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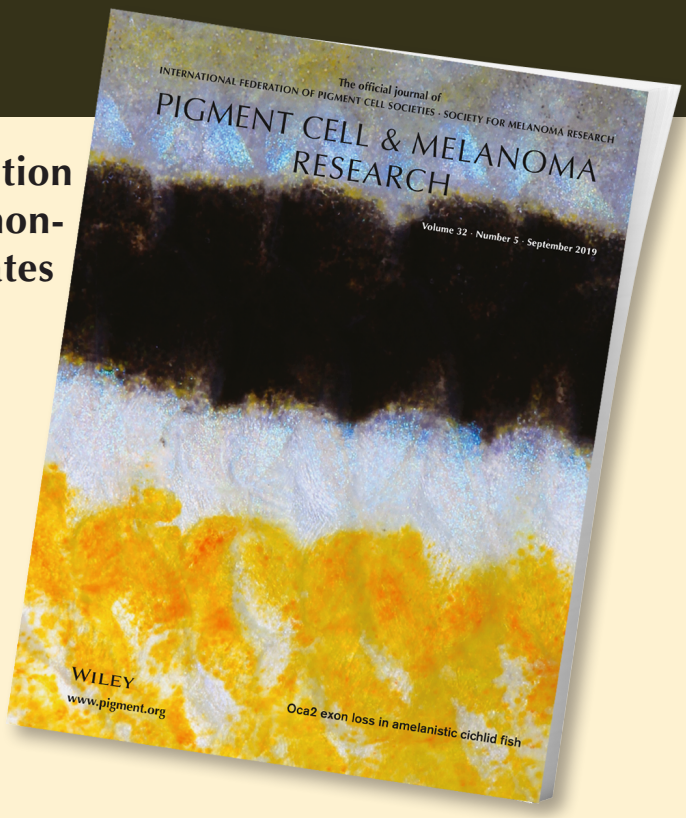

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ORIGINAL ARTICLE

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Comprehensive analysis of spectral distribution of a large cohort of Chinese patients with non-syndromic oculocutaneous albinism facilitates genetic diagnosis

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Abstract

Non-syndromic oculocutaneous albinism (nsOCA) is a group of genetically heterogeneous autosomal recessive disorders with complete lack or decrease pigmentation in skin, hair, and eyes. *TYR*, *OCA2*, *TYRP1*, *SLC45A2*, *SLC24A5*, and *LRMDA* were reported to cause OCA1-4 and OCA6-7, respectively. By sequencing all the known nsOCA genes in 114 unrelated Chinese nsOCA patients combined with In silico analyses, splicing assay, and classification of variants according to the standards and guidelines of American College of Medical Genetics and Genomics, we detected seventy-one different OCA-causing variants separately in *TYR*, *OCA2*, *SLC45A2*, and *SLC24A5*, including thirty-one novel variants (13 in *TYR*, 11 in *OCA2*, and 7 in *SLC45A2*). This study shows that OCA1 is the most common (75/114) and *OCA2* ranks the second most common (16/114) in Chinese. 99 patients of our cohort were caused by variants of all the known nsOCA genes. Cutaneous phenotypes of OCA1, OCA2, and OCA4 patients were shown in this study. The second OCA6 case in China was identified here. These data expand the spectrum of OCA variants as well phenotype and facilitate clinical implement of Chinese OCA patients.

KEYWORDS
genes, oculocutaneous albinism, phenotype, variants

1 | INTRODUCTION

Oculocutaneous albinism (OCA) is a group of autosomal recessive diseases with high heterogeneity, characterized with reduced or lost melanin in eyes, skin, and hair, often accompanied by photophobia, strabismus, poor visual acuity, and nystagmus, with an estimated worldwide prevalence of 1:17,000 (Gronskov, Ek, & Brondum-Nielsen, 2007; Hutton & Spritz, 2008b; Witkop, 1979), 1:18,000 in Chinese Han population and 3.80% are carriers based on the survey in Shandong Province (Gong, Shao, Zheng, Chen, & Guo, 1994). OCA presents either isolated or in syndromic forms in clinic (Tomita & Suzuki, 2004). Six genes (*TYR*/OCA1, *OCA2*/OCA2, *TYRP1*/OCA3, *SLC45A2*/OCA4, *SLC24A5*/OCA6, and *LRMDA*/OCA7) were identified to be associated with non-syndromic OCA (nsOCA; Boissy et al., 1996; Durham-Pierre et al., 1994; Gronskov et al., 2013; Morice-Picard et al., 2014; Newton et al., 2001; Tomita, Takeda, Okinaga, Tagami, & Shibahara, 1989; Wei et al., 2013), and a locus OCA5 is mapped to chromosome 4q24 in a consanguineous Pakistani family whose causative gene is not yet known (Kausar, Bhatti, Ali, Shaikh, & Ahmed, 2013). So far, over 125 genes were found involved in

pigmentation regulation, at least 25 of which affecting the production, metabolism, distribution, or function of melanin, including the melanosomal proteins like RAB7, RAB38, TYRP2, and SILV, have been considered as potential OCA candidate genes (Montoliu et al., 2014; Sitaram & Marks, 2012), and novel OCA genes are going to be uncovered in the near future with the advancement of high-throughput sequencing.

Among six known nsOCA genes, both *TYR* and *TYRP1* encode key melanogenic enzymes, whose defect caused OCA1 and OCA3 (MIM 203290), respectively. According to phenotype, OCA1 can be classified as two subtypes of OCA1A (MIM 203100) or less severe OCA1B (MIM 606952; Ainger, Jagirdar, Lee, Soyer, & Sturm, 2017; Dolinska et al., 2017; Tomita et al., 1989). OCA3 is reported to be rare in Chinese. Proteins encoded by *OCA2*, *SLC45A2*, and *SLC24A5* are ion transporters on melanosomal membranes to maintain melanosomes homeostasis, in which deleterious variants correspondingly lead to OCA2 (MIM 203200; Durham-Pierre et al., 1994; Park et al., 2015), OCA4 (MIM 606574; Newton et al., 2001), and OCA6 (MIM 113750; Morice-Picard et al., 2014; Wei et al., 2013). *LRMDA* is involved in melanocyte differentiation, of which the defect can cause OCA7 (MIM 615179; Gronskov et al., 2013). Less than 10 OCA6 cases have been reported worldwide (Bertolotti et al., 2016; Morice-Picard et al., 2014; Veniani et al., 2016; Wei et al., 2013) and OCA7 was currently reported only in a Lithuanian family and individuals from Faroe Islands, suggesting both types are rare (Gronskov et al., 2013). OCA clinical traits differ among patients with variants in different genes or the different variants in the same genes, while OCA patients with different variants can also have some overlap or similar phenotype. The molecular classification is more accurate in nsOCA subtype (Gronskov et al., 2007).

The prevalence of nsOCA subtypes varies with different populations. OCA1 has been previously reported to be the most common in Asian (Suzuki & Tomita, 2008; Wei et al., 2010), Dane (Gronskov et al., 2009), non-Hispanic Caucasians (Hutton & Spritz, 2008a), and a mixed population composed of Africans, Asians, and Europeans (Rooryck et al., 2008), and is very uncommon in African-Americans (Gronskov et al., 2007), while OCA2 is the most frequent in nsOCA patients of African ethnic origin (King, Hearing, Creel, & Oetting, 2001). The prevalence of other OCA subtypes differs in different populations (Gronskov et al., 2009; Hutton & Spritz, 2008a; Suzuki & Tomita, 2008; Wei et al., 2010).

In this study, 114 nsOCA patients are recruited from 18 provinces of China and comprehensive molecular analysis was conducted to reveal spectral distribution of Chinese nsOCA in all known OCA causative genes.

Significance

With comprehensive analysis of all the known nsOCA genes in 114 unrelated Chinese nsOCA patients, we identified thirty-one novel OCA-causing variant and reported the prevalence of different types of OCA in Chinese population: OCA1 (65.79%, 75/114) as the most common type, 16 OCA2 (14.03%, 16/114) as the second most common, 7 OCA4 (6.14%, 7/114), 1 OCA6 (0.88%, 1/114), and 15 OCA with unknown or unclassified variants. In this study, the second Chinese OCA6 case was identified and cutaneous phenotype of OCA1, OCA2, and OCA4 patients was present, which are helpful to facilitate clinic implement.

2 | MATERIALS AND METHODS

2.1 | Study subjects

In this study, a total of 114 unrelated subjects diagnosed as nsOCA by dermatologic specialists and ophthalmological specialists were enrolled from the 18 provinces of China. Most patients were born after 2010. White skin, white to light blond hair, pink or blue to gray irises, and mild to severe nystagmus were observed in all 114 OCA patients. Unrelated Patient 4002701, 4008301, 4008401, and 4008601 have consanguineous parents. Another cohort ascertained in this study as normal control comprises of 100 ethnically matched unrelated individuals. Detailed ocular and skin examinations for OCA diagnosis and routine physical examinations to exclude anomaly in other organ were carried out for clinical data of these participants in this study. This study adhered to the tenets of the Declaration of Helsinki and was approved by the institutional review board (IRB) of Medicine School of Tongji University in Shanghai, China (registration number: 2013YXY12). Written informed consent was obtained, and approximately 5 ml blood sample was voluntarily provided from all participating members.

