Original article

Genetic analyses of Chinese patients with digenic oculocutaneous albinism

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Oculocutaneous albinism (OCA) is a heterogeneous and autosomal recessive disorder in all populations worldwide. The mutational spectra of OCA are population-specific. Some OCA patients carry mutations from different OCA genes. In this study, we investigated the frequency of digenic mutations in Chinese OCA patients.

Methods Genomic DNAs were extracted from the blood samples of 184 clinically diagnosed OCA patients and 120 unaffected subjects. The amplified DNA segments of the exons and exon-intron boundaries were screened for mutations of TYR, OCA2, TYRP1, SLC45A2, and HPS1 by direct sequencing. To exclude the previously unidentified alleles from polymorphisms, samples from 120 unaffected controls were sequenced for the same regions of variations.

Results In all 184 patients, 134 had two pathologic mutations on one locus. Eleven cases had no apparent pathologic mutations in any of the genes studied. Among the remaining 39 patients who had only one pathologic mutation, five patients (2.7% in total) were found to carry the mutational alleles on a second locus in TYR, OCA2 or SLC45A2. Of the five digenic OCA patients, four patients were clinically diagnosed as OCA2 and one patient as OCA1. A previous unidentified allele p.G188D in SLC45A2 was identified, which was not present in the 120 unaffected controls.

Conclusions The identification of the digenic OCA patients suggests the synergistic roles among TYR, OCA2 and SLC45A2 during melanin biosynthesis, which may cause OCA under digenic mutations. This information will be useful for gene diagnosis and genetic counseling of OCA in China.

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During molecular screening of 184 OCA in the Chinese Albinism Registry by implementing the optimized strategy introduced by Wei et al., we did not find the second mutation in 39 patients after sequencing all the exons and the adjacent exon-intron boundaries of the genes containing the first mutational allele. The percentage of unidentified alleles is similar to that observed in other studies. Those “uncharacterized mutations” have been suggested to be located upstream of the gene within locus control-like regions or unsequenced intronic regions that result in functional changes of proteins, or, alternatively, the mutation may be present at a second locus (digenic scenario). The uncharacterized mutations from the missing gross deletions or insertions were possibly due to amplification of the existing wild-type allele in a regular genomic polymerase chain reaction (PCR). In our previous study, we had attempted to screen large deletional mutations on TYR or SLC45A2, but no deletions were found (data not shown). In this study, we attempted to screen the uncharacterized mutations on a second locus and we identified five patients as digenic OCA patients.

METHODS

Study subjects
One hundred and eighty-four unrelated OCA patients and 120 unaffected subjects from the Chinese Han population were recruited. The patients were from more than 20 provinces in China in the Chinese Albinism Registry. Only two OCA patients had a family history of consanguinity. Typically, among clinically diagnosed OCA1 patients, OCA1A presents with white hair that does not change with age and OCA1B is characterized by white hair at birth that later becomes light yellow or darker. OCA2 is characterized by yellow, brown or golden hair at birth with or without darkening of hair color at later age. However, the clinically diagnosed subtypes of OCA could be mixed forms of molecularly diagnosed OCA subtypes, such as clinical OCA1 could be molecularly identified as OCA1, OCA2, OCA4 or HPS1, while clinical OCA2 could be OCA1, OCA2, OCA4, or HPS1. The genotype-phenotype relationship of OCA calls for the extensive molecular screening of the known OCA genes.

DNA amplifications
Total genomic DNA was extracted from blood samples by the routine proteinase K/SDS method. The primer sequences were available upon request. The amplifications covered all the exons and exon-intron boundaries of the five OCA genes (TYR, OCA2, SLC45A2, TYRP1, and HPS1). Standard PCR amplification procedures were conducted. For most amplicons, DNA was denatured at 94°C for 4 minutes followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing from 57°C to 59°C for 30 seconds to 1 minute, followed by a final extension at 72°C for 10 minutes. All PCR product sizes were verified by 1.0%–1.2% agarose gel electrophoresis.

