Genetic analysis and prenatal diagnosis of 20 Chinese families with oculocutaneous albinism

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Abstract

Background: Oculocutaneous albinism (OCA) is a group of heterogeneous genetic disorders characterized by abnormal melanin synthesis in the hair, skin, and eyes. OCA exhibits obvious genetic and phenotypic heterogeneity. Molecular diagnosis of causal genes can be of help in the classification of OCA subtypes and the study of OCA pathogenesis.

Methods: In this study, Sanger sequencing and whole exome sequencing were used to genetically diagnose 20 nonconsanguineous Chinese OCA patients. In addition, prenatal diagnosis was provided to six OCA families.

Results: Variants of TYR, OCA2, and HPS1 were detected in 85%, 10%, and 5% of affected patients, respectively. A total of 21 distinct variants of these three genes were identified. Exons 1 and 2 were the hotspot regions of the TYR variants, and c.895C > A and c.896G > A were the hotspot variants. We also found seven novel variants: c.731G > A, c.741C > A, c.867C > A, and c.1037-2A > T in TYR, c.695dupT and c.1054A > G in OCA2, and c.9C > A in HPS1. Genetic tests on six fetuses revealed three carrier fetuses, two normal fetuses, and one affected fetus. The follow-up results after birth were consistent with the results of prenatal diagnosis (one fetus terminated during pregnancy was not followed up).

Conclusions: This study expands our understanding of the genotypic spectrum of the Chinese OCA population. The findings indicate that prenatal diagnosis can provide important information for genetic counseling.

Keywords

HPS1, OCA, prenatal diagnosis, TYR, whole exome sequencing

1 | INTRODUCTION

Albinism is a group of heterogeneous genetic disorders characterized by complete absence or reduction of melanin biosynthesis. Albinism can be categorized as either oculocutaneous albinism (OCA) or ocular albinism (OA). The main clinical features of OCA are hypopigmentation of the skin, hair, and eyes, whereas OA only affects the eyes. Ocular characteristics include reduced iris pigment, reduction
in visual acuity, nystagmus, strabismus, and photophobia. OCA is the most common form of albinism, affecting about 1 in 20 000 individuals worldwide.\(^2\) The prevalence is higher (about 1 in 18 000) in the Chinese population.\(^2\)

Based on the complexity of the system involved, OCA can be further classified as either nonsyndromic OCA or syndromic OCA. OCA1 (OCA1A, OMIM 203100; OCA1B, OMIM 606952), OCA2 (OMIM 203200), and OCA3 (OMIM 203290) are the most common subtypes of nonsyndromic OCA, caused by homozygous or compound heterozygous variants in the tyrosinase gene (TYR, OMIM 606933), OCA2 melanosomal transmembrane protein gene (OCA2, OMIM 611409), and tyrosinase-related protein 1 gene (TYRP1, OMIM 115501), respectively.\(^3,4\) In addition, four rare subtypes of syndromic OCA, such as Chediak-Higashi syndrome (CHS, OMIM 211800), Hermansky-Pudlak syndrome (HPS, OMIM 203200), Griscelli syndrome (GS, OMIM 214450), and Lasergene SeqMan software was used for DNA sequence assembly, and sequences were compared with a wild-type reference sequence.

2.2 | Sanger sequencing

The genomic DNA (gDNA) was extracted from amniocytes or peripheral blood using a Qiagen DNA Blood Midi/Mini kit (Qiagen), following the manufacturer’s protocol. Variant screening of the TYR gene in 12 families (family 1-10, 19, and 20) was performed with direct Sanger sequencing. Polymerase chain reaction (PCR) primers were designed by Primer Premier version 5.0 and contained the entire coding regions and the flanking introns of the TYR gene (Table S1). The 20 μL PCR reaction mixture contained 10-50 ng template DNA, 10 μL Premix EX Taq HS (Takara), and 1 μL of each primer. Touchdown PCR was performed as follows: 95°C for 15 minutes; 11 cycles of 95°C for 45 seconds, 60°C-0.5°C for 45 seconds, 72°C for 45 seconds; 24 cycles of 95°C for 45 s, 54°C for 45 seconds, 72°C for 45 seconds; and 72°C for 7 minutes. The PCR products were sequenced using an ABI 3130 automated DNA sequencer (Applied Biosystems). DNASTAR Lasergene SeqMan software was used for DNA sequence assembly, and sequences were compared with a wild-type reference sequence.

