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Sequence analysis of tyrosinase gene in ocular and oculocutaneous albinism patients: introducing three novel mutations

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Purpose: Albinism is a heterogeneous genetic disorder of melanin synthesis that results in hypopigmented eyes (in patients with ocular albinism) or hair, skin, and eyes (in individuals with oculocutaneous albinism). It is associated with decreased visual acuity, nystagmus, strabismus, and photophobia. The tyrosinase gene is known to be involved in both oculocutaneous albinism and autosomal recessive ocular albinism. In this study, we aimed to screen the mutations in the *TYR* gene in the nonsyndromic OCA and autosomal recessive ocular albinism patients from Iran.
Methods: The tyrosinase gene was examined in 23 unrelated patients with autosomal recessive ocular albinism or nonsyndromic OCA using DNA sequencing and bioinformatics analysis.
Results: *TYR* gene mutations were identified in 14 (app. 60%) albinism patients.
Conclusions: We found 10 mutations, 3 of which were novel. No mutation was found in our ocular albinism patients, but one of them was heterozygous for the p.R402Q polymorphism.

Albinism is a heterogeneous group of genetic disorders that affect 1 in 20,000 individuals worldwide, although the prevalence of the different subtypes of albinism varies considerably among the different ethnic backgrounds. It is caused by deficiencies in pigmentation, and clinically is divided into ocular and oculocutaneous albinism [1-3].

Oculocutaneous albinism (OCA) is a heterogeneous and autosomal recessive disorder that involves a lack of pigment in the skin, hair, and eyes, and is accompanied by optic defects such as photophobia, strabismus, poor vision, and nystagmus [2,4,5]. It is identified as nonsyndromic OCA (if the mutations occur in the *TYR*, *OCA2*, *TYRP1*, *MATP* (*SLC45A2*), *SLC24A5*, or *C10ORF11* genes) or syndromic OCA (if the mutations occur in the *HPS1*, *AP3B1*, *HPS3*, *HPS4*, *HPS5*, *HPS6*, *DTNBP1*, *BLOC1S3*, *PLDN*, *LYST*, *MYO5A*, *RAB27A*, or *MLPH* genes) [1,6]. OCA-1 is the most common subtype found in Caucasians, and accounts for about 50% of cases worldwide. It results from mutations in the *TYR* gene [7].

TYR null mutations producing inactive or incomplete polypeptides result in OCA1A (tyrosinase-negative OCA), in which melanin formation never occurs throughout the patient's life. In contrast, mutations producing a partially active or hypomorphic tyrosinase enzymes result in the OCA1B (yellow mutant OCA), in which the patients completely lack detectable pigment at birth but rapidly develop yellow hair pigment in the first few years of life and then continue to slowly accumulate pigment in the hair, eyes, and skin with time [3,8].

Ocular albinism (OA) has similar eye findings to those of OCA but does not affect the hair and skin [7]. Both X-linked recessive (OA1) and autosomal recessive forms of ocular albinism exist [9]. Previous studies have shown that autosomal recessive ocular albinism in some cases constitutes a clinically mild phenotype of OCA, due to mutations in either the *TYR* or *OCA2 (P)* genes [10].

Clinical diagnosis of albinism type is difficult, due to the observed range of phenotypic variation. Thus, genetic analysis may be helpful with respect to a more accurate diagnosis and genetic counseling [2,11]. In this study, we screened mutations in the *TYR* gene in nonsyndromic OCA and autosomal recessive ocular albinism patients from Iran.

METHODS

Twenty three Iranian albinism patients, including 2 autosomal recessive ocular albinism and 21 oculocutaneous albininsm patients from unrelated families, were recruited for this study. Features such as hypopigmentation of the hair, skin, and iris; nystagmus; strabismus; poor vision; photophobia; and foveal hypoplasia were observed in our patients. The affected individuals were examined by ophthalmologists, and syndromic forms of OCA were excluded. Informed written consent was

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Table 1. Primers designed for amplifying the *TYR* exons and intron-exon boundaries.

