Abstract. Oculocutaneous albinism (OCA) is a set of autosomal recessive disorders characterized by hypopigmented hair, skin and eyes. Homozygous or compound heterozygous mutations in the tyrosinase (TYR) gene can cause OCA1, which is the most common and severe subtype of albinism. In the present study, 17 patients with non-syndromic OCA were enrolled from eight provinces of China and were non-consanguineous, with the exception of Patient 4000301. Total genomic DNA was isolated from peripheral blood. Screening was performed for the whole exons and their flanking regions of the TYR gene using Sanger sequencing and the pathogenicity of variants was predicted using in silico analysis. In total, 12 TYR mutations were identified in 10 patients, respectively. Of these, two patients carried homozygous mutations and eight patients carried compound heterozygous mutations. Among the 12 TYR mutations, two missense mutations c.1198T>G (p.W400G) and c.819G>T (p.Q273H) were novel. The results of the present study expand the mutation spectrum of the TYR gene, which may further assist in the prenatal examination and genetic diagnosis of OCA.

Introduction

Oculocutaneous albinism (OCA) is a set of autosomal recessive disorders characterized by a reduction or complete absence of melanin in the skin, hair and eyes. The affected individuals present with signs and symptoms including photophobia, nystagmus, poor visual acuity and iris transillumination (1,2).

No effective therapy for OCA has been found previously. The worldwide prevalence of all known forms of OCA is estimated to be 1:17,000 (3). In the Chinese Han population of Shandong, the prevalence is ~1:18,000 and 3.80% of the population are carriers (4).

To date, mutations in at least 16 genes have been reported to be responsible for OCA (5). The nosology of OCA is based on classification with genetic defects in molecules, comprising 12 syndromic OCA genes and seven non-syndromic OCA genes or loci, including OCA1 (MIM 203100), OCA2 (MIM 203200), OCA3 (MIM 203290), OCA4 (MIM 606574), OCA5 (MIM 615312), OCA6 (MIM 113750) and OCA7 (MIM 615179).

Mutations in both alleles of the tyrosinase (TYR) gene can cause OCA1, which is characterized by a complete or incomplete lack of melanin. OCA1 is the most predominant subtype in the Chinese Han population (5). OCA2, associated with mutations in the OCA2 gene, is the most common type of albinism in the black African population (6). OCA3 is rare in Asiatic populations associated with tyrosinase-related protein 1 (TYRP1). OCA4, OCA6 and OCA7 are caused by mutations in SLC45A2, SLC24A5 and C10 or f11, respectively. OCA5 is a novel genetic cause mapped to chromosome 4q24 (7).

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As TYR mutations are responsible for 70.1% of cases of OCA in the Chinese population (5), the present study performed direct sequencing of TYR in 17 patients with OCA, which revealed 12 mutations in 10 patients, respectively, including two novel mutations c.1198T>G (p.W400G) and c.819G>T (p.Q273H).

Patients and methods

Patients and clinical data. A total of 17 patients with OCA were enrolled in the present study, including 6 women and 11 men, who were from 8 provinces of China (Beijing, Jiangsu, Hebei, Liaoning, Jilin, Guangdong, Sichuan and Hunan). An additional 200 unrelated healthy volunteers served as a control group. Routine screenings and complete ophthalmological examinations were performed on all participants following the provision of signed informed consent. The typical clinical features of OCA, including hypopigmented hair, skin and eyes, nystagmus, photophobia, poor vision and foveal hypoplasia, were observed.
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None of the patients had any other systemic diseases. The Institutional Review Board of the Tongji Eye Institute of Tongji University School of Medicine (Shanghai, China) approved the study, and all procedures were performed in accordance with the tenets of the Declaration of Helsinki.

Genetic analysis. Genomic DNA was extracted from peripheral leukocytes using the Tiangen RelaxGene Blood DNA system (Tiangen Biotech, Co., Ltd., Beijing, China) according to the manufacturer’s protocol. The primers (Table I) were designed for all five exons and splice junction sites of the TYR gene using Primer3 software (version 0.4.0; http://bioinfo.ut.ee/primer3-0.4.0/). All exons and flank regions of TYR were amplified using polymerase chain reaction (PCR). A total of 25 µl PCR mixture contain 40 ng genomic DNA, 1 mM each forward and reverse primers and 12.5 µl 2X Taq PCR MasterMix (Tiangen Biotech Co., Ltd.). PCR was performed on C1000 Touch Thermal Cycler (Bio-Rad Laboratories, Inc., Hercules, CA, USA) using Touchdown PCR program with 35 cycles of amplification: 95˚C for 30 sec; 64‑57˚C for 30 sec beginning at 64˚C and decreasing by 0.5˚C each cycle for 14 cycles, until finishing at a final annealing temperature of 57˚C for 21 cycles and at 72˚C for 40 sec.