2.3 | Whole exome sequencing

WES was performed in the other 10 families (family 11-20); this included two families (family 19 and 20) in which direct Sanger sequencing failed to identify any pathogenic variants. Exon-containing fragments were enriched by SureSelect Human All Exon V6 (Agilent, USA), and a HiSeq2500 sequencer was used for sample sequencing (Illumina). Paired-end sequencing was performed for each sample, and more than 95% of bases in the targeted regions were covered by at least 20 reads. Raw data generated by WES were filtered to obtain high-quality clean reads and were further aligned to the NCBI Human Reference Genome (hg19/GRCh37) using the Burrows-Wheeler Aligner (BWA). Then, SAMtools and Genome Analysis ToolKit (GATK) were used to detect single nucleotide polymorphisms or sequence variants in BAM files. Variants located in coding regions or exon-intron junctions of 33 candidate genes (Table S2) responsible for albinism or diseases whose phenotypes partially overlap with albinism were analyzed emphatically.

2.4 | Variant analysis

Data from WES or Sanger sequencing were analyzed in accordance with the following criteria. First, variants with a minor allele frequency > 0.01 in Single Nucleotide Polymorphism database (dbSNP, http://www.ncbi.nlm.nih.gov/snp), 1000 Genomes Project (http://browser.1000genomes.org), genome Aggregation Database (gnomAD, http://gnomad.broadinstitute.org/), Trans-Omics for Precision Medicine Program (TOPMed, https://www.nhlbi.nih.gov/science/trans
| Patient | Sex | Age (y) | Clinical features | Genotype |
|---------|-----|---------|-------------------|-----------|
|         |     |         | Skin color | Hair color | Iris color | Photophobia | Nystagmus | Myopia | Gene | Allele 1 | FM | Allele 2 | FM |
| 1       | M   | 12      | White      | White      | Red brown  | Positive    | Positive   | Severe  | TYR   | c.895C > A (p.R299S) | Pa | c.896G > A (p.R299H) | Ma |
| 2       | M   | 16      | White      | White      | Brown     | Negative    | Negative   | Mild    | TYR   | c.895C > A (p.R299S) | Pa | c.229C > T (p.R77W) | Ma |
| 3       | F   | 9       | White      | White      | Brown     | Positive    | Positive   | Moderate | TYR   | c.867C > A (p.C289Y) | Pa | c.929dupC (p.R311Kfs*7) | Ma |
| 4       | M   | 25      | White      | White      | Brown     | Positive    | Positive   | Moderate | TYR   | c.895C > A (p.R299S) | Pa | c.230_232dupGGG (p.E78_S79insG) | Ma |
| 5       | F   | 10      | White      | White      | Brown     | Positive    | Positive   | Severe  | TYR   | c.832C > T (p.R278*) | Pa | c.896G > A (p.R299H) | Ma |
| 6       | M   | 13      | White      | White      | Red brown  | Positive    | Positive   | Severe  | TYR   | c.731G > A (p.C244Y) | Pa | c.832C > T (p.R278*) | Ma |
| 7       | M   | 13      | White      | White      | Light yellow | Positive    | Positive   | Severe  | TYR   | c.929dupC (p.R311Kfs*7) | Pa | c.895C > A (p.R299S) | Ma |
| 8       | M   | 46      | White      | White      | Brown     | Positive    | Positive   | Severe  | TYR   | c.731G > A (p.C244Y) | NA | c.895C > A (p.R299S) | Daughter |
| 9       | M   | 30      | White      | White      | Red brown  | Positive    | Positive   | Mild    | TYR   | c.1037-2A > T | Sister, affecting brother | c.346C > T (p.R116*) | Affecting brother |
| 10      | M   | <1      | White      | Light yellow | Gray   | NA         | NA         | NA      | TYR   | c.230_232dupGGG (p.E78_S79insG) | Pa | c.895C > A (p.R299S) | Ma |
| 11      | F   | 70      | White      | White      | Red brown  | Positive    | Positive   | Severe  | TYR   | c.164G > A (p.C55Y) | NA | c.164G > A (p.C55Y) | NA |
| 12      | M   | 9       | White      | White      | Red brown  | Positive    | Positive   | Severe  | TYR   | c.1199G > T (p.W400L) | Pa | c.1199G > T (p.W400L) | Ma |
| 13      | F   | 30      | Pinkish White | Brown | Light brown | Negative    | Negative   | Moderate | TYR   | c.325G > A (p.G109R) | NA | c.741C > A (p.C247Y) | NA |
| 14      | F   | 33      | White      | White      | Red brown  | Positive    | Positive   | Severe  | TYR   | c.896G > A (p.R299H) | Pa | c.896G > A (p.R299H) | Ma |
| 15      | M   | 38      | White      | White      | Brown     | Positive    | Negative   | Moderate | TYR   | c.896G > A (p.R299H) | NA | c.896G > A (p.R299H) | NA |
| 16      | M   | 32      | White      | White      | Brown     | Positive    | Positive   | Mild    | TYR   | c.164G > A (p.C55Y) | NA | c.706T > C (p.W236R) | NA |
| 17      | M   | 26      | Pinkish White | White | Brown     | Negative    | Positive   | Mild    | TYR   | c.1204C > T (p.R402*) | NA | c.929dupC (p.R311Kfs*7) | NA |