Primer name	Sequence	Amplicon size	Annealing temperature (°C)
TYR-1F	TTAACTGGGTTTGCTTAGGTC	1230 bp	57
TYR-1R	TATACCCTGCCTGAAGAAGTG		
TYR-2F	CTCCTCAGGAGAAGTCTAAC	429 bp	58
TYR-2R	AACTCAGAAAATTCTGAATTC		
TYR-3F	ACACACTGGGTATCCAGAATG	430 bp	57
TYR-3R	ACAATAGACTACCATAACTTCTTAGC		
TYR-4F	TCAAGGCCTGAAAGAATAAACTA	570 bp	60
TYR-4R	GCCTATGTTAAGCAAAATGACC		
TYR-5F	TGTCTACTCCAAAGGACTGT	918 bp	58
TYR-5R	ACTTAGCTGGATGTGTTATAGA		

obtained from all patients and their parents for carrying out research on their specimens. The study was approved by the local ethics committee. Genomic DNA was extracted from peripheral whole blood of the patients and their parents using the salting out method.

The primers were designed for all five exons and intron-exon boundaries of the *TYR* gene (NG_008748.1) using gene runner software and also the primer blast website (NCBI) to prevent amplifying the *TYRL* pseudogene, which is similar to the 3′ half of this gene (Table1). These primers were used for PCR amplification and cycle sequencing.

The PCR condition was: 1x PCR buffer, 2 mM $MgCl_2$, 0.2 mM of each dNTPs, 0.25 µM of each primers, 0.25 unit/25 µl CinnaGen Taq DNA polymerase, and 50–100 ng template DNA in 25 µl final volume. PCR was performed as: 95 °C for 5 min (pre-denaturation), 94 °C for 40 s, annealing temperature (according to Table 1) for 40 s, 72 °C for 40 s for 26 cycles, and 72 °C for 10 min (final extension). Cycle sequencing was performed by Macrogen (Seoul, Korea). New missense mutations were analyzed using SIFT, PolyPhen, I Mutant 2, and Mutation Taster tools, and were interpreted based on American College of Medical Genetics standards and guidelines [12].

RESULTS

TYR sequences were studied in 23 albinism (2 autosomal recessive ocular albinism and 21 nonsyndromic OCA) patients. Mutations were found in 14 OCA individuals; no mutations were found in OA patients. The heterozygosity of the patients parents for the identified mutations was confirmed. Eleven patients were homozygous for *TYR* mutations, two were compound heterozygous, and one had only one detected mutation (Table 2).

One of the patients (patient 15) was heterozygous for two novel mutations that included one missense (p.G47S) and one frameshift (c.del1276–82) mutation, as well as two polymorphisms of p.S192Y and p.R402Q. One patient was homozygous for another novel mutation (p.P301L).

The p.R402Q and p.S192Y polymorphisms were detected in six and five cases, respectively.

DISCUSSION

In this study, we performed mutation analysis of the *TYR* gene on 23 unrelated albinism patients, including 2 autosomal recessive ocular albinism and 21 nonsyndromic OCA patients. We found 10 different mutations in 14 out of 23 (app. 60%) albinism patients. No mutations were found in OA patients, although one of them was heterozygous for the p.R402Q polymorphism.

Eight missense (p.M332I, p.R77Q, p.P21S, p.G419R, p.P301L, p.P406L, p.R239W, and p.G47S) and two frameshift (c.286dupA and c.del1276–82) mutations were found in this study, and no mutation was found in exons 3 and 5. The frequency percentage of the detected mutations and polymorphisms (p.S192Y and p.R402Q) are shown in Table 3.

Patient 15 had two nonreported mutations (p.G47S and c.del1276–82), as well as both p.R402Q and p.S192Y polymorphisms, all in heterozygous forms. To our knowledge, the p.G47S (c.139G>A) has not been previously reported, although pathogenic mutations have been previously reported for codon 47 (p.G47D and p.G47V) [11,13-15]. Tools such as SIFT, Polyphen-2, I Mutant 2, and Mutation Tasters predict p.G47S as a disease-causing mutation (Table 4). According to the ACMG standards and guidelines for the interpretation of sequence variants, this novel mutation is likely pathogenic. Our second novel mutation, c.del1276–82, is a frameshifting mutation and causes a termination signal in the 57th codon.

Table 2. *TYR* mutations and polymorphisms (p.S192Y and p.R402Q) in 23 OCA / OA Iranian patients.

