Available online at www.sciencedirect.com

ScienceDirect

Journal of Genetics and Genomics 42 (2015) 279−286

JGG

ORIGINAL RESEARCH

Prenatal Genotyping of Four Common Oculocutaneous Albinism Genes in 51 Chinese Families

Ai-Hua Wei [a,b,*], Dong-Jie Zang [b], Zhao Zhang [b], Xiu-Min Yang [a], Wei Li [b,c,*]

[a] *Department of Dermatology, Beijing Tongren Hospital, Capital Medical University, Beijing 100730, China*

[b] *State Key Laboratory of Molecular Developmental Biology, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, China*

[c] *Center for Medical Genetics, Beijing Children's Hospital, Capital Medical University, Beijing 100045, China*

Received 11 March 2015; revised 17 May 2015; accepted 18 May 2015
Available online 29 May 2015

ABSTRACT

Oculocutaneous albinism (OCA) is an autosomal recessive disorder characterized by hypopigmentation in eyes, hair and skin, accompanied with vision loss. Currently, six genes have been identified as causative genes for non-syndromic OCA (OCA-1～4, 6, 7), and ten genes for syndromic OCA (HPS-1−9, CHS-1). Genetic counseling of 51 Chinese OCA families (39 OCA-1 with mutations in the *TYR* gene, 6 OCA-2 with mutations in the *OCA2* gene, 4 OCA-4 with mutations in the *SLC45A2* gene, 1 HPS-1 (Hermansky−Pudlak syndrome-1) with mutation in the *HPS1* gene, and 1 mixed OCA-1 and OCA-4) led us to perform the prenatal genetic testing of OCA using amniotic fluid cells through the implementation of our optimized strategy. In our cohort, eleven previously unidentified alleles (PUAs) (5 in *TYR*, 2 in *OCA2*, and 4 in *SLC45A2*) were found. Three missense PUAs (p.C112R, p.H363R and p.G379V of *TYR*) and one in-frame deletional PUA (p.S222del of *SLC24A5*) led to fetuses with OCA when co-inherited with other disease causative alleles. Three PUAs (p.P152H and p.W272X of *TYR*, p.A486T of *SLC24A5*) identified in the OCA probands did not co-transmit with known pathological alleles and thus gave rise to unaffected fetuses. Four PUAs (p.Q83X and p.A658T of *TYR*, p.G161R and p.G366R of *SLC24A5*) did not transmit to the unaffected fetuses. In addition, the *in vitro* transfection assays showed that the p.S192Y variant of *TYR* produced less pigment compared to the wild-type allele. A fetus with a digenic carrier of OCA-1 and OCA-4 was unaffected. In combination with functional assays, the family inheritance pattern is useful for the evaluation of pathogenicity of PUAs and genetic counseling of OCA.

KEYWORDS: Oculocutaneous albinism; Prenatal genetic testing; Hermansky−Pudlak syndrome; Genotype; Previously unidentified allele

INTRODUCTION

Oculocutaneous albinism (OCA) is a heterogeneous and autosomal recessive disorder with a worldwide prevalence of about 1:17,000 (Witkop, 1979), predicted about 1 in 65 as carriers of OCA in population. It manifests as a reduction or complete loss of melanin in the skin, hair and eyes, accompanied with visual impairment (Montoliu et al., 2014). Currently, OCA has been identified to be due to mutations in six non-syndromic OCA genes (*TYR, OCA2, TYRP1*, *SLC45A2, SLC24A5* and *C10orf11*) or ten syndromic OCA genes *(HPS1, AP3B1, HPS3, HPS4, HPS5, HPS6, DTNBP1, BLOC1S3, PLDN* and *LYST*) (Li et al., 2006; Montoliu et al., 2014). In Chinese population, it has revealed that OCA-1 is the predominant type and mutations in *OCA2, TYRP1*, *SLC45A2, SLC24A5* and *HPS1* have been reported (Wei et al., 2009, 2010, 2013b; Zhang et al., 2011). In a total of 179 OCA patients from the Chinese population, OCA-1, OCA-2, OCA-4, and HPS-1 (Hermansky−Pudlak syndrome-1) account for 64.3%, 11.7%, 15.6%, and 2.2% of morbidity, respectively (Wei et al., 2010, 2011). In addition, about 100 previously

* Corresponding authors. Tel/fax: +86 10 5826 8092 (A.-H. Wei); +86 10 6480 6568 (W. Li).
E-mail addresses: weiaihua3000@163.com (A.-H. Wei); wli@genetics.ac.cn (W. Li).

http://dx.doi.org/10.1016/j.jgg.2015.05.001

unidentified alleles (PUAs) of these OCA genes have been reported in the Chinese population (Li et al., 2006), suggesting the OCA mutational spectrum is population-specific. Based on the mutational spectrum of Chinese OCA, an optimized strategy of genotyping for OCA has been implemented in the genetic testing of Chinese OCA families (Wei et al., 2011). We here reported the application of this optimized strategy in genetic testing of 51 fetuses in Chinese OCA families.