2.2 | PCR, sequencing, and in silico analysis

DNA extraction kits from Tiangen Biotech Company were used to extract total genomic DNA. Touchdown PCR amplification procedures were used with an annealing temperature of 60–57°C for all primers. Primers for screening all known nsOCA genes *TYR*, *OCA2*, *TYRP1*, *SLC45A2*, *SLC24A5*, and *LRMDA* and for constructing wild-type plasmid as well as mutant plasmid are available on request (Table S1). PCR amplified all the exons and their flanking regions, purified, and analyzed using the ABI 3730 automated sequencer (Applied Biosystems). The allele frequency data of identified variants were checked in NHLBI ExAC database and GnomAD database. The following is the access number of OCA genes—TYR (ENSP00000263321), OCA2 (ENSP00000346659), SLC45A2 (ENSP00000296589), and SLC24A5 (ENSP00000341550). Three in silico methods SIFT, PolyPhen-2, and MutationTaster were used to evaluate the pathogenicity of variants in protein level.

2.3 | In vitro splicing assay

To evaluate the impact on mRNA splicing of the variants, in vitro splicing assay was performed based on the comparative assay about the splicing pattern of genomic fragment of wild-type (WT) and mutant

(MUT), respectively, constructed into minigene plasmid pCAS2 (a kind gift from Prof. A. Martins, University of Rouen in France; Soukarieh et al., 2016). For each variant, wild-type exons were amplified from human genomic DNA together with about 150bp of flanking sequences and the fragments were inserted into the MluI and BamHI cutting sites of pCAS2. MUT minigene vectors were prepared by site-directed mutagenesis with overlap PCR method and vectors pCAS2-WT *OCA2* or *SLC45A2* as template, respectively (Table S1). Sequencing the inserts of constructs was to verify sequence accuracy. 1 μg WT or MUT plasmids were parallel and transiently transfected into cell lines at a density of about 3×10^5 per well, respectively. Total RNAs were isolated 24 hr after transfection with TRIzol reagent according to the manufacturer's instructions. Then, minigene transcripts were analyzed by reverse transcription PCR (RT-PCR) with a pair of primers (Table S1). PCR products with different sizes were separated on a 2% agarose gel by electrophoresis. Three independent experiments were carried out. In vitro splicing assay was performed in HeLa cells and ARPE-19. The detailed procedure of in vitro splicing assay was performed according to the description in Soukarieh et al. (2016).

3 | RESULTS

3.1 | Classification of variants

In our study, seventy-seven different variant alleles were identified separately in *TYR*, *OCA2*, *SLC45A2*, and *SLC24A5* genes in 107 nsOCA patients (Table 1) after comprehensive analysis of all known nsOCA genes (*TYR*, *OCA2*, *TYRP1*, *SLC45A2*/OCA4, *SLC24A5*, and *LRMDA*) in total 114 Chinese nsOCA patients, including forty-three variant alleles reported previously to be associated with nsOCA (Dolinska et al., 2017; Fokkema et al., 2011; Lasseaux et al., 2018; Wei et al., 2010, 2013) and thirty-four novel alleles. All the novel variant alleles were not found in any of our 100 Chinese normal controls. The novel 34 different variants in this study include eleven missense (*TYR*_c.636A>T, *TYR*_c.937C>A, *TYR*_c.1169A>G, *TYR*_c.1234C>A, *TYR*_c.1325C>A, *OCA2*_c.849C>A, *OCA2*_c.1342C>T, *OCA2*_c.1504G>A, *OCA2*_c.2030T>G, *OCA2*_c.2244G>A, and *SLC45A2*_c.133A>G), nine nonsense (*TYR*_c.21C>A, *TYR*_c.24C>A, *TYR*_c.324G>A, *TYR*_c.653G>A, *TYR*_c.944C>G, *OCA2*_c.247C>T, *OCA2*_c.2195C>G, *SLC45A2*_c.529G>T, and *SLC45A2*_c.844G>T), ten indels (insertions or deletions) (*TYR*_c.456delC, *TYR*_c.561_562insCATTATTATGTGTCAAATTATCCCC, *TYR*_c.572dupG, *OCA2*_c.1010dupT, *OCA2*_c.2165delT, *OCA2*_c.2204_2205insCGGT, *OCA2*_c.2373_2375delCGT, *SLC45A2*_c.152_153delTG, *SLC45A2*_c.869dupA, and *SLC45A2*_c.1273delC), and four in splicing site (*OCA2*_c.646+3A>G, *OCA2*_c.2140-2A>G, *OCA2*_c.2245-11T>G, and *SLC45A2*_c.1032+1G>T). In addition, two known variants identified in this study, *OCA2*_c.808-3C>G and *SLC24A5*_c.1361dupT, are firstly reported to be homozygous (Figure 1). The frequency of novel variants all is 0 in Exome Aggregation Consortium (ExAC) except for *OCA2*_c.849C>A (p.Ser283Arg; its frequency 0.000008238) and *SLC45A2*_c.1273delC (its frequency 0.00003295). Among the novel variants, ten are nonsense variants which could result in truncated, dysfunctional proteins and could be classified as pathogenic variants according to the standards and guidelines of American College of Medical Genetics and Genomics (ACMG; Richards et al., 2015). Among ten novel indels, nine frameshift indels can be classified as pathogenic variants and a variant *OCA2*_c.2373_2375delCGT (p.Val792del) can only be classified as a variant with uncertain significance (VUS). Eight variants are in or flank splicing site, including three reported previously to be related to OCA (Marti et al., 2018; Rimoldi et al., 2014), and five novel variants (*OCA2*_c.646+3A>G, *OCA2*_c.2140-2A>G, *OCA2*_c.2245-11T>G, *OCA2*_c.808-3C>G, and *SLC45A2*_c.1032+1G>T), four (*OCA2*_c.646+3A>G, *OCA2*_c.2140-2A>G, *OCA2*_c.808-3C>G, and *SLC45A2*_c.1032+1G>T) of which in vitro splicing assay compared with WT in HeLa and ARPE-19 cell lines, demonstrated that brought about change in splicing (Figure 2) and no change was observed between WT and MUT for analysis of variant *OCA2*_c.2245-11T>G (Data not shown). Therefore, seven splicing can be classified as pathogenic variants and *OCA2*_c.2245-11T>G can only be classified as a VUS at the current stage. Of eleven novel missense variants, ten were predicated to be pathogenic with three in silico methods while *OCA2*_c.849C>A (p.Ser283Arg) (its frequency 0.000008238) can be classified as a VUS and predicting it as the benign in protein level with three analyses. Therefore, among seventy-seven different variant alleles, seventy-four may be nsOCA-causing and three are VUS.

3.2 | Spectral distribution of variants of all known OCA genes in Chinese nsOCA patients

In our cohort of 114 Chinese nsOCA patients, 7 were identified without variants in all known nsOCA genes, 6 were identified to carry one pathogenic allele and unknown variant on second chromosome, 2 patients were identified to carry one or two compound VUS, and other 99 were identified to carry two or more pathogenic alleles including 17 patients with homozygous variants and 82 patients with compound heterozygous variants. Among 17 patients with homozygous variants, four unrelated patients 4002701 carried *OCA2*_c.2228C>T (p.Pro743Leu), 4008301 carried *OCA2*_c.808-3C>G, 4008401 carried c.929dupC (p.Arg311LysfsX7), and 4008601 carried c.229C>G (*TYR*_p.Arg77Gly) have consanguineous parents. Molecular diagnosis shows that in our cohort, there are 75 OCA1 patients (65.79%, 75/114), 16 OCA2 (14.03%, 16/114), 7 OCA4 (6.14%, 7/114), 1 OCA6 (0.88%, 1/114), and 15 OCA with unknown or unclassified variants (13.16%, 15/114; Figure 3a).

Of 79 *TRY*-related OCA patients, 75 were found to have two mutational alleles and 4 (Patient 4008901, Patient 4009601, Patient 4010701, and Patient 4011101) were found to have one mutational allele in *TYR* and second allele unknown (Table 1). We identified thirty-nine different mutational alleles of *TYR* in our cohort (Table 1), thirteen of which have not been previously reported: c.21C>A (p.Tyr7X), c.24C>A (p.Cys8X), c.324G>A (p.Trp108X), c.456delC (p.Ile153X), c.561_562insCATTATTATGTGTCAAATTATCCCC (p.Gly190CysfsX12), c.572dupG (p.Ser192IlefsX2), c.636A>T (p.Arg212Ser), c.653G>A (p.Trp218X), c.937C>A (p.Pro313Thr),