(Continues)
-omics-precision-medicine-topmed-program), and Exome Aggregation Consortium database (ExAC, http://exac.broadinstitute.org/) were filtered out. Second, databases such as the Albinism Database (http://www.ifpcs.org/albinism), ClinVar (http://www.ncbi.nlm.nih.gov/clinvar), and the Human Gene Mutation Database (HGMD, http://www.hgmd.org) were used to determine the pathogenicity of variants. Third, all whole genome variants were subjected to biological effects analysis, which included the use of programs such as MutationTaster (http://www.mutationtaster.org), Polymorphism Phenotyping v2 (PolyPhen2, http://genetics.bwh.harvard.edu/pph2), Protein Variation Effect Analyzer (PROVEAN, http://provean.jcvi.org/index.php), Sorting Intolerant From Tolerant (SIFT, http://sift.jcvi.org), and Human Splicing Finder (HSF, http://www.umd.be/HSF) to predict whether an amino acid substitution or indel was more likely to be pathogenic. Finally, all detected variants were classified as pathogenic, likely pathogenic, uncertain significance, likely benign, or benign, according to the American College of Medical Genetics and Genomics (ACMG) guidelines.

2.5 Pedigree analysis and prenatal testing

All detected (likely) pathogenic variants were subsequently tested on parents or other available family members. The relevant sequence variants identified by WES were verified by Sanger sequencing, as described above. Six of the 20 families underwent amniocentesis between 18- and 24-week gestation for prenatal diagnosis. Fetal gDNA was extracted using a QIAamp DNA Mini Kit (Qiagen), following the manufacturer’s protocol; DNA was genotyped by Sanger sequencing. Monitoring for maternal contamination was performed with the analysis of 16 short tandem repeat (STR) loci in the fetal versus maternal gDNA using an STR Identifiler PCR Amplification Kit (Genesky Biotechnologies Inc).

3 RESULTS

3.1 Clinical phenotype

In this study, all patients had typical OCA symptoms of the skin, hair, and eyes. Clinical features of these 20 patients are described in Table 1. Among these patients, there were several special cases. Specifically, patient 10 died 15 days after birth due to congenital diaphragmatic hernia. In addition, patients 9, 19, and 20 had pigmented nevi on their bodies.

3.2 Identification of sequence variants

In the current study, (likely) pathogenic variants in the TYR (GenBank: NM_000372.5), OCA2 (GenBank: NM_000275.3), and HPS1 (GenBank: NM_000195.5) genes were identified in 85% (17/20), 10% (2/20), and 5% (1/20) of OCA patients, respectively. All identified variants are shown in Table 1 and Table 2.
Among the 17 patients affected by TYR variants, four were homozygous (patient 11, 12, 14, and 15) and the other 13 were compound heterozygous. Gene analysis revealed 16 unique TYR alleles, including eight missense types, five nonsense types, one frameshift type, one in-frame type, and one splice site variant; most were located in exon 1 or 2 (13/16). c.895C > A and c.896G > A were the highest frequency variations identified, followed by c.164G > A and c.929dupC; these four variants accounted for 52.9% (18/34) of the total TYR variants. In addition, four novel variants were detected: c.731G > A, c.741C > A, c.867C > A, and c.1037-2A > T.