The PCR products were sequenced using an ABI3730 automated sequencer (PE Biosystems, Foster City, CA, USA). Benign polymorphisms were excluded using the database of the 1000 Genomes Project (http://www.1000genomes.org/) and the dbSNP National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/projects/SNP/). The albinism database (http://www.ifpcs.org/albinism/) and reported literature were then used to determine whether the mutations had been previously reported as pathogenic. To predict the pathogenicity of TYR variants in the present study, in silico analysis was performed with Sorting Intolerant From Tolerant (SIFT; http://sift.jcvi.org/), Polymorphism Phenotyping (PolyPhen)-2 (http://genetics.bwh.harvard.edu/pph2/), I-Mutant (http://folding.biofold.org/i-mutant/i-mutant2.0.html) and Human Splicing Finder (HSF) 3.0 (http://www.umd.be/HSF3/HSF.shtml), respectively.

Table I. Primers for amplification and sequence analysis of human TYR.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Primer sequence (5′-3′)</th>
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<tbody>
<tr>
<td>TYR-EXON1A-F</td>
<td>CCAGTTCTCGACGACCTTTGT</td>
</tr>
<tr>
<td>TYR-EXON1A-R</td>
<td>TCAATGGGCTAGATGCTCCCT</td>
</tr>
<tr>
<td>TYR-EXON1B-F</td>
<td>GGGACCAAACCTGCACAGAGA</td>
</tr>
<tr>
<td>TYR-EXON1B-R</td>
<td>GGTCTGAATGAGTTCCAGG</td>
</tr>
<tr>
<td>TYR-EXON2-F</td>
<td>TGATGATTCTCAGAGCATAATCCCT</td>
</tr>
<tr>
<td>TYR-EXON2-R</td>
<td>ACAACACATATTCTCGTGCAACTCTA</td>
</tr>
<tr>
<td>TYR-EXON3-F</td>
<td>TGGGATAATCATACTAGTTTTCAGT</td>
</tr>
<tr>
<td>TYR-EXON3-R</td>
<td>GGTGACACCTGATCACAGACA</td>
</tr>
<tr>
<td>TYR-EXON4-F</td>
<td>CCTGTCCTCAGATTTATATATGAC</td>
</tr>
<tr>
<td>TYR-EXON4-R</td>
<td>CACCTTCAGATTTAAAGTGTTCAAGA</td>
</tr>
<tr>
<td>TYR-EXON5-F</td>
<td>GCTTCAAAACCGGTTCTC</td>
</tr>
<tr>
<td>TYR-EXON5-R</td>
<td>CGAACCTGGACATTTACTTTGAGT</td>
</tr>
</tbody>
</table>

TYR, tyrosinase; F, forward; R, reverse.

Results

In the present study, 17 patients were diagnosed with non-syndromic OCA based on the routine screenings and complete ophthalmological examination. The parents of all patients were unaffected, which is consistent with the pattern of autosomal recessive inheritance. Using PCR combined with Sanger sequencing, a total of 12 mutations were found in TYR, including 10 previously reported mutations associated with OCA1 and two novel mutations (Fig. 1 and Table II). Patient 4000101 was a compound heterozygote for mutations c.1A>G (p.M1?) and c.896G>A (p.R299H) in the TYR gene. Patient 4000201 was a compound heterozygote for mutations c.1198T>G (p.W400G) and c.896G>A (p.R299H). Patient 4000301, from a consanguineous family, was a homozygote for mutation c.929_930insC (p.R311Kfs*7). Patient 4000701 was compound heterozygous for c.929_930insC (p.R311Kfs*7) and c.896G>A (p.R299H). Patient 4000801 was a homozygote for c.819G>T (p.Q273H). Patient 4000901 was a compound heterozygote for mutations c.758G>A (p.G253E) and c.896G>A (p.R299H). Patient 4001101 was a compound heterozygote for mutations c.231_232insGGG (p.R77_E78insG) and c.230G>A (p.R77Q), and c.230G>A was confirmed in the unaffected family member of patient 4001101 (Fig. 1). Patient 4001301 carried mutations c.346C>T (p.R116*) and c.832C>T (p.R278*), patient 4001501 carried mutations c.896G>A (p.R299H) and c.70T>C (p.C24R), and c.896G>A (p.R299H) and c.70T>C (p.C24R), and c.896G>A (p.R299H) and c.70T>C (p.C24R),
and patient 4001701 carried the mutation c.231-232insGGG (p.R77_E78insG) and the splicing mutation c.1037-7T>A.