TABLE 1 Variants identified in a Chinese cohort of OCA patients

Gene name	Patients no.	Variants info.				Pathogenicity prediction in protein level			ExAC/ GnomAD	Reported or not	OCA type
		Variant	EX.	Status	Type	Polyphen-2	SIFT	MutationTaster			
TYR	4001801	c.230_232dupGGG(p.Arg77_Glu78insGly)	EX1	HET.	Indel.	—	—	—	0.000008268/0.00002529	YES	OCA1
		c.896G>A(p.Arg299His)	EX2	HET.	Missense	PRD	D	DC	0.00007424/0.0000614	YES	
	4002001	c.230_232dupGGG(p.Arg77_Glu78insGly)	EX1	HOM.	Indel.	—	—	—	0.000008268/0.00002529	YES	
	4002401	c.230_232dupGGG(p.Arg77_Glu78insGly)	EX1	HET.	Indel.	—	—	—	0.000008268/0.00002529	YES	
		c.929dupC(p.Arg311LysfsX7)	EX2	HET.	Indel.	—	—	—	0.00004948/0.00004066	YES	
	4002601	c.229C>G(p.Arg77Gly)	EX1	HET.	Missense	PRD	D	DC	0/0	YES	
		c.896G>A(p.Arg299His)	EX2	HET.	Missense	PRD	D	DC	0.00007424/0.0000614	YES	
	4002801	c.929dupC(p.Arg311LysfsX7)	EX2	HET.	Indel.	—	—	—	0.00004948/0.00004066	YES	
		c.1037G>A(p.Gly346Glu)	EX3	HET.	Missense	PRD	D	DC	0.000008299/0.00001633	YES	
	4002901	c.230G>A(p.Arg77Gln)	EX1	HET.	Missense	PRD	D	DC	0.00009925/0.00007949	YES	
		c.929dupC(p.Arg311LysfsX7)	EX2	HET.	Indel.	—	—	—	0.00004948/0.00004066	YES	
	4003201	c.230G>A(p.Arg77Gln)	EX1	HET.	Missense	PRD	D	DC	0.00009925/0.00007949	YES	
		c.929dupC(p.Arg311LysfsX7)	EX2	HET.	Indel.	—	—	—	0.00004948/0.00004066	YES	
	4003401	c.820-3C>G	INV1	HET.	Splicing	—	—	—	0.000008264/0	YES	
		c.896G>A(p.Arg299His)	EX2	HET.	Missense	PRD	D	DC	0.00007424/0.0000614	YES	
	4003501	c.229C>G(p.Arg77Gly)	EX1	HET.	Missense	PRD	D	DC	0/0	YES	
		c.715C>T(p.Arg239Trp)	EX1	HET.	Missense	PRD	D	DC	0.00004131/0.0000285	YES	
	4004001	c.896G>A(p.Arg299His)	EX2	HOM.	Missense	PRD	D	DC	0.00007424/0.0000614	YES	
	4004101	c.230G>A(p.Arg77Gln)	EX1	HET.	Missense	PRD	D	DC	0.00009925/0.00007949	YES	
		c.1204C>T(p.Arg402X)	EX4	HET.	Nonsense	—	—	—	0.00004984/0.0000326	YES	
	4004701†	c.929dupC(p.Arg311LysfsX7)	EX2	HET.	Indel.	—	—	—	0.00004948/0.00004066	YES	
		c.944C>G(p.Ser315X)	EX2	HET.	Nonsense	—	—	—	0/0	NO	
	4004901	c.929dupC(p.Arg311LysfsX7)	EX2	HET.	Indel.	—	—	—	0.00004948/0.00004066	YES	
		c.896G>A(p.Arg299His)	EX2	HET.	Missense	PRD	D	DC	0.00007424/0.0000614	YES	
	4005001	c.229C>G(p.Arg77Gly)	EX1	HET.	Missense	PRD	D	DC	0/0	YES	
		c.929dupC(p.Arg311LysfsX7)	EX2	HET.	Indel.	—	—	—	0.00004948/0.00004066	YES	
	4005101†	**c.636A>T(p.Arg212Ser)**	EX1	HET.	Missense	PRD	D	DC	0/0.000004065	NO	
		c.1199G>T(p.Trp400Leu)	EX4	HET.	Missense	PRD	D	DC	0.00002493/0.00003622	YES	
	4005501	c.896G>A(p.Arg299His)	EX2	HOM.	Missense	PRD	D	DC	0.00007424/0.0000614	YES	

(Continues)

TABLE 1 (Continued)

Gene name	Patients no.	Variants info.				Pathogenicity prediction in protein level			ExAC/ GnomAD	Reported or not	OCA type
		Variant	EX.	Status	Type	Polyphen-2	SIFT	MutationTaster			
	4005601	c.758G>A(p.Gly253Glu)	EX1	HET.	Missense	PRD	D	DC	0/0	YES	
		c.1199G>T(p.Trp400Leu)	EX4	HET.	Missense	PRD	D	DC	0.00002493/0.00003622	YES	
	4005701	c.929dupC(p.Arg311LysfsX7)	EX2	HET.	Indel.	—	—	—	0.00004948/0.00004066	YES	
		c.1199G>T(p.Trp400Leu)	EX4	HET.	Missense	PRD	D	DC	0.00002493/0.00003622	YES	
	4005801	c.929dupC(p.Arg311LysfsX7)	EX2	HOM.	Indel.	—	—	—	0.00004948/0.00004066	YES	
	4005901	c.929dupC(p.Arg311LysfsX7)	EX2	HET.	Indel.	—	—	—	0.00004948/0.00004066	YES	
		c.425A>T(p.Lys142Met)	EX1	HET.	Missense	PRD	D	DC	0.00000824/0.00001219	YES	
	4006101†	**c.324G>A(p.Trp108X)**	EX1	HET.	Indel.	—	—	—	0/0	NO	
		c.929dupC(p.Arg311LysfsX7)	EX2	HET.	Indel.	—	—	—	0.00004948/0.00004066	YES	
	4006201†	c.346C>T(p.Arg116X)	EX1	HET.	Nonsense	—	—	—	0.00002473/0.00002887	YES	
		c.456delC(p.Ile153X)	EX1	HET.	Indel.	—	—	—	0/0	NO	
	4006301	c.929dupC(p.Arg311LysfsX7)	EX2	HOM.	Indel.	—	—	—	0.00004948/0.00004066	YES	
	4006501	c.346C>T(p.Arg116X)	EX1	HET.	Nonsense	—	—	—	0.00002473/0.00002887	YES	
		c.896G>A(p.Arg299His)	EX2	HET.	Missense	PRD	D	DC	0.00007424/0.0000614	YES	
	4006601†	c.1168C>G(p.His390Asp)	EX3	HET.	Missense	PRD	D	DC	0.000008251/0.000004068	YES	
		c.1325C>A(p.Ser442Tyr)	EX4	HET.	Missense	PRD	D	DC	0/0	NO	
	4006701†	c.346C>T(p.Arg116X)	EX1	HET.	Nonsense	—	—	—	0.00002473/0.00002887	YES	
		c.1169A>G(p.His390Arg)	EX3	HET.	Missense	PRD	D	DC	0/0	NO	
	4007101	c.832C>T(p.Arg278X)	EX2	HET.	Nonsense	—	—	—	0.00019/0.000177	YES	
		c.1168C>G(p.His390Asp)	EX3	HET.	Missense	PRD	D	DC	0.000008251/0.000004068	YES	
	4007401	c.896G>A(p.Arg299His)	EX2	HOM.	Missense	PRD	D	DC	0.00007424/0.0000614	YES	
	4007501	c.346C>T(p.Arg116X)	EX1	HET.	Nonsense	—	—	—	0.00002473/0.00002887	YES	
		c.1204C>T(p.Arg402X)	EX4	HET.	Nonsense	—	—	—	0.00004984/ 0.0000326	YES	
	4007601†	**c.653G>A(p.Trp218X)**	EX1	HET.	Nonsense	—	—	—	0/0	NO	
		c.896G>A(p.Arg299His)	EX2	HET.	Missense	PRD	D	DC	0.00007424/0.0000614	YES	
	4007801	c.346C>T(p.Arg116X)	EX1	HET.	Nonsense	—	—	—	0.00002473/0.00002887	YES	
		c.929dupC(p.Arg311LysfsX7)	EX2	HET.	Indel.	—	—	—	0.00004948/0.00004066	YES	
	4007901	c.929dupC(p.Arg311LysfsX7)	EX2	HET.	Indel.	—	—	—	0.00004948/0.00004066	YES	
		c.896G>A(p.Arg299His)	EX2	HET.	Missense	PRD	D	DC	0.00007424/0.0000614	YES	
	4008201	c.929dupC(p.Arg311LysfsX7)	EX2	HET.	Indel.	—	—	—	0.00004948/0.00004066	YES	
		c.896G>A(p.Arg299His)	EX2	HET.	Missense	PRD	D	DC	0.00007424/0.0000614	YES	
	4008401	c.929dupC(p.Arg311LysfsX7)	EX2	HOM.	Indel.	—	—	—	0.00004948/0.00004066	YES	

(Continues)

TABLE 1 (Continued)