#P	Clinical diagnosis	Female/ Male	Proband				Type of OCA	Consan-guinity
			Mutation 1	Mutation 2	Polymorphisms			
					p.S192Y	p.R402Q		
1	OCA	F	c.286dupA	c.286dupA	-	-	OCA1A	+
2	OCA	F	p.P406L (Maternal)	-	-	-	ND	+
3	OA	F	-	-	-	Hetero (Maternal)	ND	+
4	OCA	F	-	-	Hetero (Maternal)	Hetero (Paternal)	ND	+
5	OCA	M	p.M332I	p.M332I	-	-	OCA1B	+
6	OCA	M	-	-	Hetero	-	ND	+
7	OCA	F	p.M332I	p.M332I	-	-	OCA1B	+
8	OCA	F	-	-	-	Homo	ND	+
9	OCA	M	p.R239W (Paternal)	p.M332I (Maternal)	-	-	ND	ND
10	OCA	F	-	-	-	-	ND	+
11	OCA	M	c.286dupA	c.286dupA	-	-	OCA1A	+
12	OCA	F	-	-	-	-	ND	+
13	OCA	F	c.286dupA	c.286dupA	-	-	OCA1A	+
14	OCA	M	p.R77Q	p.R77Q	-	Homo	OCA1A	+
15	OCA	F	p.G47S (Paternal)	c.del1276–82 (Maternal)	Hetero	Hetero (Paternal)	OCA1A	+
16	OCA	F	p.P21S	p.P21S	-	-	OCA1A	+
17	OCA	M	p.R77Q	p.R77Q	-	Homo	OCA1A	+
18	OCA	M	p.G419R	p.G419R	-	-	OCA1A	+
19	OCA	F	p.P301L	p.P301L	-	-	OCA1A	+
20	OCA	M	-	-	Hetero	-	ND	ND
21	OCA	F	c.286dupA	c.286dupA	-	-	OCA1A	+
22	OCA	F	-	-	Homo	-	ND	+
23	OA	F	-	-	-	-	ND	+

ND: not determined. The type of OCA1 is delineated based on the hair color with increasing age (white in OCA1A and yellow/blonde in OCA1B).

According to the ACMG standards and guidelines, this frameshift mutation is interpreted to be pathogenic.

Patient 19 was homozygous for another novel mutation (p.P301L). No mutation has been reported in this codon before. Mentioned bioinformatics tools predicted p.P301L to be a disease-causing mutation (Table 4). According to the ACMG standards and guidelines, this novel mutation is likely pathogenic.

In this study, nine patients did not show any causative mutations in the *TYR* gene. This may be due to the involvement of other OCA genes, variants in the promoter or other regulatory elements that were not covered in this study, or deletions/duplications of the complete gene or single exons that are not detectable by the cycle sequencing procedure.

The contribution of the p.R402Q variant to the albinism phenotype has been heavily disputed in the literature [7,16]. According to our study, it doesn't seem to be pathogenic. In Figure 1, Figure 2, and Figure 3, the pedigrees of our three OCA patients (patients 4, 9, and 15) are in support of this phenomenon. Nonpathogenicity of p.S192Y can also be inferred from these pedigrees. Since the parents of patient 4 are both homozygous for p.R402Q and p.S192Y, respectively, and do not show the albinism phenotype, none of these variants could be considered pathogenic in the homozygous state

