Hereditary Genetics

Tóth et al., Hereditary Genetics 2015, S7
DOI: 10.4172/2161-1041.S7-006

Research Article Open Access

Identification of Two Novel Mutations in the *SLC45A2* Gene in a Hungarian Pedigree Affected by Unusual OCA Type 4

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Abstract

Oculocutaneous Albinism (OCA) is a clinically and genetically heterogenic group of pigmentation abnormalities. OCA type IV (OCA4, OMIM 606574) develops due to homozygous or compound heterozygous mutations in the *solute carrier family 45, member 2* (*SLC45A2*) gene. This gene encodes a membrane-associated transport protein, which regulates tyrosinase activity and, thus, melanin content by changing melanosomal pH and disrupting the incorporation of copper into tyrosinase. Here we report two Hungarian siblings affected by an unusual OCA4 phenotype. Direct sequencing of the *SLC45A2* gene revealed two novel, heterozygous mutations, one missense (c.1226G/A p.Gly411Asp) and one nonsense (c.1459C/T p.Gln437X), which were present in both patients, suggesting the mutations were compound heterozygous. The identified novel mutations affect the transmembrane domains of the protein, indicating that they might impair transport function, resulting in decreases in both melanosomal pH and tyrosinase activity. Our study provides new insights to the genetic background of OCA4 by reporting an unusual OCA4 phenotype and expanding the mutation spectrum of the *SLC45A2* gene.

Keywords: Oculocutaneous albinism type 4; Unusual phenotype; *SLC45A2* gene; Compound heterozygous state; Novel mutations

Introduction

Oculocutaneous Albinism (OCA) is a clinically and genetically heterogenic group of rare monogenic diseases characterized by reduced melanin production in the skin, hair and/or eyes [1]. OCA symptoms can include poor visual acuity, nystagmus, iris transillumination, strabismus, photophobia, foveal hypoplasia and misrouting of optic nerve fibers at the chiasm [2]. All OCA forms exhibit autosomal recessive inheritance [1].

OCA type 4 (OCA4, OMIM 606574) is a rare form of OCA caused by mutations in the solute carrier family 45, member 2 (*SLC45A2*) gene on chromosome 5p13 [3]. The *SLC45A2* gene encodes a Membrane-Associated Transport Protein (MATP), which is located in melanosomes and shows high sequence and structural similarity to *Drosophila melanogaster* and plant sucrose transporters containing an RxGRR motif [4,5]. *SLC45A2* knockdown reduced melanin content and tyrosinase activity by acidifying the pH of melanosomes in a human melanoma cell line, MNT-1 [6]. It has been suggested that, as a proton/sugar symporter, MATP transports sugars from the melanosomes to the cytoplasm using a proton gradient generated by a proton pump. Thus, normal protein function ensures elevated melanosomal pH, allowing proper binding of copper to tyrosinase and resulting in normal tyrosinase activity [6].

To date, 78 of the mutations identified in the *SLC45A2* gene are related to OCA4 [7]. In this study, we report a Hungarian family with two members affected by OCA4. Our genetic investigation identified that these members carried two novel heterozygous mutations in a compound heterozygous state, expanding the mutational spectrum of OCA4.

Patients and Methods

Patients

A Hungarian family with two affected siblings was investigated (Figure 1). The affected individuals were 30 (Patient II/1) and 27 years (Patient II/2) old at the time of investigation. Both exhibited pale skin, complete absence of hair pigment, pink nevi and blue eyes with nystagmus. This complete absence of pigmentation is unusual for OCA4. Patient II/1 has been suffering from Crohn's disease for 9 years and hypothyreosis for 4 years. Patient II/2 was not aware of any known concomitant diseases. The parents (I/1 and I/2) of the affected siblings are clinically unaffected by OCA4. The investigated patients declined publication of their clinical pictures.

Genetic investigation

Blood was taken from the affected patients as well as from unrelated, healthy Hungarian individuals without pigmentation abnormality (n=30), and genomic DNA was isolated using a BioRobot EZ1 DSP Workstation (QIAGEN; Godollo, Hungary). The entire coding region of the *SLC45A2* gene and the flanking introns were amplified and sequenced (primer sequences used were taken from the UCSC Genome Browser www.genome.ucsc.edu). The investigation was approved by the Internal Review Board of the University of Szeged. Written informed consent was obtained from the patient and the study was conducted according to the Principles of the Declaration of Helsinki. After identifying the causative mutations in the patients, further genetic screening of the parents was declined.

