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# Functional characterization of two novel splicing mutations in the *OCA2* gene associated with oculocutaneous albinism type II

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ABSTRACT

Oculocutaneous albinism (OCA) is characterized by hypopigmentation of the skin, hair and eye, and by ophthalmologic abnormalities caused by a deficiency in melanin biosynthesis. OCA type II (OCA2) is one of the four commonly-recognized forms of albinism, and is determined by mutation in the *OCA2* gene.

In the present study, we investigated the molecular basis of OCA2 in two siblings and one unrelated patient. The mutational screening of the *OCA2* gene identified two hitherto-unknown putative splicing mutations. The first one (c.1503+5G>A), identified in an Italian proband and her affected sibling, lies in the consensus sequence of the donor splice site of *OCA2* intron 14 (IVS14+5G>A), in compound heterozygosity with a frameshift mutation, c.1450_1451insCTGCCCTGACA, which is predicted to determine the premature termination of the polypeptide chain (p.I484Tfs*19). *In-silico* prediction of the effect of the IVS14+5G>A mutation on splicing showed a score reduction for the mutant splice site and indicated the possible activation of a newly-created deep-intronic acceptor splice site.

The second mutation is a synonymous transition (c.2139G>A, p.K713K) involving the last nucleotide of exon 20. This mutation was found in a young African albino patient in compound heterozygosity with a previously-reported *OCA2* missense mutation (p.T404M). *In-silico* analysis predicted that the mutant c.2139G>A allele would result in the abolition of the splice donor site.

The effects on splicing of these two novel mutations were investigated using an *in-vitro* hybrid-minigene approach that led to the demonstration of the causal role of the two mutations and to the identification of aberrant transcript variants.



## 1. Introduction

Oculocutaneous albinism (OCA) is characterized by general hypopigmentation of the skin, hair and eye, and by ophthalmologic abnormalities caused by a deficiency in melanin biosynthesis. This inherited disorder can affect all ethnic groups with an overall prevalence of approximately 1/17,000 people (Grønskov et al., 2007). Eye and optic system abnormalities include various degrees of congenital nystagmus, hypopigmentation of iris leading to iris translucency, reduced pigmentation of the retinal pigment epithelium, foveal hypoplasia, reduced visual acuity (usually in the range 20/60–20/400), and refractive errors. Photophobia may be prominent. A characteristic finding is the misrouting of the optic nerves, consisting in an excessive crossing of the fibers in the optic chiasm, which can result in strabismus and reduced stereoscopic vision (Kirkwood, 2009). These features are common to all types of albinism and are probably related to melanin reduction during embryonic development and early postnatal life (King et al., 2001). OCA is commonly subdivided into four types (OCA1–4), due to mutations in the *TYR*, *OCA2*, *TYRP1*, and *SLC45A2* genes, respectively (Boissy et al., 1996; Tomita et al., 1989; Newton et al., 2001; Rinchik et al., 1993). Recently, at least three additional loci involved in albinism have been reported. First, in 2012, a new OCA locus mapped on chromosome 4q24 has been found in a consanguineous Pakistani family, but the corresponding gene (termed OCA5) has not yet been identified (Kausar et al., 2013). Subsequently, two new genes involved in non-syndromic OCA have been discovered: *SLC24A5* (Morice-Picard et al., 2013; Wei et al., 2013) and *C10orf11* (Grønskov et al., 2013) named OCA6 and OCA7, according to the recent review of Montoliu (Montoliu et al., 2013). In addition, an X-linked form of ocular albinism

*Abbreviations:* OCA, Oculocutaneous Albinism; OA, Ocular Albinism; ENT, Ears, Nose and Throat; OCT, Optical Coherence Tomography; VEP, Visual Evoked Potential; ERG, Electroretinogram; PCR, Polymerase Chain Reaction; MLPA, Multiplex Ligation-dependent Probe Amplification; IVS, Intervening Sequences; RT-PCR, Reverse Transcription PCR; TMD, Trans-Membrane Domain; mRNA, Messenger RNA; ECD, Extra-Cellular Domain.

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(OA1), in which the phenotype is mainly restricted to eyes and optic system, is associated with mutations in the *GPR143* gene (Schiaffino et al., 1995).

Oculocutaneous albinism type II (OCA2—OMIM *611409, #203200) has been described in all major ethnic groups; its prevalence is approximately 1:36,000 in Caucasians (Grønskov et al., 2007). It is the most common type of albinism in the southern African population, in which the prevalence is estimated as 1:1500–1:3900 due to consanguinity issues (Cruz-Inigo et al., 2011), and in the African–American population, in which the prevalence is estimated to be 1:10,000 (King and Oetting, 2006). The *OCA2* gene, formerly known as the *P* gene (official HGNC name, *OCA2*), a human homologue of the mouse pink-eye dilution (p) gene, is located on chromosome 15q11.2–q12 and contains 24 exons, the first being a non-coding one (Lee et al., 1995). Although its function is not precisely characterized, the p protein is characterized by 12 transmembrane spanning regions and is an integral component of the melanosomal membrane (King et al., 2001; Lee et al., 1995). It appears to have multiple functions in the biosynthesis of melanin including a key role in the maturation and transport of tyrosinase and in the regulation of pH into the melanosome (Brilliant, 2001; Kushimoto et al., 2003; Suzuki and Tomita, 2008).