Optimized strategy for mutational screening
The optimized strategy for the DNA amplifications of the five OCA genes, TYR, OCA2, TYRP1, SLC45A2 and HPS1, was followed as described. For all the OCA patients, mutations on the TYR gene were first screened. When no mutation in the TYR gene was found, the SLC45A2 and OCA2 genes were screened sequentially for clinically diagnosed OCA1 patients, and OCA2 and SLC45A2 were screened sequentially for clinically diagnosed OCA2 patients. When no point mutation was found on TYR and SLC45A2, a set of combinational primers was applied to screen deletional mutations. When mutations on TYR, OCA2, and SLC45A2 were not found after the above efforts, TYRP1 and HPS1 were screened sequentially by direct sequencing. We sequenced all the 5 exons of the TYR gene, the 7 exons of the SLC45A2 gene, the 24 exons of the OCA2 (the first of which is noncoding), the 7 exons of the SLC45A2, the 7 exons of the TYRP1, the 20 exons of the HPS1 gene, and the adjacent intronic and flanking sequences in all patients. When blood samples were collected from a patient’s parents, paternal and maternal origins of the mutational alleles were verified to exclude de novo germline mutations and to identify biallelism or triallelism.

PCR product sequencing and identification of novel mutations
For direct sequencing, all purified PCR products were sequenced using the ABI PRISM 3700 automated sequencer (Applied Biosystems, USA). When a potential novel mutation was considered after careful check with the HGMD (http://www.hgmd.cf.ac.uk/ac/), HPDS (http://liweilab.genetics.ac.cn/HPDS/) and the SNP (http://www.ncbi.nlm.nih.gov/SNP/) databases, direct sequencing of the amplified PCR products from the same region of the 120 unaffected subjects was applied to exclude the possibility of polymorphism. For any possible base pair change in the sequencing results, the MutConv (http://liweilab.genetics.ac.cn/mutconv/) tool was used to check the possible changes at the protein level.

RESULTS

We studied 184 unrelated Chinese patients referred with
the clinical diagnosis of OCA. In all these patients, 134 (72.8%) had two pathologic mutations on one locus and were identified as definitive cases of OCA1, OCA2, OCA4 or HPS1. Eleven (6%) cases had no apparent pathologic mutations in any of the genes studied. The remaining 39 patients who had only one pathologic mutation were further examined for a second mutation on another OCA gene which is different from the first identified OCA gene.

In the 39 patients, five patients (2.7% (5/184) of the total) were found mutations on two loci and were identified as digenic OCA patients, including four patients clinically diagnosed as OCA2 (Patients 1–4) and one patient clinically diagnosed as OCA1 (Patient 5). We did not find the second mutation in the other 34 (18.5%) patients, after sequencing all exons and the adjacent exon-intron boundaries of the \( \text{TYR} \), \( \text{OCA2} \), \( \text{TYRP1} \), \( \text{SLC45A2} \) and \( \text{HPS1} \).

Patients 1, 2 and 4 were all digenic OCA1 and OCA4 although they carried different mutational alleles. Patient 1 carried the p.R77Q (c.230G>A) of \( \text{TYR} \) and p.G349R (c.1045G>A) of \( \text{SLC45A2} \); Patient 2 carried p.R278X (c.832C>T) of \( \text{TYR} \) and p.D160H (c.478G>C) of \( \text{SLC45A2} \); Patient 4 carried p.R402Q (c.1205G>A) of \( \text{TYR} \) and p.G188D (c.563G>A) of \( \text{SLC45A2} \). The p.G188D allele is a previously unreported allele which does not exist in the 120 unaffected controls. The normally pigmented mother of patient 4 had the same variation in the heterozygous state. Patient 3 was a digenic OCA2 and OCA4 who carried p.V833L (c.2497G>T) of \( \text{OCA2} \) and p.D160H (c.478G>C) of \( \text{SLC45A2} \). In addition, this patient was a homozygote of p.D160H. Patient 5 was a digenic OCA1 and OCA2 who carried p.R116X (c.346C>T) of \( \text{TYR} \) and p.A481T (c.1441G>A) of \( \text{OCA2} \) by manifesting atypical white skin color as OCA1A.

We screened all the five patients’ parents and verified the transmission of the alleles. The sequencing results are shown in Figure 1. Clinical phenotypes and mutational alleles of the five digenic OCA patients are shown in Table 1 and Figure 2. All the patients had typical OCA symptoms on the skin, hair and iris.

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mild variants of OCA1 and OCA2. On the other hand, digenic mutations on TYR and OCA2 were identified in two AROA patients. Patients with OCA1TS yielding only 25% of the tyrosinase catalytic activity of wild-type enzyme at 37°C and the enzyme activity increased when the temperature decreases. Patients with OCA1MP produce a small amount of pigment only expressed in the eyes, other phenotypic characteristics are similar with OCA1A. This subtype of OCA has been reported only in Caucasians. Other atypical OCA patients may manifest as white hair and blue iris with nearly normal skin color, or white skin and blue iris with dark hair color. Due to the residual tyrosinase in producing partial pigment, these cases are not diagnosed as typical OCA. To date, no extensive studies have been conducted on the genotype-phenotype relationship of the atypical or digenic Chinese OCA patients.

