

Identification of a missense mutation in the tyrosinase gene in a Chinese family with oculocutaneous albinism type 1

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Abstract. Oculocutaneous albinism (OCA) is a group of heterogeneous and autosomal recessive disorders characterized by a reduction or complete loss of melanin biosynthesis in melanocytes. OCA type 1 (OCA1) is the most severe and common form of OCA, and is caused by mutations in the tyrosinase gene (*TYR*). The present study aimed to identify the genetic cause of OCA1 in a four-generation consanguineous Chinese Han family. Complete physical examinations were performed and blood samples were collected from five members of the family and 100 unrelated healthy controls. Exome sequencing was conducted in the proband, followed by verification in other family members, using Sanger sequencing. Patients in the family presented with typical OCA1 features, including hypopigmentation of the skin and hair, and distinctive ocular changes. A homozygous missense variant, c.896G>A (p.R299H), in the *TYR* gene was identified in two patients, which co-segregated with disease in the family. This variant was not present in the 100 healthy controls. These results expand the number of mutations identified to be responsible for OCA1 in the Chinese Han

population, and may have implications for genetic counseling and clinical management of the disease.

Introduction

Oculocutaneous albinism (OCA) is a group of heterogeneous and autosomal recessive disorders characterized by a reduction or complete loss of melanin biosynthesis in melanocytes (1). All types of OCA demonstrate a lack of melanin pigment in the skin, hair and eyes, and are often accompanied by ocular abnormalities, including varying degrees of nystagmus, hypopigmentation of the iris, foveal hypoplasia, poor vision and refractive errors, photophobia and occasionally color vision impairment (2). The prevalence of OCA subtypes varies considerably between ethnicities. OCA has a prevalence of ~1:20,000 individuals worldwide, or ~1:18,000 in the Chinese Han population (3). At least 17 genes, including four non-syndromic and 13 syndromic, have been identified to be associated with OCA. The tyrosinase (*TYR*), oculocutaneous albinism II, tyrosinase-related protein 1 and solute carrier family 45, member 2 genes are the four non-syndromic OCA genes and are responsible for OCA type 1 (OCA1; MIM 203100), type 2 (OCA2; MIM 203200), type 3 (OCA3; MIM 203290), and type 4 (OCA4; MIM 606574), respectively. The 13 syndromic OCA genes include the Hermansky-Pudlak syndrome 1 (*HPS1*), adaptor-related protein complex 3, β 1 subunit, *HPS3*, *HPS4*, *HPS5*, *HPS6*, dystrobrevin binding protein 1, biogenesis of lysosomal organelles complex-1 subunit 3 (*BLOC1S3*), *BLOC1S6*, lysosomal trafficking regulator, myosin VA, ras-related protein and melanophilin genes (4,5). Due to the phenotypic variation and overlap in clinical presentation among different OCA subtypes, genetic analysis is essential to identify the gene defect and the subtype of OCA (6).

OCA1 is the most severe and common form of OCA, and is caused by mutations of the *TYR* gene (7). It is often divided into two categories: OCA1A and OCA1B. Patients with OCA1A are characterized by a lifelong and complete absence of pigment in the skin, hair and eyes. Patients with OCA1B

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may have a reduced activity of tyrosinase and may produce some melanin over time, resulting in a darkening of hair (3,8). OCA1 affects ~1 in 40,000 individuals in the majority of populations, and has been reported to be the most common subtype of OCA in Japanese, non-Hispanic Caucasian and Danish populations (9). It accounts for ~64.3% of patients with OCA in China. However, OCA1 is rare among African-Americans, reflecting a population-specific distribution of different OCA subtypes (6).

The present study aimed to identify the genetic mutation responsible for OCA1 in a four-generation family of Chinese origin. Patients in the family presented with typical OCA1 characteristics, including white hair and skin, nystagmus, impaired visual acuity, photophobia, color vision impairment, refractive errors, foveal hypoplasia and iris hypopigmentation and translucency. The p.R299H variant of the *TYR* gene, predicted to disrupt the overall integrity of tyrosinase, was revealed to co-segregate with patients in this family and was absent in healthy controls, indicating that it is a pathogenic mutation.

Materials and methods

Subjects. A four-generation, 11-member consanguineous Chinese Han family with OCA from Hunan, China was recruited from The Third Xiangya Hospital, Central South University (Hunan, China; Fig. 1). Blood samples from the five living members of the family, including two affected patients (IV:2 and IV:3) and three unaffected members (III:1, III:2 and IV:1) were collected. Additionally, blood samples were collected from 100 unrelated healthy controls (male, $n=50$; female, $n=50$; age, 45.8 ± 3.4 years) with normal pigmentation and with no family history of ocular abnormalities, from the same region of China. All participants provided written informed consent. The present study was approved by the Institutional Review Board of The Third Xiangya Hospital, Central South University.