Results

Direct sequencing of the coding regions and the flanking introns of the *SLC45A2* gene revealed two heterozygous mutations, one missense mutation (c.1226G/A p.Gly411Asp) in the sixth exon (Figure 2A)

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Received October 12, 2015; **Accepted** October 24, 2015; **Published** October 27, 2015

Citation: Tóth L, Fábos B, Farkas K, Sulák A, Tripolszki K, et al. (2015) Identification of Two Novel Mutations in the *SLC45A2* Gene in a Hungarian Pedigree Affected by Unusual OCA Type 4. Genetics S7: 006. doi:10.4172/2161-1041.S7-006

and one nonsense mutation (c.1459C/T p.Gln437X) in the seventh exon (Figure 2B). Both patients carried both mutations, suggesting a compound heterozygous state. Unrelated healthy controls carried the wild type sequence.

Discussion

Here we report two Hungarian siblings affected by OCA4 and carrying two heterozygous mutations of the *SLC45A2* gene, indicating a compound heterozygous state. These mutations, missense c.1226G/A p.Gly411Asp and nonsense c.1459C/T p.Gln437X, are novel. Both are situated in transmembrane domains of the MATP protein (Uniprot: Q9UMX9: the c.1226G/A p.Gly411Asp missense mutation is located within the ninth domain and the c.1459C/T p.Gln437X nonsense mutation within the tenth (Figure 2C). The locations of the mutations suggest that they impair the transport function of the MATP protein. MATP dysfunction might cause an acidic melanosomal lumen, leading to improper incorporation of copper into typrosinase. The reduced tyrosinase activity could, in turn, lead to the development of the OCA phenotype [6]. The c.1226G/A p.Gly411Asp missense mutation affects an evolutionary conserved region of the MATP protein (Figure 2D), further emphasizing the putative pathogenic role of this mutation in the development of the observed pigmentation abnormalities of the affected patients.

Taken together, the OCA4 symptoms of the affected siblings are likely the consequence of the identified mutations of the *SLC45A2* gene. It is still unclear whether the concomitant diseases of Patient II/1 are related to the identified *SLC45A2* mutations. A Canadian patient affected by both OCA4 and Crohn's diseases has been reported [7]. High-throughput genetic investigation of this Canadian patient identified two pathogenic homozygous mutations, one in the *SLC45A2* gene and another in the *G6PC3* gene, which encodes the third subunit of the glucose-6-phosphatase enzyme [7]. The authors concluded that the patient suffers from two distinct diseases, OCA4 and severe congenital neutropenia type 4 (SCN4), and the presence of Crohn's disease was considered a manifestation of SCN4 [7]. No mutation screening of the *SCN4* gene was undertaken for the Hungarian siblings, as their clinical symptoms did not support this diagnosis. We hypothesize that the

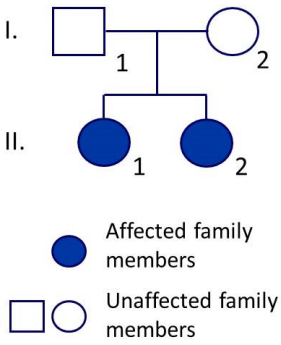

Figure 1: Pedigree of the patients. The investigated Hungarian pedigree spans two generations and includes two affected siblings (II/1 and II/2).