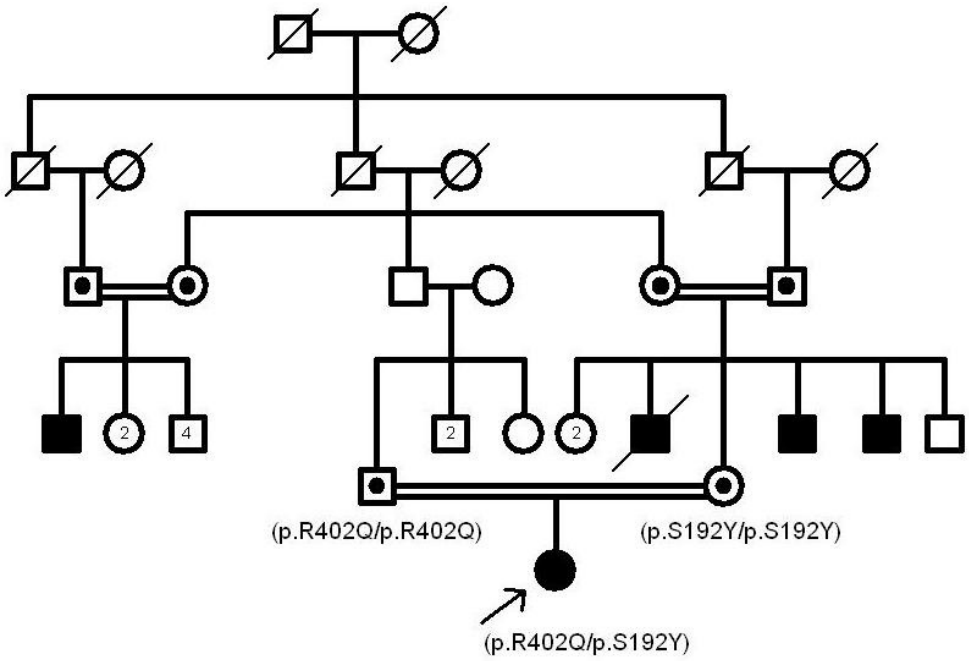

Figure 1. The pedigree of patient 4. The nonpathogenic nature of p.R402Q and p.S192Y can be inferred from the above pedigree in which the patient's parents do not show any albinism features.

(Figure 1). Also, p.S192Y is not pathogenic in heterozygous form in combination with other mutations such as p.R239W or p.M332I in the parents of patient 9 (Figure 2). Moreover, according to the pedigree of patient 15, both p.S192Y and p.R402Q, together with p.G47S, are not pathogenic (Figure 3).

In Table 2, the patients with identified mutations in the *TYR* gene are classified in two types of OCA1A and OCA1B

Table 3. The frequency of the 10 mutations and the p.R402Q and p.S192Y polymorphisms in our 23 albinism patients.

Nucleotide change	Amino acid change	Exon No.	Status (Number of the patients)	Frequency percentage
c.286dupA	Frameshift	Ex 1	Homo (4)	17.39
c.61C>T	p.P21S	Ex 1	Homo (1)	4.34
c.139G>A	p.G47S	Ex 1	Hetero (1)	2.17
c.230G>A	p.R77Q	Ex 1	Homo (2)	8.69
c.715C>T	p.R239W	Ex 1	Hetero (1)	2.17
c.902C>T	p.P301L	Ex 2	Homo (1)	4.34
c.0996G>A	p.M332I	Ex 2	Homo (2), Hetero (1)	10.86
c.1217C>T	p.P406L	Ex 4	Hetero (1)	2.17
c.1255G>A	p.G419R	Ex 4	Homo (1)	4.34
c.del1276–82	Frameshift	Ex 4	Hetero (1)	2.17
c.575C>A	p.S192Y	Ex 1	Homo (1), Hetero (4)	13.04
c.1205G>A	p.R402Q	Ex 4	Homo (3), Hetero (3)	19.56

Table 4. Results of the analysis of the novel missense mutations with the bioinformatics tools.

Novel missense mutations	Polyphen-2		SIFT		I Mutant 2	Mutation Taster
	Prediction	Score	Prediction	Score	Prediction (sign of DDG)	
p.G47S	Probably damaging	1.000	damaging	0	Decrease stability	disease causing
p.P301L	Probably damaging	1.000	damaging	0	Decrease stability	disease causing

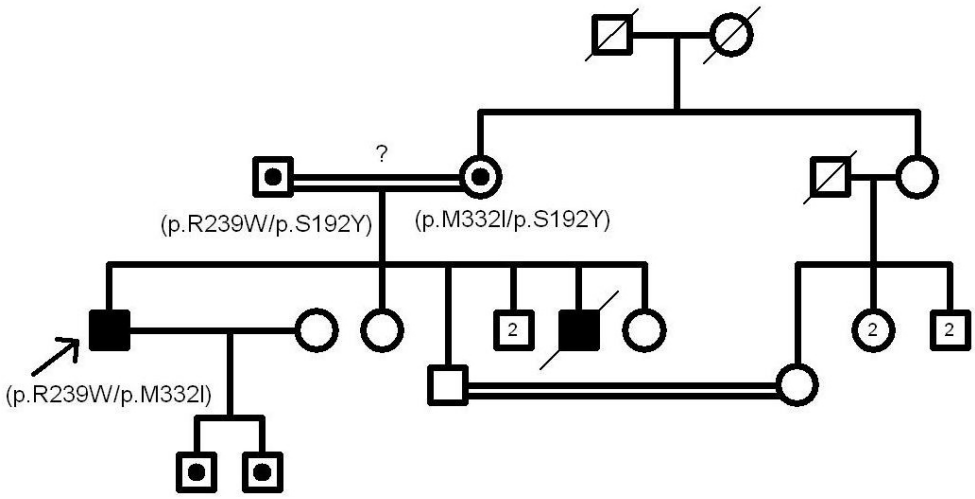

Figure 2. The pedigree of patient 9. p.S192Y is not pathogenic in heterozygous form in combination with p.R239W or p.M332I in the patient's parents.