Gene name	Patients no.	Variants info.				Pathogenicity prediction in protein level			ExAC/ GnomAD	Reported or not	OCA type
		Variant	EX.	Status	Type	Polyphen-2	SIFT	MutationTaster			
	4008601	c.229C>G(p.Arg77Gly)	EX1	HOM	Missense	PRD	D	DC	0/0	YES	
	4009001	c.896G>A(p.Arg299His)	EX2	HOM.	Missense	PRD	D	DC	0.00007424/0.0000614	YES	
	4009101	c.230_232dupGGG(p.Arg77_Glu78insGly)	EX1	HET.	Indel.	—	—	—	0.000008268/0.00002529	YES	
		c.1199G>T(p.Trp400Leu)	EX4	HET.	Missense	PRD	D	DC	0.00002493/ 0.00003622	YES	
	4009201	c.71G>A(p.Cys24Tyr)	EX1	HET.	Missense	PRD	D	DC	0.000008238/0.000004061	YES	
		c.820-3C>G	INV1	HET.	Splicing	—	—	—	0.000008264/0	YES	
	4009301	c.832C > T (p.Arg278X)	EX2	HET.	Nonsense	—	—	—	0.00019/0.000177	YES	
		c.896G>A(p.Arg299His)	EX2	HET.	Missense	PRD	D	DC	0.00007424/0.0000614	YES	
	4009401	c.929dupC(p.Arg311LysfsX7)	EX2	HET.	Indel.	—	—	—	0.00004948/0.00004066	YES	
		c.1204C>T(p.Arg402X)	EX4	HET.	Nonsense	—	—	—	0.00004984/0.0000326	YES	
	4009501†	**c.24C>A(p.Cys8X)**	EX1	HET.	Nonsense	—	—	—	0/0	NO	
		c.895C>A(p.Arg299Ser)	EX2	HET.	Missense	PRD	D	DC	0.000008249/0.00002439	YES	
	4009701	c.929dupC(p.Arg311LysfsX7)	EX2	HET.	Indel.	—	—	—	0.00004948/0.00004066	YES	
		c.1425G>A(p.Trp475X)	EX5	HET.	Nonsense	—	—	—	0.00001648/0.000008131	YES	
	4009801	c.929dupC(p.Arg311LysfsX7)	EX2	HOM.	Indel.	—	—	—	0.00004948/0.00004066	YES	
	4009901	c.895C>T(p.Arg299Cys)	EX2	HET.	Missense	PRD	D	DC	0.00002475/0.00001626	YES	
		c.896G>A(p.Arg299His)	EX2	HET.	Missense	PRD	D	DC	0.00007424/0.0000614	YES	
	4010001	c.655G>A(p.Glu219Lys)	EX1	HET.	Missense	PRD	D	DC	0/0	YES	
		c.832C>T(p.Arg278X)	EX2	HET.	Nonsense	—	—	—	0.00019/0.000177	YES	
	4010101	c.820-3C>G	INV1	HET.	Splicing	—	—	—	0.000008264/0	YES	
		c.896G>A(p.Arg299His)	EX2	HET.	Missense	PRD	D	DC	0.00007424/0.0000614	YES	
	4010601	c.929dupC(p.Arg311LysfsX7)	EX2	HET.	Indel.	—	—	—	0.00004948/0.00004066	YES	
		c.896G>A(p.Arg299His)	EX2	HET.	Missense	PRD	D	DC	0.00007424/0.0000614	YES	
	4010801	c.655G>A(p.Glu219Lys)	EX1	HET.	Missense	PRD	D	DC	0/0	YES	
		c.832C>T(p.Arg278X)	EX2	HET.	Nonsense	—	—	—	0.00019/0.000177	YES	
	4011001†	c.230_232dupGGG(p.Arg77_Glu78insGly)	EX1	HET.	Indel.	—	—	—	0.000008268/0.00002529	YES	
		c.1234C>A(p.Pro412Thr)	EX4	HET.	Missense	PRD	D	DC	0/0	NO	
	4011201	c.346C>T(p.Arg116X)	EX1	HET.	Nonsense	—	—	—	0.00002473/0.00002887	YES	
		c.896G>A(p.Arg299His)	EX2	HET.	Missense	PRD	D	DC	0.00007424/0.0000614	YES	

(Continues)

TABLE 1 (Continued)

Gene name	Patients no.	Variants info.				Pathogenicity prediction in protein level			ExAC/ GnomAD	Reported or not	OCA type
		Variant	EX.	Status	Type	Polyphen-2	SIFT	MutationTaster			
	4011301	c.929dupC(p.Arg311LysfsX7)	EX2	HET.	Indel.	—	—	—	0.00004948/0.00004066	YES	
		c.1037-2A>T	INV2	HET.	Splicing	—	—	—	0/0	YES	
	4011601	c.655G>A(p.Glu219Lys)	EX1	HET.	Missense	PRD	D	DC	0/0	YES	
		c.929dupC(p.Arg311LysfsX7)	EX2	HET.	Indel.	—	—	—	0.00004948/0.00004066	YES	
	4011801	c.71G>A(p.Cys24Tyr)	EX1	HET.	Missense	PRD	D	DC	0.000008238/0.000004061	YES	
		c.164G>A(p.Cys55Tyr)	EX1	HET.	Missense	PRD	D	DC	0/0.00003231	YES	
	4011901	c.1A>G(p.Met1?)	EX1	HET.	Missense	—	—	—	0.00003299/0.00006494	YES	
		c.929dupC(p.Arg311LysfsX7)	EX2	HET.	Indel.	—	—	—	0.00004948/0.00004066	YES	
	4012001	c.1199G>T (p.Trp400Leu)	EX4	HET.	Missense	PRD	D	DC	0.00002493/0.00003622	YES	
		c.1204C>T (p.Arg402X)	EX4	HET.	Nonsense	—	—	—	0.00004984/0.0000326	YES	
	4012101	/0(p.Gly253Glu)	EX1	HET.	Missense	PRD	D	DC	0/0	YES	
		c.1199G>T(p.Trp400Leu)	EX4	HET.	Missense	PRD	D	DC	0.00002493/0.00003622	YES	
	4012201	c.832C>T(p.Arg278X)	EX2	HET.	Nonsense	—	—	—	0.00019/0.000177	YES	
		c.929dupC(p.Arg311LysfsX7)	EX2	HET.	Indel.	—	—	—	0.00004948/0.00004066	YES	
	4012401	c.230_232dupGGG(p.Arg77_Glu78insGly)	EX1	HOM.	Indel.	—	—	—	0.000008268/0.00002529	YES	
	4001901	c.703T>C(p.Tyr235His)	EX1	HET.	Missense	PRD	D	DC	0/0	YES	
		c.896G>A(p.Arg299His)	EX2	HET.	Missense	PRD	D	DC	0.00007424/0.0000614	YES	
	4003301	c.895C>T(p.Arg299Cys)	EX2	HET.	Missense	PRD	D	DC	0.00002475/0.00001626	YES	
		c.1199G>T(p.Trp400Leu)	EX4	HET.	Missense	PRD	D	DC	0.00002493/ 0.00003622	YES	
	4003601†	**c.572dupG(p.Ser192IlefsX2)**	EX1	HET.	Indel.	—	—	—	0/0	NO	
		c.820-3C>G	INV1	HET.	Splicing	—	—	—	0.000008264/0	YES	
	4003701	c.71G>A(p.Cys24Tyr)	EX1	HET.	Missense	PRD	D	DC	0.000008238/0.000004061	YES	
		c.1265G>A(p.Arg422Gln)	EX4	HET.	Missense	PRD	D	DC	0.00004968/0.00005709	YES	
	4003801	c.896G>A(p.Arg299His)	EX2	HET.	Missense	PRD	D	DC	0.00007424/0.0000614	YES	
		c.1037-7A>T	INV2	HET.	Splicing	—	—	—	0.0008838/0.0008789	YES	
		c.1037-10_11delTT	INV2	HET.	Splicing	—	—	—	0/0	YES	
	4004201†	**c.561_562insCATTATTATGTGTCAAATTATCCCC (p.Gly190CysfsX12)**	EX1	HET.	Indel.	—	—	—	0/0	NO	
		c.929dupC(p.Arg311LysfsX7)	EX2	HET.	Indel.	—	—	—	0.00004948/0.00004066	YES	
	4004301	c.230_232dupGGG(p.Arg77_Glu78insGly)	EX1	HET.	Indel.	—	—	—	0.000008268/0.00002529	YES	
		c.896G>A(p.Arg299His)	EX2	HET.	Missense	PRD	D	DC	0.00007424/0.0000614	YES	

(Continues)

TABLE 1 (Continued)