Table 2: Variants detected in this study

| Gene | Exon | Variant | Type | Frequency |
|------|------|---------|------|-----------|
| TYR  | EX1  | c.164G > A (p.C55Y) | Missense | 3/34 |
|      | EX1  | c.229C > T (p.R777W) | Missense | 1/34 |
|      | EX1  | c.230_232dupGGG (p.E78_579insG) | In-frame | 2/34 |
|      | EX1  | c.325G > A (p.G109R) | Missense | 1/34 |
|      | EX1  | c.346C > T (p.R116*) | Nonsense | 1/34 |
|      | EX1  | c.706T > C (p.W236R) | Missense | 1/34 |
|      | EX1  | c.731G > A (p.C244Y) | Missense | 2/34 |
|      | EX1  | c.741C > A (p.C247*) | Nonsense | 1/34 |
|      | EX2  | c.832C > T (p.R278*) | Nonsense | 1/34 |
|      | EX2  | c.867C > A (p.C289*) | Nonsense | 1/34 |
|      | EX2  | c.895C > A (p.R299S) | Missense | 6/34 |
|      | EX2  | c.896G > A (p.R299H) | Missense | 6/34 |
|      | EX2  | c.929dupC (p.R311Kfs*7) | Frameshift | 3/34 |
|      | IVS2 | c.1037-2A > T | Splicing | 1/34 |
|      | EX4  | c.1199G > T (p.W400L) | Missense | 2/34 |
|      | EX4  | c.1204C > T (p.R402*) | Nonsense | 1/34 |
| OCA2 | EX7  | c.695dupT (p.A233Gfs*26) | Frameshift | 1/4 |
|      | EX10 | c.1054A > G (p.R352G) | Missense | 1/4 |
|      | EX13 | c.1255C > T (p.R419W) | Missense | 1/4 |
|      | EX14 | c.1426A > G (p.N476D) | Missense | 1/4 |
| HPS1 | EX3  | c.9C > A (p.C3*) | Nonsense | 2/2 |

Two patients were found to carry four distinct OCA2 alleles. Patient 18 was compound heterozygous for c.695dupT (p.A233Gfs*26), a novel frameshift insertion, and c.1255C > T (p.R419W). We also found that c.695dupT was a maternal variant, and c.1255C > T was a paternal variant. Patient 19 was compound heterozygous for the novel missense variant c.1054A > G (p.R352G) and c.1426A > G (p.N476D).

Patient 20 was homozygous for the c.9C > A (p.C3*) variant in HPS1. This novel nonsense variant was located in exon 1 and predicted to result in the shortest truncated protein, only containing three residues.

3.3 | Pathogenicity analysis of novel variants

Seven novel variants were identified in this study, including c.731G > A, c.741C > A, c.867C > A, and c.1037-2A > T of the TYR gene, c.695dupT and c.1054A > G of the OCA2 gene, and c.9C > A of the HPS1 gene (Figure 1 and Table 3). None of these were present in the ClinVar, HGMD, or Albinism databases. Almost all of these seven variants were absent from the 1000 Genomes Project, gnomAD database, TOPMed, and ExAC, except for c.731G > A and c.741C > A, which were present at extremely low frequencies in TOPMed and gnomAD, respectively. When the in silico prediction tool was applied to the variant types, all results revealed the potential harmfulness of these variants with respect to function. Based on these results and with consideration for changes in amino acids, variant frequency, segregation analysis, and the occurrence of variants in trans, four variants (c.741C > A, c.867C > A, and c.1037-2A > T of TYR, and c.695dupT of OCA2) were classified as pathogenic. The remaining variants (c.731G > A of TYR, c.1054A > G of OCA2, and c.9C > A of HPS1) were classified as likely pathogenic.

3.4 | Prenatal genetic diagnosis

Among the 20 families, six pregnant women (family 2, 3, 5, 7, 17, and 18) underwent amniocentesis for prenatal diagnosis. Genetic tests on six fetuses revealed three carrier fetuses, two normal fetuses, and one affected fetus. The fetus of family 5 carried compound heterozygous alleles. Patient 18 was compound heterozygous for c.695dupT (p.A233Gfs*26), a novel frameshift insertion, and c.1255C > T (p.R419W). We also found that c.695dupT was a maternal variant, and c.1255C > T was a paternal variant. Patient 19 was compound heterozygous for the novel missense variant c.1054A > G (p.R352G) and c.1426A > G (p.N476D).