Exome capture. Genomic DNA was extracted from peripheral blood samples according to the standard phenol-chloroform extraction method (10). Exome capture was performed on genomic DNA from the proband (Fig. 1) by the Novogene Bioinformatics Institute (Beijing, China). The SureSelect Human All Exon V5 kit (Agilent Technologies, Inc., Santa Clara, CA, USA) was used for exome capture and the HiSeq 2000 platform (Illumina, Inc., San Diego, CA, USA) was used for sequencing, following the manufacturer's protocol. A total of 1.5 μ g genomic DNA was used to construct the exome library and the genomic DNA was sheared into 180–280 bp for enrichment. Enrichment libraries for target regions were sequenced by the HiSeq 2000 platform, which generated 100 bp pair-end reads.

Read mapping and variant analysis. All the clean reads were aligned to the human reference genome (University of California, Santa Cruz; Build 37.1, hg19) using Burrows-Wheeler Aligner (bio-bwa.sourceforge.net). High quality alignment was required to guarantee variant calling accuracy (>0) to detect single nucleotide polymorphisms (SNPs) and insertions-deletions (indels). Picard software

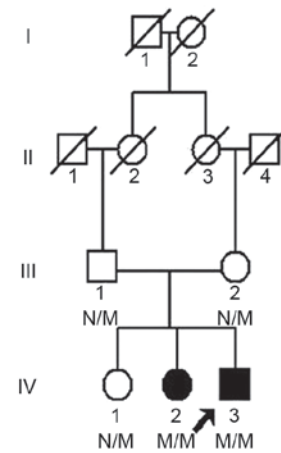


Figure 1. Pedigree of the family with oculocutaneous albinism type 1. Filled symbols represent affected members; empty symbols represent unaffected members. Squares, males; circles, females. Symbols with a diagonal line represent deceased family members. The proband is indicated by an arrow. N, normal; M, *TYR* c.896G>A (p.R299H) mutation.

(sourceforge.net/projects/picard/), the Genome Analysis Toolkit software version 2.1 (software.broadinstitute.org/gatk/) and SAMtools (samtools.sourceforge.net) were used for base quality recalibration. Following this, the binary alignment/map results were obtained, ready for analysis, and ANNOVAR software (Annotate Variation; annovar.openbioinformatics.org/en/latest/user-guide/download) was used to annotate SNPs and indels. Variant lists were filtered against the dbSNP build 137 (dbSNP137), database of SNPs (www.ncbi.nlm.nih.gov/projects/SNP/snp_summary.cgi), the 1000 Genomes Project (1000genomes release_20100804; www.1000genomes.org/) and the National Heart, Lung and Blood Institute Exome Sequencing Project (NHLBI ESP) 6500. Polymorphism Phenotyping version 2 (PolyPhen-2; genetics.bwh.harvard.edu/pph2/) and Sorting Intolerant from Tolerant (SIFT; sift.jcvi.org/) software were used to predict protein functions.

Mutation validation. Sanger sequencing was used to confirm the presence and identity of potential disease-causing variants, using the 3500 Genetic Analyzer sequencer (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Polymerase chain reaction amplification and Sanger sequencing were conducted as described previously (11), using the following primers: Forward, 5'-GTCTGTAGC CGATTGGAGGA-3' and reverse, 5'-GCAGCTTTATCC ATGGAACC-3'.

The National Centre for Biotechnology Information Basic Local Alignment Search Tool (blast.ncbi.nlm.nih.gov/Blast.cgi) was used to perform multiple sequence alignments. The pathogenic potential of amino acid changes as possible disease-causing mutations was further examined using the MutationTaster online tool (<http://www.mutationtaster.org/>).

Results

Clinical findings. Complete physical examinations, including detailed ophthalmic examination (best-corrected visual acuity

Table I. Clinical and genetic data of the two patients with the tyrosinase gene c.896G>A (p.R299H) mutation.

