OCA patients with unusual OCA4 phenotypes, since the investigated patients have developed complete absence of pigmentation, which is more common in OCA1 caused by mutations in the *TYR* gene. To rule out any other putative genetic modifier variant, which might

be responsible for the identified unusual phenotype, the mutation screening of the *TYR* and the *OCA2* genes have also been performed, but mutation has not been identified, however both patients carry the p.Ser192Tyr common, non-pathogenic polymorphism of the *TYR* gene, which might have any phenotype modifying role if pathogenic *SLC45A2* mutations are present. Our report further contributes not only to the mutation spectrum of the *SLC45A2* gene but also to the spectrum of the observed unusual clinical symptoms and hopefully it will add novel data to future studies characterizing genotype-phenotype correlations in OCA4.

OCA has been considered for many years as a group of monogenic rare diseases without cure. However, in case of OCA4, accumulating knowledge regarding the underlying mechanism of the disease might alter this viewpoint. It has been recently demonstrated in MNT-1 cell lysates that the reduced tyrosinase activity, induced by the knockdown of the *SLC45A2* can be recovered by exogenously introduced copper treatment (Bin *et al.,* 2015).

In conclusion, two novel heterozygous mutations are detected, a missense and a nonsense one, in the *SLC45A2* gene in two Hungarian patients affected by OCA4. The location of the mutations and the evolutionary conservation of the missense one suggest their putative pathogenic role in the development of OCA4 in the investigated patients. Our study provides new insights to the genetic background of OCA4 and might serve as a basis for future studies aiming to develop novel therapeutic approaches to OCA patients.

In addition to the pathogenic *SLC45A2* mutations (the p.Gly411Asp missense and the p.Gln487X nonsense mutations) described above, the p.Leu374Phe polymorphism was detected in nine Hungarian OCA patients. This latter variant has a striking population distribution and exists almost exclusively in Europeans (Graf *et al.,* 2005). Additionally, this variant has also been implicated in the development of different shades of hair, skin and eye color (Graf *et al.,* 2005).

5. SUMMARY

In my PhD dissertation, my aim was to elucidate the genetic background of a clinically and genetically heterogeneous group of genodermatoses, the OCA. OCA is characterized by decreased pigmentation or by complete loss of pigmentation in the skin, the hairs and the eyes. Here I have performed complete clinical and genetic workup and summarized the results of my investigations in OCA. Regarding its genetic heterogeneity, since all the patients were considered as having non-syndromic OCA variants, I have performed the concomitant analysis of multiple genes - *TYR, OCA2* and *SLC45A2* -, which are implicated in the majority of the isolated OCA variants. After the study got ethical approval, I have enrolled and investigated a Hungarian OCA population, which involved 12 patients. The number of the enrolled patients might seem to be small, but considering that OCA is a rare disease, the enrollment of 12 individuals with similar clinical phenotype was already enormous work.

During my investigations, I would like to highlight, that I have identified two novel *SLC45A2* mutations in two Hungarian OCA patients with unusual phenotype of OCA4 (Tóth *et al.,* 2017). These novel heterozygous mutations were the followings: a missense one (c.1226G/A p.Gly411Asp) and a nonsense one (c.1459C/T p.Gln437X). These mutations were present in both patients suggesting their compound heterozygous states. The identified novel mutations affect the transmembrane domains of the protein suggesting that they might impair its transport function leading to decreased melanosomal pH and decreased tyrosinase activity. My investigations provide new insights to the genetic background of OCA4 by reporting an unusual OCA4 phenotype and expanding the mutation spectrum of the *SLC45A2* gene.

Besides the identification of novel mutations, the other significant results of my study are the followings: Although, the clinical features of the investigated Hungarian OCA patients (n=12) were identical, the molecular genetic data suggested an OCA1 subtype in nine cases and an OCA4 subtype in three cases (Fábos *et al.*, 2017). In five patients, two different heterozygous pathogenic mutations were present, whereas seven patients had only one pathogenic mutation and associated non-pathogenic variants (Fábos *et al.*, 2017). Therefore, my results suggest that the concomitant screening of the non-pathogenic variants — which alone do not cause the development of OCA, but might have clinical significance in association with a pathogenic variant — is important (Fábos *et al.*, 2017).

Based on the results of my scientific work, these investigations has promoted the understanding of the heterogeneity of OCA, assessed the independent and cumulative contributions of the *TYR, OCA2* and *SLC45A2* genes to the development of OCA, and compared relative and cumulative frequencies of the clinical variants of OCA in a representative Hungarian OCA population.

My results confirm that the concomitant analysis of OCA genes is critical, providing new insights to the phenotypic diversity of OCA and expanding the mutation spectrum of OCA genes in Hungarian patients.

6. ACKNOWLEDGEMENT

First and foremost I would like to thank to my supervisor, Dr. Nikoletta Nagy her great supervising activity.

My sincere thank also goes to Prof. Dr. Márta Széll, who provided me an opportunity to join her team and who gave access to perform the genetic investigations of this study at the Department of Medical Genetics, University of Szeged.

Special thanks to all my colleagues for their kind help in the Department of Medical Genetics, University of Szeged.

Last but not the least, I would like to thank to my family for supporting me for everything and encouraging me throughout this experience.

These investigations were supported by the Hungarian TÁMOP-4.2.2.A-11/1/KONV-2012-0035, TÁMOP-4.2.4.A/2-11-1-2012-0001, TÁMOP-4.2.2.A3 grants and GINOP-2.3.2-15-2016-00039.