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appearance at birth; thus, the follow-up results were consistent with the results of prenatal diagnosis.

4 | DISCUSSION

This study successfully identified the genotypes of 20 OCA patients by Sanger sequencing and WES. It should be noted that Sanger sequencing performed on family 19 and family 20 failed to identify any pathogenic variations due to the atypical phenotypes, while subsequent WES revealed pathogenic variants in OCA2 in family 19 and HPS1 in family 20. This indicates that new techniques such as WES can help to identify causal variants in rare or atypical subtypes of OCA, which are likely to be missed by Sanger sequencing.

The distribution of OCA genes and the variant alleles of these genes vary according to ethnicity. TYR is the most common OCA gene throughout the world. Sequence variants in the OCA2 gene have been described in all major ethnic groups and are most common in African and African American populations.10 In contrast, TYRP1 variants and HPS1 variants are prevalent in Southern Africa and Northwestern Puerto Rico, respectively.11,12 In this study of Chinese OCA patients, variants in TYR, OCA2, and HPS1 were found in 85%, 10%, and 5%, of patients, respectively, which is similar to the report of Wei et al.13 In contrast, SLC45A2 variants were totally absent here but have been reported to account for 10%-20% of Chinese OCA cases.13,14 This highlights the genetic heterogeneity of OCA even in patients from the same ethnic group. In addition, differences in the included number of cases may be another explanation.

Sixteen distinct variants in the TYR gene were identified in this study. Aside from one splice site variant (c.1037-2A > T) in the junction region of exon 3 and intron 2, all other variants were located in exon 1, 2, and 4. Among these, variants in exon 1 and 2 collectively accounted for 81% (13/16), highlighting the hotspot regions of TYR variants. This finding is consistent with a previous study.13 Of the 34 TYR gene variant alleles, c.895C > A (p.R299S) and c.896G > A (p.R299H), both of which changed codon 299 in the CuA region, were the most common variations in our study, with each identified in six probands (17.6%, 6/34). The latter is a known hotspot variant and the former is common in Chinese OCA patients as well.13,15

More than 400 pathogenic variants in TYR have been identified to date, and here, we identified four additional novel variants in five families. The c.731G > A variant was detected in two unrelated patients (patient 6 and 8) diagnosed as OCA type 1. This variant caused cysteine to be substituted by tyrosine at codon 244, showing a high level of sequence conservation; thus, the p.C244Y variant may have deleterious effects on tyrosinase. c.741C > A (p.C247*) in patient 13

FIGURE 1 Sanger sequencing result of seven novel variants. A, Heterozygous for c.731G > A in TYR; B) heterozygous for c.741C > A in TYR; C) heterozygous for c.867C > A in TYR; D) heterozygous for c.1037-2A > T in TYR; E) heterozygous for c.695dupT in OCA2; F) heterozygous for c.1054A > G in OCA2; G) homozygous for c.9C > A in HPS1
Table 3. Pathogenicity analysis of novel variants

| Gene     | Type        | Variant          | Minor allele frequency (gnomAD/ExAC/1000G) | Pathogenicity prediction (SIFT/PolyPhen-2/Provean/MutationTaster/HSF) | Evidence criterion | Classification       |
|----------|-------------|------------------|------------------------------------------|---------------------------------------------------------------------|-------------------|----------------------|
|          | OCA2        | c.695dupT        | —/—/—                                  | Damage/Probably Damaging/Deleterious/disease causing/missing        | —                 | Likely pathogenic    |
|          |             | (p.A233Gfs*26)   |                                         | —/—/—/—                                                              |                   |                      |
|          | HPS1        | c.9C > A         | p.C3                                  | Damage/Probably Damaging/Deleterious/disease causing/missing        | —                 | Likely pathogenic    |
|          |             | (p.C3*)         |                                         | —/—/—/—                                                              |                   |                      |
| OCA2     |             | c.1054A > G      | p.R352G                                | Damage/Probably Damaging/Deleterious/disease causing/missing        | —                 | Likely pathogenic    |
|          |             | (p.R352G)       |                                         | —/—/—/—                                                              |                   |                      |
|          |             | c.346C > T       | p.R116*                                | Damage/Probably Damaging/Deleterious/disease causing/missing        | —                 | Likely pathogenic    |
|          |             | (p.R116*)       |                                         | —/—/—/—                                                              |                   |                      |