In this study, we have characterized five digenic Chinese OCA patients with different combinations of mutational alleles. It accounts for 2.7% of the Chinese OCA patients. The p.D160H allele in patient 2 and patient 3 is the most common allele of SLC45A2 in Chinese OCA4 patients. In OCA4, the p.D160H allele accounts for more than half of the mutational SLC45A2 alleles, suggesting a founder mutational allele in the Chinese Han population. The p.D160H heterozygotes or homozygotes were clinically diagnosed as either OCA1A or OCA2 on the basis of hair pigmentation in our patients. It will be interesting to know how this mutation disrupts the function of MATP.

The p.G188D allele of SLC45A2 is a previously unidentified allele which did not exist in 120 unrelated normally pigmented Chinese subjects. This mutation is located at the fifth transmembrane domain. The p.G188V at the same position was found to have some functional activity in melanogenesis in a Japanese patient. To date, 273 mutations of the SLC45A2 gene have been reported in different populations (http://liweilab.genetics.ac.cn/HPSD/), and most of them were missense mutations located within transmembrane domains, including G349R of patient 2 in this study. In contrast, most mutations of OCA2 cause the missense substitutions within the loops between the transmembrane domains of the P protein. Both the MATP and the P protein have 12 transmembrane domains which behave as integral membrane proteins of the melanosomes. The functional relevance of these mutations remains to be investigated.

There is some controversy concerning the status of the p.R402Q allele of TYR in patient 4. It is often regarded as a polymorphism (rs1126809) in Caucasians. But several patients with OCA1B have been found to carry two TYR variants, one is a known pathogenic mutation and the other is the p.R402Q variant. In vitro expression studies, the Q402 variant protein has been shown to have reduced enzyme activity at 37°C but has normal activity when the transfected cells are incubated at lower temperatures (31–32°C). It has also been reported to be associated with autosomal recessive ocular albinism (AROA). In this study, we did not find the A allele at nucleotide 1205 in the 120 unaffected controls. Likewise, this locus presents exclusively the G allele (allelic frequency =1.0) in the Asian and Sub-Saharan African populations in the HapMap project (http://www.ncbi.nlm.nih.gov/SNP/snpr_ref.cgi?rs=1126809). This renders the p.R402Q may contribute to the hypopigmentation in the Chinese OCA patients, which is similar to the p.S192Y allele of TYR gene.

The p.A481T allele of OCA2 in patient 5 is regarded as a pathologic mutation in the Caucasians and a common OCA2 allele in Japanese OCA patients. A transfection study reveals that the T481 allele has approximately 70% function of the A481 allele in melanogenesis, suggesting it is a relatively mild OCA2 allele contributing to the hypopigmentation in the OCA2 patients. However, Population study shows the T481 allele is relatively common in the northeastern Asian populations with low ultraviolet radiation. Likewise, the T481 allele has an allelic frequency of 0.068 in the Asian population in the HapMap project (http://www.ncbi.nlm.nih.gov/projects/SNP/snpr_ref.cgi?rs=74653339). We did not find the T481 allele in the 120 unaffected controls. Taken together, the T481 allele may also contribute to the hypopigmentation in the digenic OCA patients.

In summary, we have characterized five digenic Chinese OCA patients with different combinations of mutational alleles. It remains to be clarified how the pathological conditions occur in the state of digenic mutations of different OCA genes although several cases of digenic OCA have been reported. Similarly, in autosomal recessive Parkinson disease (PD) with digenic mutations on both DJ-1 and PINK1 gene, it has demonstrated that DJ-1 and PINK1 physically associate and collaborate to protect cells against stress via complex formation, contributing to the development of early-onset PD in the digenic mutational condition. It has shown the synergistic roles among TYR, OCA2, TYRP1 and SLC45A2 during melanin biosynthesis, which may explains the pathogenesis of OCA under digenic mutations. This information will be useful for gene diagnosis and genetic counseling of OCA in China. Functional studies for some interesting alleles are needed to elucidate the pathogenesis of OCA and the genotype-phenotype relationship.

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