Parameter	IV:2	IV:3
Gender	Female	Male
Age (years)	42	39
Genotype	Homozygous	Homozygous
Hair color (at birth/at present)	White/white	White/white
Skin color	White	White
Nystagmus	+	+
Iris hypopigmentation and translucency	+	+
Foveal hypoplasia	+	+
Refractive errors	+	+
Color vision impairment	+	+
Photophobia	+	+
Impaired visual acuity	+	+

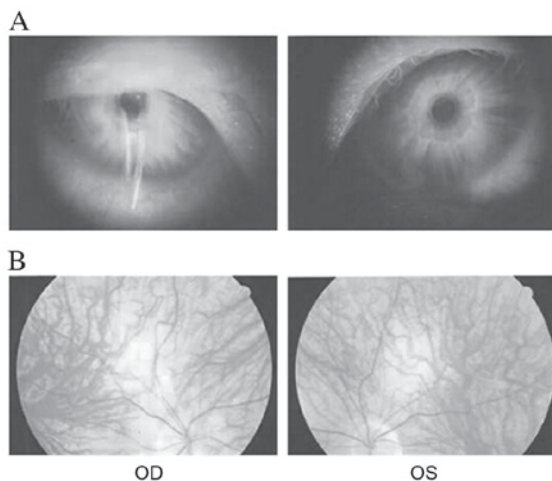


Figure 2. Representative images of the clinical characteristics of the proband (IV:3). Images demonstrate (A) iris hypopigmentation and translucency in the anterior segment and (B) sunset-glow fundus with visible choroidal vessels in the fundus. OD, Oculus Dexter; OS, Oculus Sinister.

testing, slit-lamp examination, dilated fundus examination and optical coherence tomography) were performed. The two patients had typical OCA skin, hair and eye symptoms; they presented with white hair that had not altered in color over time. More detailed descriptions of clinical features of the two OCA patients are presented in Table I. The proband (IV:3) presented with evident horizontal nystagmus, photophobia, impaired visual acuity, absence of the foveal pit, visible choroidal macula vessels, and iris hypopigmentation and translucency (Fig. 2). Additionally, he had refractive errors (-3.00D) and color vision impairment.

Mutation screening. Exome sequencing of the proband (IV:3; Fig. 1) was performed in the Chinese family with OCA. A total of 46.37 million reads with an average read length of 100 bp were generated from the patient; 46.33 million bases (99.92%) were aligned to the human reference sequence. A total of

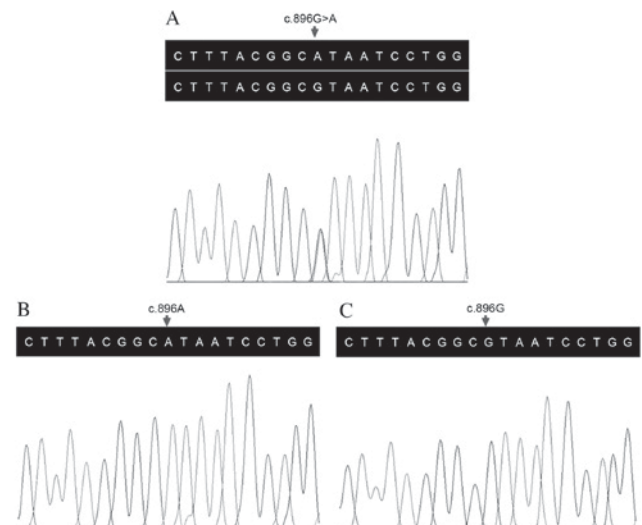


Figure 3. Tyrosinase gene DNA sequencing analysis of p.R299H mutation. (A) A heterozygous p.R299H mutation in the parent of the patient (III:1) and (B) a homozygous p.R299H mutation in the proband (IV:3). (C) Unrelated healthy control.

	p.R299H
Human	NGTPEGPLRRNPGNHDKSR
Chimpanzee	NGTPEGPLRRNPGNHDKSR
Rhesus monkey	NGMPEGPLRRNPGNHDKSR
Cattle	NGTSEGPLLRNPGNHDKAR
Sheep	NGTSEGPLLRNPGNHDKAR
Dog	DGTPEGPLLRNPGNHDKAR
House mouse	DGTPEGPLLRNPGNHDKAK
Norway rat	DGTPEGPLLRNPGNHDKAK
Chicken	NATSEGPILRNPGNNDKSR
Zebrafish	DGSPEGPLLRNPGDHDRIR

Figure 4. Conservation analysis of the tyrosinase p.R299 amino acid residue. The arrow indicates that arginine at position 299 was phylogenetically conserved among various species.

38,626 genetic variants in the coding regions and splice sites, and 36,091 SNPs, including 17,241 in the exon regions and 1,595 in the splice sites, were identified. Furthermore, 2,535 indels, including 410 in the exon regions and 190 in the splice sites, were detected. A prioritization scheme was used to detect the potential pathogenic mutation in the patient, as previously described (12). Known variants identified in the dbSNP137, 1,000 Genomes Project and NHLBI ESP 6500 with a minor allele frequency >0.50% were excluded. SIFT and PolyPhen-2 were used to predict the function of non-synonymous variants. By applying the above filtration criteria, the number of candidate genes was reduced by >99.03%; only 238 novel variants were identified to be potentially disease-causing, therefore were selected for further analysis.