Gene name	Patients no.	Variants info.				Pathogenicity prediction in protein level			ExAC/ GnomAD	Reported or not	OCA type
		Variant	EX.	Status	Type	Polyphen-2	SIFT	MutationTaster			
	4005201	c.346C>T(p.Arg116X)	EX1	HET.	Nonsense	—	—	—	0.00002473/0.00002887	YES	
		c.819G>C(p.Gln273His)	EX1	HET.	Missense	PRD	D	DC	0/0	YES	
	4005301	c.896G>A(p.Arg299His)	EX2	HET.	Missense	PRD	D	DC	0.00007424/0.0000614	YES	
		c.1199G>T(p.Trp400Leu)	EX4	HET.	Missense	PRD	D	DC	0.00002493/ 0.00003622	YES	
	4006001	c.929dupC(p.Arg311LysfsX7)	EX2	HET.	Indel.	—	—	—	0.00004948/0.00004066	YES	
		c.1265G>A(p.Arg422Gln)	EX4	HET.	Missense	PRD	D	DC	0.00004968/0.00005709	YES	
	4008101†	c.446A>G(p.Tyr149Cys)	EX1	HET.	Missense	PRD	D	DC	0/0	YES	
		c.937C>A(p.Pro313Thr)	EX2	HET.	Missense	PRD	D	DC	0/0	NO	
	4008501	c.896G>A(p.Arg299His)	EX2	HET.	Missense	PRD	D	DC	0.00007424/0.0000614	YES	
		c.1199G>T(p.Trp400Leu)	EX4	HET.	Missense	PRD	D	DC	0.00002493/0.00003622	YES	
	4008801	c.896G>A(p.Arg299His)	EX2	HOM.	Missense	PRD	D	DC	0.00007424/0.0000614	YES	
	4010201	c.229C>T(p.Arg77Trp)	EX1	HET.	Missense	PRD	D	DC	0.00003307/0.00003251	YES	
		c.346C>T(p.Arg116X)	EX1	HET.	Nonsense	—	—	—	0.00002473/0.00002887	YES	
	4010301	c.346C>T(p.Arg116X)	EX1	HET.	Nonsense	—	—	—	0.00002473/0.00002887	YES	
		c.895C>A(p.Arg299Ser)	EX2	HET.	Missense	PRD	D	DC	0.000008249/0.00002439	YES	
	4011401	c.832C>T(p.Arg278X)	EX2	HET.	Nonsense	—	—	—	0.00019/0.000177	YES	
		c.1199G>T(p.Trp400Leu)	EX4	HET.	Missense	PRD	D	DC	0.00002493/0.00003622	YES	
	4012301	c.230G>A(p.Arg77Gln)	EX1	HET.	Missense	PRD	D	DC	0.00009925/0.00007949	YES	
		c.1199G>T(p.Trp400Leu)	EX4	HET.	Missense	PRD	D	DC	0.00002493/0.00003622	YES	
	4008901	c.896G>A(p.Arg299His)	EX2	HET.	Missense	PRD	D	DC	0.00007424/0.0000614	YES	OCA1?
		?	?	?	?	—	—	—	—	—	
	4009601	c.346C>T(p.Arg116X)	EX1	HET.	Nonsense	—	—	—	0.00002473/0.00002887	YES	
		?	?	?	?	—	—	—	—	—	
	4010701	c.929dupC(p.Arg311LysfsX7)	EX2	HET.	Indel.	—	—	—	0.00004948/0.00004066	YES	
		?	?	?	?	—	—	—	—	—	
	4011101†	**c.21C>A(p.Tyr7X)**	EX1	HET.	Nonsense	—	—	—	0/0	NO	
		?	?	?	?	—	—	—	—	—	
OCA2	4000401†	c.1426A>G(p.Asn476Asp)	EX14	HET.	Missense	PRD	D	DC	0/0	YES	OCA2
		c.2030T>G(p.Val677Gly)	EX19	HET.	Missense	PRD	D	DC	0/0	NO	
	4000601	c.1262G>C(p.Arg421Pro)	EX13	HET.	Missense	PRD	D	DC	0/0	YES	
		c.632C>T(p.Pro211Leu)	EX6	HET.	Missense	PRD	D	DC	0.0001494/0.0001228	YES	

(Continues)

TABLE 1 (Continued)

Gene name	Patients no.	Variants info.				Pathogenicity prediction in protein level			ExAC/ GnomAD	Reported or not	OCA type
		Variant	EX.	Status	Type	Polyphen-2	SIFT	MutationTaster			
	4001201†	**c.1010dupT(p.Leu338ProfsX11)**	EX9	HET.	Indel.	—	—	—	0/0	NO	
		c.1504G>A(p.Gly502Ser)	EX15	HET.	Missense	PRD	D	DC	0/0	NO	
	4002701	c.2228C>T(p.Pro743Leu)	EX21	HOM	Missense	PRD	D	DC	0.00009078/0.0001263	YES	
	4003001†	**c.1342C>T(p.Leu448Phe)**	EX13	HET.	Missense	PRD	D	DC	0/0	NO	
		c.2204_2205insCGGT(p.Ser736GlyfsX6)	EX21	HET.	Indel.	—	—	—	0/0	NO	
	4003101†	c.406C>T(p.Arg136X)	EX4	HET.	Nonsense	—	—	—	0.00003295/0.000004061	YES	
		c.1342C>T(p.Leu448Phe)	EX13	HET.	Missense	PRD	D	DC	0/0	NO	
	4003901†	c.406C>T(p.Arg136X)	EX4	HET.	Nonsense	—	—	—	0.00003295/0.000004061	YES	
		c.646+3A>G	INV6	HET.	Splicing	—	—	—	0/0	NO	
	4004601†	**c.2195C>G(p.Ser732X)**	EX21	HET.	Nonsense	—	—	—	0/0	NO	
		c.1441G>A(p.Ala481Thr)	EX14	HET.	Missense	POD	T	DC	0.007751/0.008502	YES	
	4005401†	**c.247C>T(p.Gln83X)**	EX3	HET.	Nonsense	—	—	—	0/0	NO	
		c.2344G>A(p.Gly782Arg)	EX23	HET.	Missense	PRD	D	DC	0/0.000007215	YES	
	4007301†	**c.2165delT(p.Ile722LysfsX17)**	EX21	HET.	Indel.	—	—	—	0/0	NO	
		c.2244G>A(p.Met748Ile)	EX21	HET.	Missense	PRD	D	DC	0/0	NO	
	4007701	c.1255C>T(p.Arg419Trp)	EX13	HET.	Missense	PRD	D	DC	0.0002452/0.000264	YES	
		c.1349C>T(p.Thr450Met)	EX13	HET.	Missense	PRD	D	DC	0.00001678/0.00003231	YES	
	4008301*	**c.808-3C>G**	INV7	HOM.	Splicing	—	—	—	0/0.000004061	NO	
	4010501	c.1182+1G>A	INV11	HOM.	Missense	—	—	—	0.00009144/0.00005775	YES	
	4010901	c.1441G>A(p.Ala481Thr)	EX14	HET.	Missense	POD	T	DC	0.007751/0.008502	YES	
		c.1503+5G>A	INV14	HET.	Splicing	—	—	—	0.000008237/0.00001082	YES	
	4011501†	**c.2140-2A>G**	INV20	HET.	Splicing	—	—	—	0/0	NO	
		c.632C>T(p.Pro211Leu)	EX6	HET.	Missense	PRD	D	DC	0.0001494/0.0001228	YES	
	4002501	c.1182+1G>A	INV11	HET.	Splicing	—	—	—	0.00009144/0.00005775	YES	
		c.1714C>T(p.Arg572Cys)	EX16	HET.	Missense	PRD	D	DC	0.00006945/0.00005862	YES	
	4006901†	**c.2245-11T>G**	INV21	HET.	Splicing	—	—	—	0/0	NO	OCA2?
		c.2373_2375delCGT(p.Val792del)	EX23	HET.	Indel.	—	—	—	0/0.000008121	NO	
	4004501†	**c.849C>A(p.Ser283Arg)**	EX8	HET.	Missense	Benign	T	P	0.000008238/0.00002525	NO	
		?	?	?	?	—	—	—	—	—	
	4008001	c.406C>T(p.Arg136X)	EX4	HET.	Nonsense	—	—	—	0.00003295/0.000004061	YES	
		?	?	?	?	—	—	—	—	—	

(Continues)

TABLE 1 (Continued)

Gene name	Patients no.	Variants info.				Pathogenicity prediction in protein level			ExAC/ GnomAD	Reported or not	OCA type
		Variant	EX.	Status	Type	Polyphen-2	SIFT	MutationTaster			
	4008701	c.808-3C>G	INV7	HET.	Splicing	—	—	—	0/0.000004061	NO	
		?	?	?	?	—	—	—	—	—	
SLC45A2	4000501†	**c.1032+1G>T**	INV4	HET.	Splicing	—	—	—	0/0	NO	OCA4
		c.1045G>A(p.Gly349Arg)	EX5	HET.	Missense	PRD	D	DC	0.0001318/0.00007311	YES	
	4001001†	**c.133A > G(p.Arg45Gly)**	EX1	HOM.	Missense	PRD	D	DC	0/0	NO	
	4001601†	**c.529G>T(p.Glu177X)**	EX2	HET.	Nonsense	—	—	—	0/0	NO	
		c.844G>T(p.Glu282X)	EX3	HET.	Nonsense	—	—	—	0/0	NO	
	4002201†	**c.152_153delTG(p.Val51GlyfsX82)**	EX1	HET.	Indel.	—	—	—	0/0	YES	
		c.1045G>A(p.Gly349Arg)	EX5	HET.	Missense	PRD	D	DC	0.0001318/0.00007311	YES	
	4004801†	**c.133A>G(p.Arg45Gly)**	EX1	HET.	Missense	PRD	D	DC	0/0	NO	
		c.478G>C(p.Asp160His)	EX2	HET.	Missense	PRD	D	DC	0/0.000004061	YES	
	4007201†	c.478G>C(p.Asp160His)	EX2	HET.	Missense	PRD	D	DC	0/0.000004061	YES	
		c.1273delC(p.Leu425TrpfsX9)	EX6	HET.	Indel.	—	—	—	0.00003295/0.00001218	NO	
	4011701†	c.478G>C(p.Asp160His)	EX2	HET.	Missense	PRD	D	DC	0/0.000004061	YES	
		c.869dupA(p.Asn290LysfsX6)	EX3	HET.	Indel.	—	—	—	0/0.000008145	NO	
SLC24A5	4007001*	**c.1361dupT(p.Leu454PhefsX33)**	EX9	HOM.	Indel.	—	—	—	0/0	NO	OCA6

Note: The proband marked with † sign carries novel variant or variants; the proband with * sign carries known variants but its homozygosity reported firstly in this study. The variants reported to be associated with OCA at first time are in the bold. The items without data available are marked with backslash. Variants marked with hyphen are not necessary to be predicted or improper to be predicted their pathogenicity in protein level via SIFT, Polyphen-2, and MutationTaster. Grayish lattices were splicing mutations and non-pathogenic results in protein level with all or two of three in silico approaches. The allele frequency of ExAC or GnomAD data here refers to all individuals.