Among the mutations identified, two mutations c.819G>T (p.Q273H) and c.1198T>G (p.W400G) were novel, and their...
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Pathogenicity were predicted via in silico analysis. The SIFT and PolyPhen-2 scores for the c.819G>T (p.Q273H) mutation were 0.030 and 0.981, respectively, which predicted that the mutation was ‘probably damaging’. The I-Mutant online server predicted that the mutation may decrease protein stability with a reliability index (RI) of 6. The c.1198T>G mutation in the TYR gene was not detected in the 200 normal controls enrolled in the present study, nor was it present within the dbSNP database, the 1000 Genomes Project or the albinism database (http://www.ifpcs.org/albinism/). This mutation, resulting in the conversion from tryptophan to glycine at codon 400, was predicted to be ‘probably damaging’ with a score of 0.99 by PolyPhen2 and a score of 0 by SIFT, and it was predicted that the protein stability may decrease with an RI of 9 by the I-Mutant online server. Multiple sequence alignment showed that residues W241, I237 and H245 in the TYR protein of Bacillus megaterium are highly conserved across species, and correspond to W400, I393 and H404 in the human TYR protein, respectively (Fig. 2A). The crystal structure of wild-type TYR (PDB ID: 5I3B) showed that W241 interacts with I237 and H245, and this interaction may be impaired when W241 is substituted by amino acid G (Fig. 2B).

Discussion

TYR is a glycoprotein containing four regions, a signal sequence (SP; amino acid residues 1-18), an intramelanosomal domain (IMD; residues 19-476) with a binuclear copper binding site, a single α-helical trans-membrane domain (STMD; residues 477-497), and a flexible C-terminal domain (CTD; C-terminal domain; TYR, tyrosinase).
mutations of TYR have been found to account for almost 62.5\% of all reported TYR mutations, whereas the insertion and deletion mutations account for 25\% (12).

Of the 12 mutations identified in the present study, 10 mutations were reported as causative for OCA in previous literature. Among these 10 mutations, R299H is the most frequent mutation in the present study (5/12). R299H is the most frequent mutation in Chinese, Caucasian, Korean, and Christian Arab populations, and this mutation may affect the function of TYR by disrupting copper binding and different substitutions occurring in R299 (5.13-15). c.231-232insGGG is another frequent mutation in the Chinese OCA1 population, resulting in an insertion of glycine residue between R7 and E7 (13). Mutation c.230G>A, resulting in an amino acid change from arginine to glutamine at position 77, has been reported in the Japanese, Korean, Chinese, German and Pakistan populations (16). The mutation c.929_930insC, resulting in a truncated polypeptide, is the most frequent mutation allele in far East Asian OCA1 populations (13,17). The missense mutation c.1A>G (p.M1?) has been found in British and Chinese patients with OCA1, and this may alter codons initiating translation to M31, resulting in a skipping of the putative SP (18,19). The splicing mutation IVS2-7T>A is frequently found in patients with OCA1 worldwide and can result in pathological splicing sites (19-22). The amino acid residue W400 of the TYR protein is highly conserved from bacteria to humans (Fig. 2A). Residues W241, I237 and H245 in the TYR protein of Bacillus megaterium corresponded with W400, I237 and H410 in the human TYR protein, respectively (Fig. 2A). Structural analysis of wild-type TYR from Bacillus megaterium (PDB: 5I3B) showed that W241 interacts with I237 and H245, and the predicted structure of the mutated protein shows that this substitution disrupted hydrogen bonds in these two amino acids, impairing the spatial conformation of TYR (Fig. 2B).

In conclusion, mutation analysis of the TYR gene in 17 patients with non-syndromic OCA revealed that 12 mutations of TYR may have caused OCA in 10 patients. Among these mutations, two novel mutations c.1198T>G (p.W400G) and c.819G>T (p.Q273H) were identified, expanding on the mutation spectrum of TYR in OCA. This may assist in the prenatal examination and genetic diagnosis of OCA, and offers novel insight into the molecular mechanism of OCA1.

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