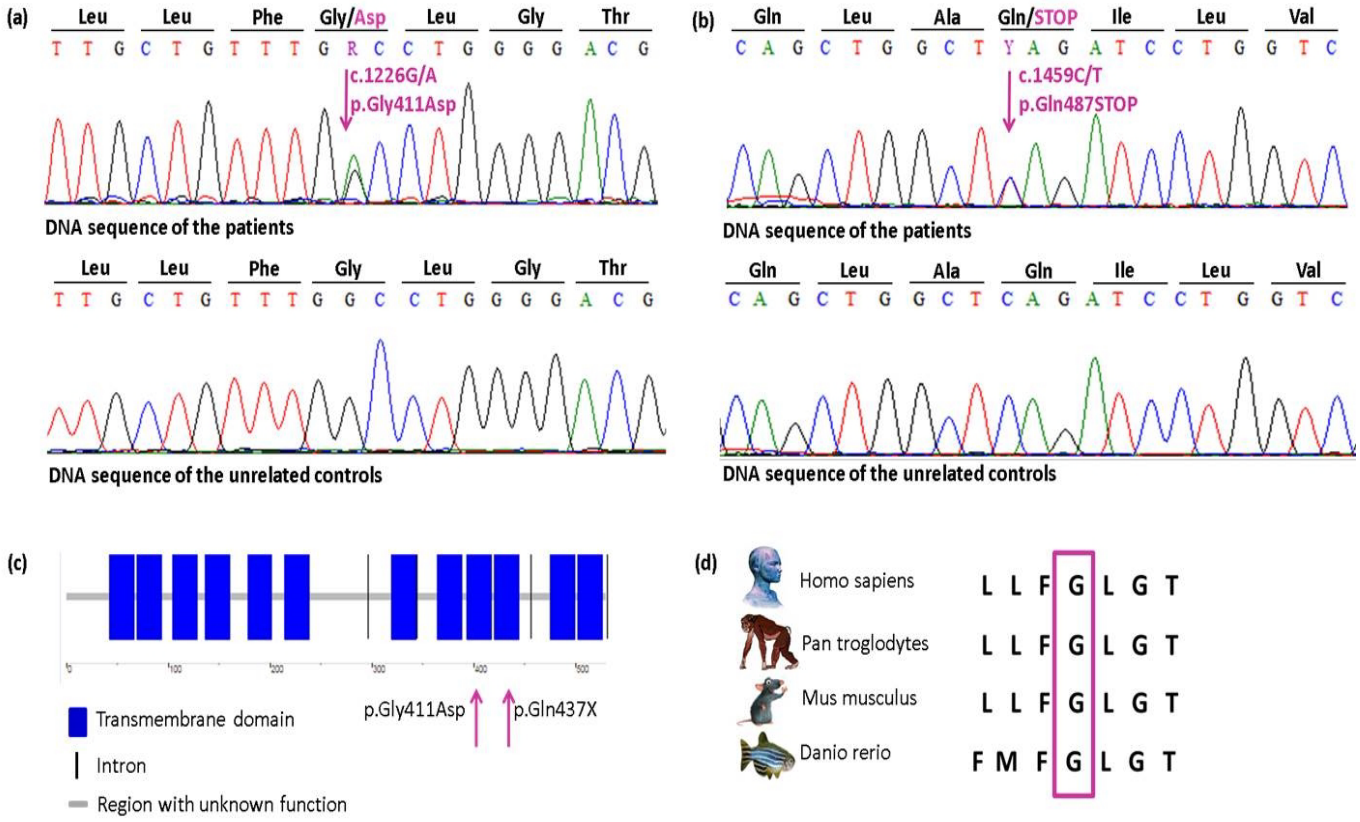

Figure 2: Identification of two novel mutations of the *SLC45A2* gene. (A) Direct sequencing revealed a heterozygous missense mutation (c.1226G/A p.Gly411Asp) in the sixth exon and (B) a heterozygous nonsense mutation (c.1459C/T p.Gln437X) in the seventh exon of the gene. Both mutations were present in both affected patients. Unrelated controls (n=30) carried the wild type sequence. (C) The identified mutations are located within the transmembrane domains of the MATP protein. (D) The identified missense mutation is situated within an evolutionary conserved region.

concomitant diseases of Patient II/1 are not related to the identified *SLC45A2* mutations.

Mutations of the *SLC45A2* gene have been reported to cause complete or partial loss of pigmentation, thus contributing to the development of several different OCA phenotypes [8]. However, a genotype–phenotype correlation based the *SLC45A2* mutations and the patients' clinical symptoms has not yet been established for OCA4 [9]. Mutations of the *SLC45A2* gene are typically associated with partial loss of pigmentation, referred to as the "brown OCA" phenotype [9]. The two siblings reported here exhibited an unusual OCA4 phenotype, as they developed the complete absence of pigmentation. This phenotype is more common in type 1 OCA, which is caused by mutations in the tyrosinase *(TYR)* gene. To rule out the influence of other putative genetic-modifier variants responsible for the unusual phenotype, the mutation screening of the *TYR* and the *OCA2* genes was also performed; however, no mutations were identified. Our report, which further contributes to the mutation spectrum of the *SLC45A2* gene as well as to the spectrum of the observed unusual clinical symptoms, will hopefully contribute 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. Accumulating knowledge regarding the underlying mechanism of the OCA4 might alter this viewpoint: it has been recently demonstrated in MNT-1 cell lysates that exogenously applied copper recovers reduced tyrosinase activity resulting from *SLC45A2* knockdown [6].

In conclusion, we report two novel heterozygous mutations, one missense and one nonsense, of the *SLC45A2* gene in two Hungarian sisters affected by OCA4. The location of the mutations as well as the evolutionary conservation of the missense mutation suggest a pathogenic role in the development of OCA4. 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 for OCA patients.

Acknowledgements

This study was supported by the Hungarian TÁMOP-4.2.2.A-11/1/KONV-2012-0035 grant, TÁMOP-4.2.4.A/2-11-1-2012-0001 grant and TÁMOP-4.2.2.A3 grant.

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This article was originally published in a special issue, **Genetic Diseases** handled by Editor(s). Dr. Steven J. Fliesler, Buffalo VA Medical Center, USA; Dr. Jijing Pang, University of Florida, USA