B, benign; D, Damaging; DC, Disease causing; P, polymorphism; POD, possibly damaging; PRD, Probably damaging; T, tolerated.

FIGURE 1 Sequence chromatograms of novel variants in *TYR*, *OCA2*, *SLC45A2*, and *SLC24A5*

c.944C>G (p.Ser315X), c.1169A>G (p.His390Arg), c.1234C>A (p.Pro412Thr), and c.1325C>A (p.Ser442Tyr; Figure 1, Table 1). In OCA1, 27 of 39 different mutational alleles are clustered on exon 1 and exon 2 of *TYR*, accounting for 81.1% (131 of 155) of the total TYR mutational alleles (Figure 3b). Among these mutational alleles of *TYR*, c.929dupC and p.Arg299His account for 20.00% (31 of 155) and 18.71% (29 of 155), respectively (Figure 3c,d), the variant in exon 4 p.Trp400Leu ranks third with 7.10% (11 of 155; Figure 3b), and other variants with higher sequence are p.Arg116X (6.45%, 10 of 155), p.Arg77_Glu78insGly (5.81%, 9 of 155), p.Arg77Gly (3.87%, 6 of 155), and p.Arg278X (3.87%, 6 of 155; Figure 3c,d). The above seven alleles account for 65.81% (102 of 155) of the mutational *TYR* alleles in our cohort of Chinese OCA patients.

In our cohort, sixteen nsOCA patients were diagnosed as OCA2, and four are uncertain including Patient 4006901 identified to carry two compound VUS (*OCA2*_c.2245-11T>G and *OCA2*_c.2373_2375delCGT), Patient 4004501 with one VUS (*OCA2*_c.849C>A) plus Patient 4008001 and Patient 4008001 only identified to carry one pathogenic variant in OCA2 (Table 1). Twenty-four different OCA2-causing variants identified in 16 patients, of which 11 mutational alleles were novel: c.247C>T (p.Gln83X), c.646+3A>G, c.1010dupT (p.Leu338ProfsX11), c.1342C>T (p.Leu448Phe), c.1504G>A (p.Gly502Ser), c.2030T>G (p.Val677Gly), c.2140-2A>G, c.2195C>G (p.Ser732X), c.2165delT (p.Ile722LysfsX17), c.2204_2205insCGGT (p.Ser736GlyfsX6), and c.2244G>A (p.Met748Ile), plus 13 were recurrent (Table 1). Among 13 known variants, homozygous variants c.808-3C>G are firstly reported.

Seven nsOCA patients were diagnosed as OCA4, and total fourteen variant alleles are identified in *SLC45A2*. All OCA4 patients in this study carried at least one novel variant. Together, nine different OCA4 causative variants were found in this study including two recurrent variants—c.478G>C (p.Asp160His) and c.1045G>A, and seven novel variants—c.133A>G (p.Arg45Gly), c.152_153delTG (p.Val51GlyfsX82), c.529G>T (p.Glu177X), c.844G>T (p.Glu282X), c.869dupA (p.Asn290LysfsX6), c.1032+1G>T, and c.1273delC (p.Leu425TrpfsX9).

In addition, homozygous variants c.1361dupT (p.Leu454PhefsX33) in *SLC24A5* were detected in Patient 4007001—the second molecularly

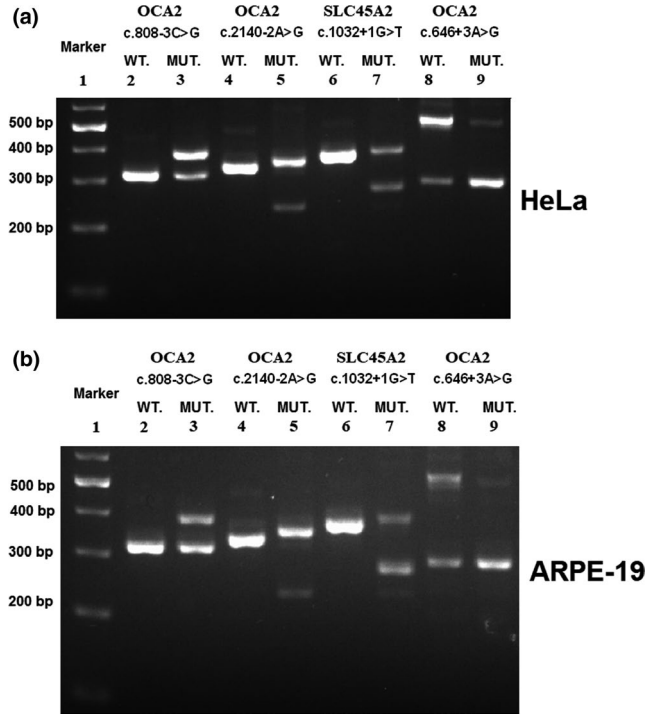

FIGURE 2 Splicing assay shows variant-induced change in *OCA2* or *SLC45A2* splicing. Gel electrophoresis of RT-PCR products for all tested constructs. Lane 1: 100bp marker, splicing assay is based on comparative assay about the splicing pattern of genomic fragment of wild-type (WT) and mutant (MUT), respectively. Lane 2 and lane 3 as a group are to evaluate the change in splicing which the variant *OCA2*_c.808-3C>G brought about. Lane 4 and lane 5 are for *OCA2*_c.2140-2A>G; lane 6 and lane 7 are for *SLC45A2*_c.1032+1G>T; lane 8 and lane 9 are for *OCA2*_c.646+3A>G. The differences in the size and composition of band(s) between WT and MUT demonstrate the variant-induced aberrant in mRNA level. (a) In vitro splicing assay in HeLa cell line. (b) In vitro splicing assay in ARPE-19 cell line

diagnosed as OCA6 case in Chinese population. Homozygous variants *SLC24A5*_c.1361dupT are firstly reported here (Figure 1). No OCA3 patient and OCA7 patient were identified in our cohort.

3.3 | Cutaneous phenotype of OCA1, OCA2, and OCA4 patients

Patient 4005001, diagnosed as OCA1 with compound variants c.229C>G (p.Arg77Gly) and c.929dupC (p.Arg311LysfsX7) in *TYR*, presents milky white hair and skin (Figure 4), and does not tan, and his irises are light blue as well as full transillumination, photophobia, has poor visual acuity (Figure 4). Patient 4003001, diagnosed as OCA2 with two novel heterozygous variants c.1342C>T (p.Leu448Phe) and c.2204_2205insCGGT (p.Ser736GlyfsX6) in *OCA2*, had blond hair at birth, and hair darken into brown at four-year age (Figure 4), plus light blue irises at birth were recorded in the medical history and brown irises were observed currently. Patient 4003101, diagnosed as OCA2 with a reported variant c.406C>T (p.Arg136X) and a novel variant c.1342C>T (p.Leu448Phe) in *OCA2*, had blond hair (Figure 4) and brown irises at the time when she was recruited. Patient 4003901, diagnosed as OCA2 with a reported variant c.406C>T (p.Arg136X) and a novel variant c.646+3A>G in *OCA2*, had golden hair. Patient 4004801, diagnosed as OCA4 with a reported variant c.478G>C (p.Asp160His) and a novel variant c.133A>G (p.Arg45Gly) in *SLC45A2*, had light blond hair, white skin (Figure 4), and irises translucency.

4 | DISCUSSION

The oxidation and polymerization of tyrosine synthesized in epidermal melanocytes originate a macromolecular biopolymer of melanin. Melanin is found in several tissues such as skin, hair and iris, choroid, and retina of the eye. Melanin can be transferred to the surrounding cells to protect them from the effect of UV radiation at sun exposure. Two types of melanin are produced in melanosomes, including brown or black photoprotective eumelanins, and yellow or red phototoxic pheomelanins, whose ratio of each type depends on the enzymatic activity of TYR (tyrosinase) and the availability of cysteine which is one of the rate-limiting factors in glutathione metabolism. Melanosomal ion transport proteins and pH are crucial for the genesis and function of melanosome. Low TYR activity presents in melanosomes from individuals with fair skin color displaying more acidic. In addition, low TYR activity and/or low concentrations of cysteine lead to phototoxic pheomelanins and high TYR activity and/or high concentrations of cysteine lead to photoprotective eumelanins. Any defect in melanocytes, dysfunction of melanocytes, and impairment in producing and transferring of melanin can cause albinism, and its specific involvement of skin, hair, and eyes is called as nsOCA. To date, *TYR*, *OCA2*, *TYRP1*, *SLC45A2*, *SLC24A5*, and *LRMDA* are six known nsOCA-causing genes, corresponding to OCA1, OCA2, OCA3, OCA4, OCA6, and OCA7 (Boissy et al., 1996; Durham-Pierre et al., 1994; Gronskov et al., 2013; Newton et al., 2001; Tomita et al., 1989; Wei et al., 2013).