based on their hair color with increasing age. Patients with permanent white hair are diagnosed to have OCA1A, and patients with white hair at birth and yellow/blonde coloration later in their life are classified as OCA1B. In patient 2, the hair color was white at birth and yellow afterwards, but because we did not find her second mutation and digenic OCA is reported [4], we couldn't determine her type of OCA.

Here we found homozygous *TYR* mutations in 11 patients whose parents had consanguineous marriage (Table 2). This suggests that the disease-causing mutations in these patients are identical by descent. As reported previously, the overall rate of consanguineous marriage is about 38.6% in Iran, and this can increase the probability of incidence of genetic disorders due to common ancestors [17,18].

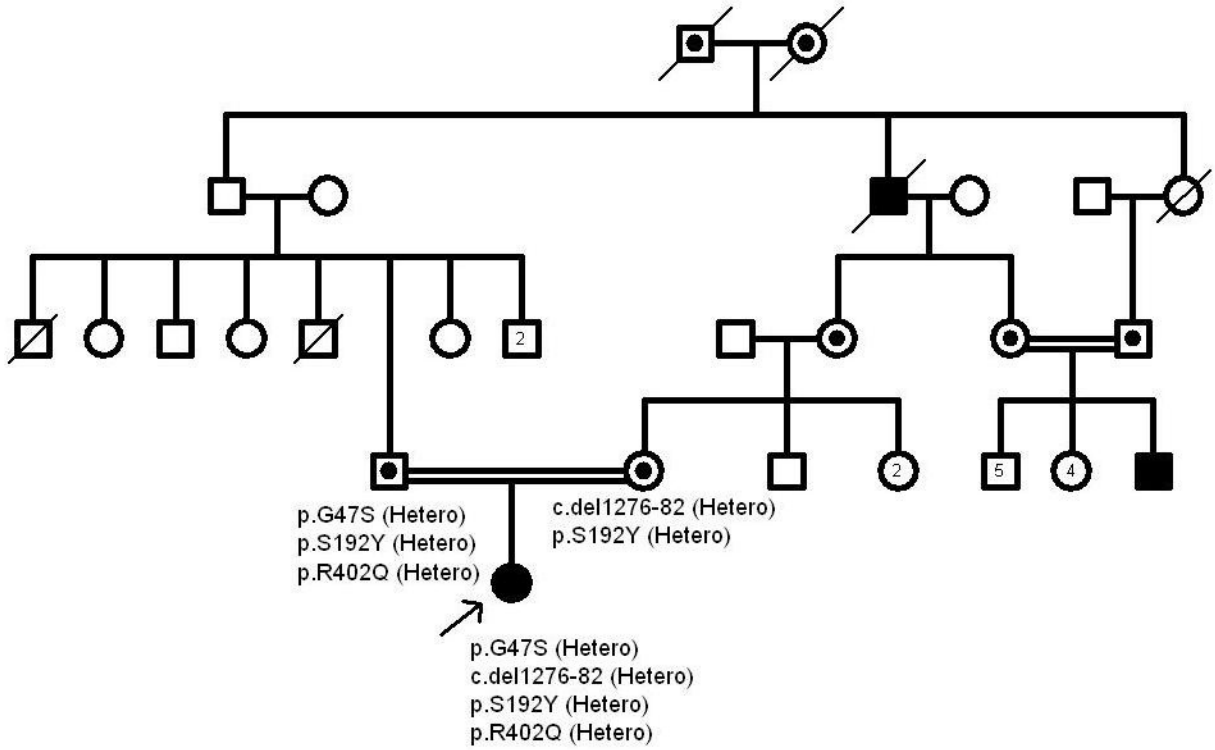

Figure 3. The pedigree of patient 15. Both p.S192Y and p.R402Q together with the p.G47S are not pathogenic in the patient's father according to the above pedigree.

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