The parents of the proband were identified to carry a heterozygous variant in the *TYR* gene (Fig. 3A). A c.896G>A (p.R299H) variant in the two alleles of the *TYR* gene was identified in the proband (Fig. 3B) following validation by Sanger sequencing. One female patient (IV:2) in the family was subsequently identified to carry the same homozygous

mutation. This variant co-segregated with disease phenotype. The variant was absent in the control cohort, consisting of 100 ethnicity-matched unrelated controls (Fig. 3C). Arginine at position 299 was phylogenetically conserved among various species (Fig. 4). MutationTaster predicted that the substitution was disease-causing. These data indicated that the *TYR* p.R299H variant may be the disease-associated variant in the family investigated in the present study.

Discussion

Albinism was one of the earliest genetic disorders to be studied. In 1903, Farabee (13) first suggested that human albinism may be recessively inherited. The mouse and human *TYR* genes were isolated in 1987. In 1989, the first mutation (nonsense mutation) in the *TYR* gene responsible for human OCA was reported by Tomita *et al* (14). There are 349 *TYR* sequence variants recorded in the Human Gene Mutation Database (www.hgmd.cf.ac.uk/ac/index.php). A wide spectrum of mutations, including gross and small deletions, small insertions, small indels, and splicing, missense and nonsense mutations, have been described, with missense mutations being the most common.

The *TYR* gene (MIM 606933) is located on chromosome 11q14.3, and contains 5 exons spanning ~65 kb of genomic DNA. It encodes a 529-amino acid protein, tyrosinase. Tyrosinase consists of an 18-amino acid signal peptide, two copper binding sites, a transmembrane region at the C-terminal end and an epidermal growth factor-like motif (15). It is a glycoprotein and a copper-containing oxidase, and is expressed in melanocytes. Tyrosinase serves an important role in melanin biosynthesis by catalyzing the rate-limiting conversions of tyrosine to dihydroxy-phenylalanine (DOPA) and from DOPA to DOPA-quinone. In addition, it may catalyze the conversion of 5,6-dihydroxyindole to indole-5,6 quinone (16). Mutations in the *TYR* gene lead to decreased or even absent tyrosinase enzyme activity, and subsequently, a decreased or complete loss of melanin synthesis. Numerous missense mutations are located in or adjacent to the copper binding sites, and interrupt the normal function of tyrosinase, by affecting copper binding or by disrupting the substrate binding site (17).

The present study examined a Chinese Han family with OCA1, characterized by white hair that had not altered in color with age, white skin, nystagmus, impaired visual acuity, photophobia, color vision impairment, refractive errors, foveal hypoplasia and iris hypopigmentation and translucency. The homozygous c.896G>A variant (p.R299H, rs61754375) in the *TYR* gene was identified in the two patients in the family and was absent in the 100 unrelated ethnicity-matched controls. The mutation was predicted to be 'probably damaging' by PolyPhen-2, 'damaging' by SIFT and 'disease-causing' by MutationTaster. These results suggested that the c.896G>A variant (p.R299H) may be pathogenic. Multiple sequence alignment demonstrated that the arginine residue is highly conserved among vertebrates, suggesting that it may be important for function. The parents of the patients, with a heterozygous p.R299H mutation in the *TYR* gene, presented a typical phenotype with no symptoms of OCA. Notably, homozygous and heterozygous p.R299H

mutations in the *TYR* gene have been frequently identified in Chinese, Caucasian, Korean and Arabian patients with OCA1, indicating that this location is a mutation hotspot in OCA1 (7,17-20).

Albinism is understood to occur in the majority of fish, birds, reptiles, amphibians and mammals (21). Molecular abnormalities in the *Tyr* gene that are associated with OCA have been described in whales, chickens, mice, rats, cats, rabbits, cattle, buffalo, American mink, ferrets and rhesus monkeys (22). In 2005, Blaszczyk *et al* (23) identified the mutation p.R299H in the albino Wistar rat, emphasizing the functional significance of this mutation leading to OCA.

In conclusion, the results of the present study confirmed the clinical diagnosis of OCA1 in a Chinese family by identifying a p.R299H mutation in the *TYR* gene. These findings suggested that exome sequencing may be a cost-effective tool for accurate diagnosis of the disease, therefore providing genetic counseling options for individuals with OCA. Further studies using appropriate *TYR*-deficient animal models may facilitate the development of novel therapeutic strategies for the treatment of this disease.

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