In this study, comprehensive analysis of all currently known nsOCA genes (*TYR*, *OCA2*, *TYRP1*, *SLC45A2*, *SLC24A5*, and *LRMDA*) in 114 nsOCA patients recruited from 18 provinces in China shows the prevalence of OCA1, OCA2, OCA4, and OCA6 is 65.79%, 14.03%, 6.14%, and 0.88%, respectively, and the left nsOCA with uncertain causative defect of molecule (13.16%). In our cohort, OCA1 is the most common type of nsOCA and OCA2 ranks as the second most common type of nsOCA, which are in accordance with what reported in Japanese (Suzuki & Tomita, 2008), non-Hispanic Caucasians (Hutton & Spritz, 2008a), Danes (Gronskov et al., 2009), in the population of a European setting at the albino day hospital (Marti et al., 2018), and in the group of the patients mainly from France who were originated from different countries worldwide (Lasseaux et al., 2018). In this study, we failed to find the second variants in 7 patients and after sequencing all the exons and their flanking regions, which could be ascribed to the following possibilities of an undetected large indel in another allele or variants in deep-intronic regions or in regulatory elements located far away from those six known nsOCA genes. Plus, additional 8 patients without found pathogenic variants in all known nsOCA genes might suggest the possibility of uncovering novel OCA genes.

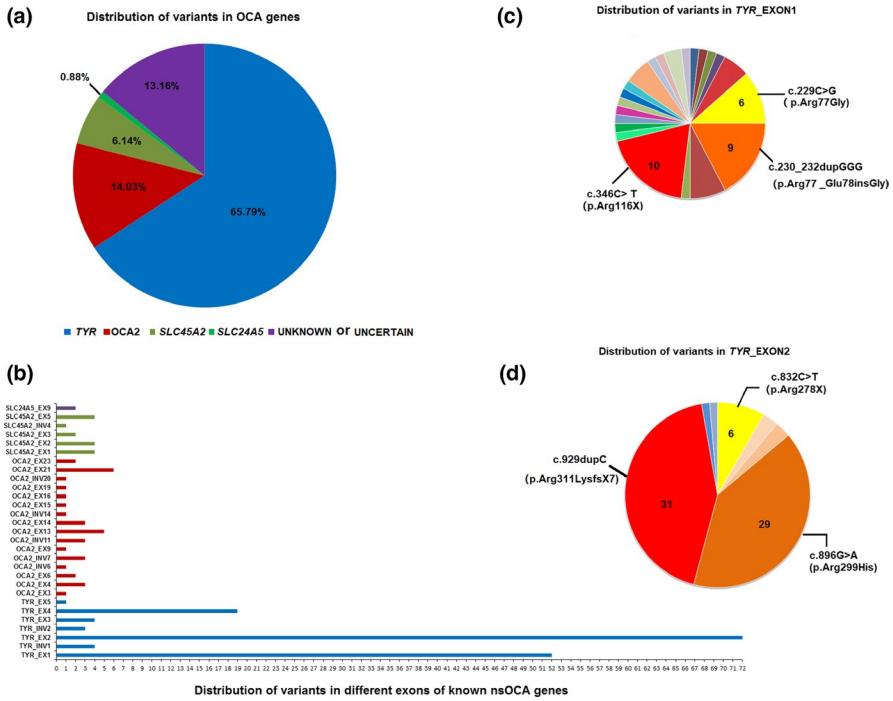

FIGURE 3 Spectral distribution of variants of all known OCA genes in Chinese nsOCA patients. (a) The prevalence of OCA types in our cohort. (b) Distribution of variants in exons of known OCA genes identified in this study. (c) Distribution of variants in exon 1 of *TYR*. (d) Distribution of variants in exon 2 of *TYR*

In addition, if *OCA2*_c.2245-11T>G, *OCA2*_c.2373_2375delCGT, and *OCA2*_c.849C>A were confirmed to be pathogenic in further function study, two patients with those variants would be OCA2.

The spectrum of variants in each nsOCA genes has been reported to vary with populations. In OCA1, 27 of 39 different mutational alleles are clustered on exon 1 and exon 2 of *TYR*, accounting for 81.1% (131 of 155) of the total TYR mutational alleles (Figure 3b), suggesting exon 1 and exon 2 is mutational hotspots in Chinese nsOCA, which is consistent with the report of Wei et al.'s study (Wei et al., 2010). In addition, both c.929idupC and p.Arg299His, which is the most frequent alleles in this study, are on exon 2. In this study, twenty-four different OCA2-causing variants identified in 16 patients are sparsely distributed in *OCA2*, and no apparent mutational hotspots can be observed in our cohort, which has also been observed in Wei et al's study (Wei et al., 2010). Although we supported the recommendation about prioritizing the sequencing of hotspots in exons 1 and 2 of *TYR* in diagnosis of OCA pointed out by Wei et al. (2010), the priority of *SLC45A2* over *OCA2* in sequencing may be uncertain. The frequency of a *SLC45A2* mutational allele c.478G>C (p.Asp160His) was 3/14 in this study while Wei et al. (2010) described that it accounts for 55.6% (15/27) of the mutational alleles of *SLC45A2* in their study, which may suggest that it is possible that kind of difference exists between the ancestors of patients in this study and those in Wei *et al.*' study although all the patients in both studies are Chinese.

The defect in OCA2, SLC45A2, and SLC24A5 functioning as ion transporters on melanosomal membranes to maintain melanosomes homeostasis (Durham-Pierre et al., 1994; Morice-Picard et al., 2014; Newton et al., 2001; Park et al., 2015; Wei et al., 2013) can cause OCA2, OCA4, and OCA6, respectively. The hair color of Patient 4003001 who was molecularly diagnosed as OCA2 darkened with age from blond hair at birth to brown at four years old (Figure 4, Table 1) and the color of irises darken too. Patient 4003001 and Patient 4003101 of whom both carry one same novel variant allele of *OCA2*_c.1342C>T (p.Leu448Phe) have different hair color, which might be due to difference in impact between c.2204_2205insCGGT (p.Ser736GlyfsX6) and c.406C>T (p.Arg136X) (Figure 4, Table 1). Similarly, the difference in hair color also exists between Patient 4003101 and Patient 4003901 of whom both carry one same variant allele *OCA2*_c.406C>T (p.Arg136X) but the second allele is different between them (Figure 4, Table 1). The hair of Patient 4005001 and Patient 4004801 look to have less pigment than that of other 3 OCA2 patients. To be sure, genotype–phenotype correlation analysis based on more patients with different types of OCA is helpful to answer whether there is correlation between genotype and phenotype in OCA1, OCA2, and OCA4. The defect in *SLC24A5* can cause OCA6, and less than 10 OCA6 cases have been reported worldwide (Bertolotti et al., 2016; Morice-Picard et al., 2014; Veniani et al., 2016; Wei et al., 2013). Skin phenotype of OCA6 cases is heterogeneous, and their hair color ranges from dark brown to white. Ophthalmologic anomalies of OCA6 cases have been reported to include severe hypopigmentation in retina and foveal hypoplasia and extensive iris transillumination (Bertolotti et al., 2016; Montoliu et al., 2014; Morice-Picard et al., 2014; Veniani et al., 2016; Wei et al., 2013). In this study, Patient 4007001 has been firstly diagnosed as OCA2 with light brown hair and white skin, and without severe ocular problems except for nystagmus and photophobia when being recruited at toddler stage, actually caused by homozygous variants in *SLC24A5* and was molecularly diagnosed as OCA6 in our study. This OCA6 case expands the currently limited spectrum of OCA6 since less than ten OCA6 cases had been reported.

FIGURE 4 Phenotypes in skin and hairs of patients with different types of nsOCA

Herein, we described the prevalence of nsOCA types in Chinese population and thirty-one different novel OCA causative variants, which expands the spectrum of nsOCA variants. In addition, the second OCA6 case in Chinese population was detected in our cohort. These findings may facilitate molecular diagnosis and genetic counseling for OCA patients.

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CONFLICT OF INTEREST

The authors declare no competing financial interests.

REFERENCES

Ainger, S. A., Jagirdar, K., Lee, K. J., Soyer, H. P., & Sturm, R. A. (2017). Skin Pigmentation Genetics for the Clinic. *Dermatology*, *233*(1), 1–15. https://doi.org/10.1159/000468538

Bertolotti, A., Lasseaux, E., Plaisant, C., Trimouille, A., Morice-Picard, F., Rooryck, C., … Arveiler, B. (2016). Identification of a homozygous mutation of SLC24A5 (OCA6) in two patients with oculocutaneous albinism from French Guiana. *Pigment Cell and Melanoma Research*, *29*(1), 104–106. https://doi.org/10.1111/pcmr.12425

Boissy, R. E., Zhao, H., Oetting, W. S., Austin, L. M., Wildenberg, S. C., Boissy, Y. L., … Nordlund, J. J. (1996). Mutation in and lack of expression of tyrosinase-related protein-1 (TRP-1) in melanocytes from an individual with brown oculocutaneous albinism: A new subtype of albinism classified as "OCA3". *American Journal of Human Genetics*, *58*(6), 1145–1156.