RESULTS

Genotyping of 51 OCA families

In this study, we have recruited 51 OCA families with the first child as OCA probands. The parents came for genetic counseling as they concerned about having OCA for a new birth. We screened the mutations of common OCA genes in these families including *TYR, OCA2, SLC45A2* and *HPS1*. The genotypes of the probands and their parents are shown in Table 1. For the new pregnancies in these families, we performed direct sequencing of the PCR amplicons of the targeted mutational alleles by amplifying the genomic DNA from amniotic fluid cells. In our cohort, we identified 14 fetuses with two normal alleles (+/+), 9 with two pathological alleles (OCA/OCA), 23 carriers (+/OCA), and 5 with unidentified alleles (3, ?/OCA; 2, ?/+). For the pregnancy outcome, 11 OCA and 40 unaffected fetuses were verified by fetoscopy, abortion or child birth (Table 1). In the probands, we verified paternal or maternal origin of the mutational alleles. With the allelic information, we analyzed the transmission pattern in the fetuses, which will be used to evaluate the pathogenicity of newly identified alleles.

Pathogenicity of previously unidentified alleles (PUAs)

We used the following step-by-step evaluation when a potential novel mutational allele was considered. A sequence variant was evaluated by the MutConv tool (http://liweilab.genetics.ac.cn/mutconv/) to obtain the sequence change at the protein level (Wei et al., 2010). We first excluded the variant from known single nucleotide variant (SNV) with allelic frequency larger than 0.5% by using the SNP database (http://www.ncbi.nlm.nih.gov/SNP/), 1000 Genomes Project database, and HapMap database (Wei et al., 2013b). We then excluded the variant from known mutations of the OCA genes in the databases such as Hermansky−Pudlak syndrome database (HPSD, http://liweilab.genetics.ac.cn/HPSD/) (Li et al., 2006), human gene mutation database (HGMD, http://www.hgmd.cf.ac.uk/ac/), and literatures in PubMed (http://www.ncbi.nlm.nih.gov/pubmed/). Furthermore, direct sequencing of the amplified PCR products from the targeted region of 120 unaffected subjects was applied to exclude the possibility of polymorphism in Chinese population. To predict whether a protein variant is deleterious to the gene function, PROVEAN algorithm (http://provean.jcvi.org) is run. Finally, transfection assays are performed to evaluate pigment production when necessary.

After going through the above procedures, we have identified 11 PUAs, which are predicted as deleterious to protein function (Table 2). To further evaluate the pathogenicity of these PUAs, we analyzed the allele transmission pattern to the probands and fetuses. Three missense PUAs (p.C112R (proband ID T40), p.G379V (T149), and p.H363R (T181) of *TYR*) and one in-frame deletional PUA (p.S222del (X55) of *SLC24A5*) led to fetuses with OCA when co-inherited with other disease causative alleles, confirming the pathological effects of these PUAs. Two PUAs (p.W272X (T44) and p.P152H (T88) of *TYR*) did not co-transmit with known pathological alleles and thus gave birth to unaffected fetuses, suggesting their pathological effects on the probands. Likewise, four PUAs (p.Q83X (T58) and p.A658T (T150) of *TYR*, p.G366R (T115) and p.G161R (T30) of *SLC24A5*) did not transmit to the fetuses which were confirmed by unaffected newborns, suggesting their pathological effects on the probands.

During the mutational screen, five alleles in the probands were unidentified. In the T53 family, the proband inherited the paternal p.A486T allele (a PUA) of *SLC24A5* but the maternal allele was unknown. In the fetus, the paternal p.A486T was transmitted and it is uncertain whether the maternal pathological allele was transmitted or not. However, the fetus was shown unaffected after abortion, suggesting that the maternal allele is normal in this fetus. In the T150 family, the proband showed a hemizygous p.A658T which was inherited from the father but not shown in the mother, suggesting that the unknown maternal allele is a large deletion. However, the paternal p.A658T did not transmit to the unaffected baby although it was unknown whether the fetus carried the maternal deletional allele or not. A similar paternal large deletion was predicted in both the proband and fetus of the X55 family as they both showed hemizygous p.S222del inherited only from their mother. In the T115 family, the maternal allele was unidentified in the proband and it is uncertain whether this allele is transmitted to the fetus. However, the paternal p.G366R did not transmit to the unaffected fetus, further supporting that this allele is pathogenic in the proband. Finally, in the T161 family, the unknown paternal allele was likely transmitted to the fetus which was confirmed to be OCA by fetoscopy and abortion while the pathological p.N476D was co-transmitted.

Pigment production is reduced in cells transfected with the *TYR*-S192Y plasmid

In Chinese OCA-1 patients, we proposed that the p.S192Y allele of *TYR*, a well-known SNP in Caucasians (Hutton and Spritz, 2008a), may produce OCA when co-existing with another pathological allele (Wei et al., 2010, 2011) as shown in the X77 family (Table 1). To investigate whether the p.S192Y allele and other predicted deleterious alleles of *TYR* have any effects on pigment production, we adopted a transfection assay of tyrosinase expression to observe pigment production in the transfected cells. In the HEK293T cells transfected with GFP-*TYR* plasmids, the green signals from GFP are used as a tracer for expression. The dark color of cell