More than 140 mutations have been identified in the *OCA2* gene (HGMD Professional http://biobase-international.com/hgmd/pro/all.php). The most common mutation is a 2.7-kb deletion encompassing exon 7 that is found in many affected individuals of sub-Saharan African heritage, as well as in unrelated African Americans, Haitian, and Africans, suggesting a founder effect (Durham-Pierre et al., 1994; Spritz et al., 1995; Stevens et al., 1995, 1997).

In this study, we report the identification of the genetic defects underlying OCA2 in two siblings and one unrelated proband. The molecular screening disclosed two hitherto-unknown putative splice defects, whose functional consequences were elucidated by *in-vitro* expression experiments.

## 2. Patients and methods

### 2.1. Patients

Patients were recruited at the Pediatric Ophthalmology Department and the Genetic Service of the Niguarda Ca' Granda Hospital through a multidisciplinary diagnostic workup that included ophthalmologic exams, dermatological and ENT (Ears, Nose and Throat) visits, and genetic counseling.

This study was conducted according to the Declaration of Helsinki and to the Italian legislation on sensible data recording. A signed informed consent for the genetic analysis was obtained from the parents of the probands, who are younger than 18 years.

### 2.2. Ophthalmologic evaluation

The patients underwent full ophthalmological and instrumental evaluations, including: motor and sensory status, visual acuity, cycloplegic refraction, iris transillumination with slit lamp biomicroscope, and macular translucency with indirect ophthalmoscope. The visual acuity assessment for distance and near was evaluated in monocular and binocular vision with appropriate tests (Pigassou figures, E test and Snellen charts).

The following instrumental examinations were performed: optical coherence tomography (OCT), visual evoked potential (VEP) and electroretinogram (ERG). Through Spectral Domain OCT (Heidelberg, Germany) we evaluated the macular morphological characteristics with particular reference to the presence or absence of foveal depression. Electrophysiological tests (Retimax, CSO, Italy) were performed to evaluate the optic nerve fiber decussation.

### 2.3. Genetic analysis

DNA was extracted from peripheral blood according to standard protocols.

The coding regions (exon and adjacent flanking regions) of the *TYR*, *OCA2*, *TYRP1*, and *SLC45A2* genes were PCR amplified and the products sequenced from both ends (primer sequences and experimental conditions are available on request). Sequence analysis was performed on an ABI Prism 3730 genetic analyzer (Life Technologies Corporation, Carlsbad, CA, USA) and the SeqScape software was used for mutation detection.

To analyze the 2.7-kb deletion of the *OCA2* gene, we used three specific primers according to Durham-Pierre protocols (Durham-Pierre et al., 1994). To detect the presence of *TYR* and *OCA2* exon rearrangements (deletion/duplication) the MLPA (multiplex ligation-dependent probe amplification) assay Kit "SALSA P325" (MRC-Holland, Amsterdam, The Netherlands) was used, according to the manufacturer's instructions. The individual peak corresponding to each exon was identified based on the difference in migration relative to the size standards 500 LIZ™ (Life Technologies Corporation). The peak area of each fragment was compared to that of three control samples. Raw data were analyzed using Coffalyser software v.9 (MRC-Holland). Each positive result was confirmed by two independent experiments and on two different DNA extractions.

### 2.4. In-vitro analysis of splicing mutations

Computer-assisted analysis for splice-site prediction was accomplished using the NNSPLICE 0.9 program (http://www.fruitfly.org/seq_tools/splice.html).

The relevant genomic DNA region was cloned in the hybrid alpha-globin–fibronectin minigene plasmid (pBS-KS_modified; Figs. 1A and 2A), in which the alternatively-spliced extra-domain-B exon of fibronectin has been removed to generate a site for the insertion of the *OCA2* exons under study (Baralle et al., 2003). For the functional study of the c.1503+5G>A variant, a 480-bp region of *OCA2*, including exon 14 and the flanking intronic sequences was PCR amplified from the proband's genomic DNA, using the primer couple *OCA2*-IVS13_NdeI_F 5′-GGAATTCCATATGgggggttgttgcagttgtt and *OCA2*-IVS14_NdeI_R 5′-GGAATTCCATATGggattgaaggaccagtcacc, and cloned into the pBS-KS vector (the sequences in capital letter were added to the primers to introduce the *Nde*I restriction site).

For the analysis of the c.2139G>A substitution, a 1137-bp fragment of *OCA2* (introns 19 to 21) was PCR amplified from the patient's genomic DNA using the following primers: *OCA2*_IVS19_*Nde*I_F 5′-GGAATTCCATATGatgagggatttctgccactg and *OCA2*_IVS21_*Nde*I_R 5′-GGAATTCCATATGgctctgctcactttcgtcct and cloned into the pBS-KS_modified vector.

The obtained wild-type (pBS-KS-exon14-wt and pBS-KS-exon20_exon21-wt) and mutant (pBS-KS-IVS14+5G>A-mut and pBS-KS-c.2139G>A-mut) plasmids were isolated by the PureYield Plasmid Miniprep System (Promega, Madison, WI, USA). The correct orientation of the insert, as well as the presence/absence of the c.1503+5G>A or of the c.2139G>A variant were verified by DNA sequencing.

The wild-type and mutant transcripts were produced in HeLa cells by transient transfection experiments as previously described (Costantino et al., 2013), and aberrant splicing events evidenced by RT-PCR assays (Dall'Osso et al., 2008).

## 3. Results

### 3.1. Clinical features of the patients

Three young patients (P1, P2, P3) with oculocutaneous albinism, belonging to two unrelated families, were recruited. P1 and P2 are