Dolinska, M. B., Kus, N. J., Farney, S. K., Wingfield, P. T., Brooks, B. P., & Sergeev, Y. V. (2017). Oculocutaneous albinism type 1: Link between mutations, tyrosinase conformational stability, and enzymatic activity. *Pigment Cell and Melanoma Research*, *30*(1), 41–52. https://doi.org/10.1111/pcmr.12546

Durham-Pierre, D., Gardner, J. M., Nakatsu, Y., King, R. A., Francke, U., Ching, A., … Brilliant, M. H. (1994). African origin of an intragenic deletion of the human P gene in tyrosinase positive oculocutaneous albinism. *Nature Genetics*, *7*(2), 176–179. https://doi.org/10.1038/ng0694-176

Fokkema, I. F., Taschner, P. E., Schaafsma, G. C., Celli, J., Laros, J. F., & den Dunnen, J. T. (2011). LOVD v.2.0: The next generation in gene variant databases. *Human Mutation*, *32*(5), 557–563. https://doi.org/10.1002/humu.21438

Gong, Y., Shao, C., Zheng, H., Chen, B., & Guo, Y. (1994). Study on genetic epidemiology of albinism. *Yi Chuan Xue Bao*, *21*(3), 169–172.

Grønskov, K., Dooley, C. M., Østergaard, E., Kelsh, R. N., Hansen, L., Levesque, M. P., … Rosenberg, T. (2013). Mutations in c10orf11, a melanocyte-differentiation gene, cause autosomal-recessive albinism. *American Journal of Human Genetics*, *92*(3), 415–421. https://doi.org/10.1016/j.ajhg.2013.01.006

Gronskov, K., Ek, J., & Brondum-Nielsen, K. (2007). Oculocutaneous albinism. *Orphanet Journal of Rare Diseases*, *2*, 43. https://doi.org/10.1186/1750-1172-2-43

Grønskov, K., Ek, J., Sand, A., Scheller, R., Bygum, A., Brixen, K., … Rosenberg, T. (2009). Birth prevalence and mutation spectrum in Danish patients with autosomal recessive albinism. *Investigative Ophthalmology and Visual Science*, *50*(3), 1058–1064. https://doi.org/10.1167/iovs.08-2639

Hutton, S. M., & Spritz, R. A. (2008a). Comprehensive analysis of oculocutaneous albinism among non-Hispanic caucasians shows that OCA1 is the most prevalent OCA type. *The Journal of Investigative Dermatology*, *128*(10), 2442–2450. https://doi.org/10.1038/jid.2008.109

Hutton, S. M., & Spritz, R. A. (2008b). A comprehensive genetic study of autosomal recessive ocular albinism in Caucasian patients. *Investigative Ophthalmology and Visual Science*, *49*(3), 868–872. https://doi.org/10.1167/iovs.07-0791

Kausar, T., Bhatti, M. A., Ali, M., Shaikh, R. S., & Ahmed, Z. M. (2013). OCA5, a novel locus for non-syndromic oculocutaneous albinism, maps to chromosome 4q24. *Clinical Genetics*, *84*(1), 91–93. https://doi.org/10.1111/cge.12019

King, R. A., Hearing, V. J., Creel, D. J., & Oetting, W. S. (2001). Albinism. In A. L. Beaudet, C. R. Scriver, D. Valle, & W. S. Sly (Eds.), *The metabolic & molecular basis of inherited disease* (8th ed., pp. 5587–5627). New York, NY: McGraw-Hill.

Lasseaux, E., Plaisant, C., Michaud, V., Pennamen, P., Trimouille, A., Gaston, L., … Arveiler, B. (2018). Molecular characterization of a series of 990 index patients with albinism. *Pigment Cell and Melanoma Research*, *31*(4), 466–474. https://doi.org/10.1111/pcmr.12688

Marti, A., Lasseaux, E., Ezzedine, K., Léauté-Labrèze, C., Boralevi, F., Paya, C., … Morice-Picard, F. (2018). Lessons of a day hospital: Comprehensive assessment of patients with albinism in a European setting. *Pigment Cell and Melanoma Research*, *31*(2), 318–329. https://doi.org/10.1111/pcmr.12651

Montoliu, L., Grønskov, K., Wei, A.-H., Martínez-García, M., Fernández, A., Arveiler, B., … Li, W. (2014). Increasing the complexity: New genes and new types of albinism. *Pigment Cell and Melanoma Research*, *27*(1), 11–18. https://doi.org/10.1111/pcmr.12167

Morice-Picard, F., Lasseaux, E., François, S., Simon, D., Rooryck, C., Bieth, E., … Arveiler, B. (2014). SLC24A5 mutations are associated with non-syndromic oculocutaneous albinism. *The Journal of Investigative Dermatology*, *134*(2), 568–571. https://doi.org/10.1038/jid.2013.360

Newton, J. M., Cohen-Barak, O., Hagiwara, N., Gardner, J. M., Davisson, M. T., King, R. A., & Brilliant, M. H. (2001). Mutations in the human orthologue of the mouse underwhite gene (uw) underlie a new form of oculocutaneous albinism, OCA4. *American Journal of Human Genetics*, *69*(5), 981–988. https://doi.org/10.1086/324340

Park, S., Morya, V. K., Nguyen, D. H., Singh, B. K., Lee, H. B., & Kim, E. K. (2015). Unrevealing the role of P-protein on melanosome biology and structure, using siRNA-mediated down regulation of OCA2. *Molecular and Cellular Biochemistry*, *403*(1–2), 61–71. https://doi.org/10.1007/s11010-015-2337-y

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., … Rehm, H. L. (2015). Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genetics in Medicine*, *17*(5), 405–424. https://doi.org/10.1038/gim.2015.30

Rimoldi, V., Straniero, L., Asselta, R., Mauri, L., Manfredini, E., Penco, S., … Primignani, P. (2014). Functional characterization of two novel splicing mutations in the OCA2 gene associated with oculocutaneous albinism type II. *Gene*, *537*(1), 79–84. https://doi.org/10.1016/j.gene.2013.11.102

Rooryck, C., Morice-Picard, F., Elcioglu, N. H., Lacombe, D., Taieb, A., & Arveiler, B. (2008). Molecular diagnosis of oculocutaneous albinism: New mutations in the OCA1-4 genes and practical aspects. *Pigment Cell & Melanoma Research*, *21*(5), 583–587. https://doi.org/10.1111/j.1755-148X.2008.00496.x

Sitaram, A., & Marks, M. S. (2012). Mechanisms of protein delivery to melanosomes in pigment cells. *Physiology (Bethesda)*, *27*(2), 85–99. https://doi.org/10.1152/physiol.00043.2011

Soukarieh, O., Gaildrat, P., Hamieh, M., Drouet, A., Baert-Desurmont, S., Frébourg, T., … Martins, A. (2016). Exonic Splicing Mutations Are More Prevalent than Currently Estimated and Can Be Predicted by Using In Silico Tools. *PLoS Genetics*, *12*(1), e1005756. https://doi.org/10.1371/journal.pgen.1005756

Suzuki, T., & Tomita, Y. (2008). Recent advances in genetic analyses of oculocutaneous albinism types 2 and 4. *Journal of Dermatological Science*, *51*(1), 1–9. https://doi.org/10.1016/j.jdermsci.2007.12.008

Tomita, Y., & Suzuki, T. (2004). Genetics of pigmentary disorders. *American Journal of Medical Genetics*, *131C*(1), 75–81. https://doi.org/10.1002/ajmg.c.30036

Tomita, Y., Takeda, A., Okinaga, S., Tagami, H., & Shibahara, S. (1989). Human oculocutaneous albinism caused by single base insertion in the tyrosinase gene. *Biochemical and Biophysical Research Communications*, *164*(3), 990–996. https://doi.org/10.1016/0006-291X(89)91767-1

Veniani, E., Mauri, L., Manfredini, E., Gesu, G. P., Patrosso, M. C., Zelante, L., … Primignani, P. (2016). Detection of the first OCA6 Italian patient in a large cohort of albino subjects. *Journal of Dermatological Science*, *81*(3), 208–209. https://doi.org/10.1016/j.jdermsci.2015.11.012

Wei, A., Wang, Y. U., Long, Y., Wang, Y. I., Guo, X., Zhou, Z., … Li, W. (2010). A comprehensive analysis reveals mutational spectra and common alleles in Chinese patients with oculocutaneous albinism. *The Journal of Investigative Dermatology*, *130*(3), 716–724. https://doi.org/10.1038/jid.2009.339

Wei, A.-H., Zang, D.-J., Zhang, Z., Liu, X.-Z., He, X., Yang, L., … Li, W. (2013). Exome sequencing identifies SLC24A5 as a candidate gene for nonsyndromic oculocutaneous albinism. *The Journal of Investigative Dermatology*, *133*(7), 1834–1840. https://doi.org/10.1038/jid.2013.49

Witkop, C. J. (1979). Albinism: Hematologic-storage disease, susceptibility to skin cancer, and optic neuronal defects shared in all types of oculocutaneous and ocular albinism. *Alabama Journal of Medical Sciences*, *16*(4), 227–330.

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