and c.867C > A (p.C289*) in patient 3 resulted in a truncated protein and led to a loss of tyrosinase activity. These two nonsense variants are reported here for the first time, while missense variants on the same amino acid, p.C247R, p.C289R, p.C289G, and p.C289Y, have been previously reported.16-19 Patient 9 was found to have compound heterozygosity of the novel c.1037-2A > T and the known pathogenic variant c.346C > T (p.R116*). Through segregation analysis, these two variants were also identified in his affected brother who exhibited a similar phenotype, while their normal sister carried the single variant c.1037-2A > T. The splicing variant c.1037-2A > T was predicted to most likely affect the splicing pattern of exons.

OCA2 (previously called P) is the only causal gene implicated in OCA type 2; about 300 variants implicated in OCA2 have been reported to date. In this study, patient 18 and 19 were both compound heterozygotes, with each possessing a known variant and a novel variant, c.695dupT (p.A233Gfs*26) and c.1054A > G (p.R352G), respectively. The frameshift c.695dupT introduces a 1-bp duplication in exon 7 of OCA2, resulting in a frameshift distal to codon 233 with the termination of the nonsense polypeptide at codon 258. Several nonsense variants downstream of codon 258 have been reported to be pathogenic,13,20, and c.695dupT (p.A233Gfs*26) is suggested to be a loss-of-function variant and might lead to inactivity of the P protein. The c.1054A > G (p.R352G) variant of OCA2 in patient 19 caused arginine to be substituted by glycine at codon 352 and was predicted to be deleterious by all in silico tools. Patient 19 also carried another known pathogenic variant, c.1426A > G (p.N476D).21 We further determined that the c.1054A > G variant was in trans (different copy of OCA2 gene) with the known variant by testing the next generation of the patient.

HPS is a rare genetic disorder characterized by oculocutaneous albinism, bleeding tendency and, in some cases, lysosomal storage disease.22,23 As of April 2019, 68 variants in HPS1 were listed in the HGMD. Here, we found patient 20 was homozygous for a novel nonsense variant c.9C > A (p.C3*) in the HPS1 gene. Compared with various HPS1 variants in the known databases, including the ClinVar, HGMD, and Albinism databases, this variant is predicted to cause the shortest truncated protein to lose most of the wild-type HPS1 sequence, keeping only three amino acid residues. The female patient with this novel variant showed obvious hypopigmentation and characteristic ophthalmological findings, but no hemorrhagic problems; all coagulation tests and routine blood tests were normal. Similar characteristics were reported in two HPS1 patients in the study by Luo et al24 and one female patient in the study by Wei et al25; these three patients were described as compound heterozygote for p.E63* and p.Q603*, compound heterozygote for c.399-14G > A and p.M325Hfs*128, and homozygous for p.D644Dfs*79, respectively. The phenotypes of these four patients were different from the majority of HPS individuals who experience reduced platelet aggregation abilities besides OCA.26 As Oh et al27 suggested, differentially truncated HPS1 polypeptides may have different consequences for subcellular function. The c.9C > A (p.C3*) variant, reflecting truncated alleles located near the N-terminus, might result in a serious OCA phenotype of HPS but no clear hemorrhagic
diathesis. Further studies on the function of this variant will reveal the underlying mechanism.

In summary, this study successfully detected causal variants in 20 OCA families. A total of 21 distinct variants of the TYR, OCA2, and HPS1 genes were identified by direct Sanger sequence and WES, of which, seven variants were novel. In addition, effective prenatal diagnosis and genetic counseling were provided to six OCA families to assist them to avoid the birth of affected children. This study expands our understanding of the genotypic spectrum of the Chinese OCA population and highlights that the development of new genetic testing technologies can contribute to more accurate and efficient clinical diagnoses.

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AUTHORS’ CONTRIBUTIONS
All authors contributed to the study conception and design. Chenyang Xu and YanBao Xiang designed the study, interpreted the clinical data, and wrote the article. Huanzheng Li, Yunzhi Xu, Xueqin Xu, and Yijian Mao collected samples, genotyped the cases, and finished the follow-up. Lili Zhou and Shaohua Tang performed genetic counseling. Chenyang Xu and Shaohua Tang helped in the statistical analysis. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT
The data that provided the evidence for the study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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