Am. J. Hum. Genet. 54:586–594, 1994

Mutations of the Tyrosinase Gene in Patients with Oculocutaneous Albinism from Various Ethnic Groups in Israel

Ruth Gershoni-Baruch,* Ada Rosenmann,† Sara Droetto,‡ Stuart Holmes,‡ Ram K. Tripathi,‡ and Richard A. Spritz‡

*Department of Pediatrics and Medical Genetics, Rambam Medical Center, Technion-Israel Institute of Technology, Haifa; †Michaelson Institute for the Prevention of Blindness, Hadassah Medical Center, Jerusalem; and ‡Departments of Medical Genetics and Pediatrics, Laboratory of Genetics, University of Wisconsin, Madison

Summary

We have analyzed the tyrosinase (TYR) gene in 38 unrelated patients with oculocutaneous albinism (OCA), derived from several different ethnic groups of the diverse population of Israel. We detected TYR gene mutations in 23 of the 34 patients with apparent type I (i.e., tyrosinase-deficient) OCA and in none of the patients with other clinical forms of albinism. Among Moroccan Jews with type IA (i.e., tyrosinase-negative) OCA, we detected a highly predominant mutant allele containing a missense substitution, Gly47Asp (G47D). This mutation occurs on the same haplotype as in patients from the Canary Islands and Puerto Rico, suggesting that the G47D mutation in these ethnically distinct populations may stem from a common origin.

Introduction

Oculocutaneous albinism (OCA) is a heterogeneous group of genetic disorders of melanin pigmentation that have been observed in virtually all human populations. Deficient melanin pigmentation of the skin results in severe photosensitivity and predisposition to skin cancer. Deficient melanin pigment in the developing visual neural pathways results in foveal hypolasia and reduced visual acuity, nystagmus, and strabismus (reviewed in Witkop et al. 1989). In type I (i.e., tyrosinase-deficient) OCA, reduced or absent melanin biosynthesis is the result of deficient catalytic activity of tyrosinase (monophenol monooxygenase; monophenol, L-dopa:oxygen oxidoreductase; E.C.1.14.18.1). Tyrosinase is a copper-containing enzyme that catalyzes the rate-limiting first two steps in the melanin biosynthetic pathway, the oxidation of tyrosine to dopa, and the subsequent dehydrogenation of dopa to dopaquinone (Lerner and Fitzpatrick 1950). In classic, type IA (i.e., tyrosinase-negative) OCA, tyrosinase activity and melanin biosynthesis are entirely absent, whereas in type IB (i.e., "yellow") OCA, tyrosinase activity and melanin biosynthesis are greatly reduced (reviewed in Spritz 1993).

Tyrosinase is a 58-kD glycoprotein composed of 529 amino acids (Kwon et al. 1987; Shibahara et al. 1988). The human tyrosinase (TYR) gene consists of five exons spanning ~50 kb of DNA (Giebel et al. 1991*b*) in chromosome segment 11q14-q21 (Barton et al. 1988). To date, at least 44 different pathologic mutations of the TYR gene have been reported in patients with OCA from several different ethnic groups (reviewed in Oetting and King 1993; Spritz 1993; Tripathi et al. 1993), although the great majority of these patients have been Caucasian.

We have analyzed the TYR gene in 38 unrelated patients with OCA from Israel. Among these patients, we identified 10 different TYR gene mutations, 7 of which are novel, and 1 novel silent polymorphism. Among Moroccan Jews with type I OCA, one mutant allele is highly predominant, greatly simplifying efforts at car-

Received September 30, 1993; accepted for publication December 7, 1993.

Address for correspondence and reprints: Dr. Ruth Gershoni-Baruch, Department of Pediatrics and Medical Genetics, Rambam Medical Center, P.O. Box 9602, Haifa, 31096 Israel.

0002-9297/94/5404-0002$02.00

Table 1

TYR Gene Mutation Analysis in 38 Israeli Patients with OCA

Patient	OCA Type	Ethnic Group	Age (years)	Sex	Consanguinity	Mutation[a]
1	IA	Moroccan Jew	7	F	+	G47D/G47D
2	IA	Moroccan Jew	33	M	+	G47D/G47D
3	IA	Moroccan Jew	15	M	−	G47D/G47D
4	IA	Moroccan Jew	5	M	−	G47D/G47D
5	IA	Moroccan Jew	37	M	+	G47D/G47D
6	IA	Moroccan Jew	29	M	+	G47D/G47D
7	IA	Moroccan Jew	29	F	−	G47D/G47D
8	IA	Moroccan Jew	28	M	−	G47D/G47D
9	IA	Moroccan Jew	17	F	−	G47D/G47D
10	IA	Moroccan Jew	21	F	−	G47D/None
11	IA	Moroccan/Tunisian Jew	5	M	−	R278X/None
12	IA	Tunisian Jew	16	M	+	None
13	IA	Tunisian Jew	31	F	−	None
14	IA	Libyan Jew	42	M	−	T373K/None
15	IA	Libyan Jew	34	M	−	T373K/None
16	IA	Libyan Jew	29	F	+	None
17	IA	Ashkenazi/Tunisian/Sephardic Jew	14	M	−	G47D/217ΔC
18	IA	Ashkenazi Jew	26	M	−	None
19	IA	Moroccan/Iraqi Jew	19	F	−	None
20	IA	Arab Christian	17	M	+	R299H/R299H
21	IA	Arab Christian	42	M	+	None
22	IA	Arab Christian	31	M	+	R402X/R402X
23	IA	Arab Christian	13	F	+	R402X/R402X
24	IA	Arab Christian	2	M	+	S50X/S50X
25	IA	Arab Moslem	7	F	+	None
26	IA	Arab Moslem	7	M	+	None
27	IB	Moroccan Jew	14	F	−	G47D/None
28	IB	Moroccan Jew	4	M	−	G47D/None
29	IB	Moroccan Jew	32	F	−	G47D/None
30	IB	Moroccan/Sephardic Jew	8	M	−	E294K/IVS2 −7A
31	IB	Ashkenazi Jew	35	M	+	None
32	IB	Ashkenazi Jew	11	F	−	P152S/A355A
33	IB	Arab Moslem	40	F	−	None
34	IB	Arab Moslem	13	M	−	None
35	II	Ashkenazi Jew	10	F	−	None
36	II	Ashkenazi Jew	22	F	−	None
37	II	Arab Moslem	4	M	+	None
38	OA	Ashkenazi Jew	45	M	−	None

[a] Novel mutations are indicated by underlining.

rier detection and prenatal diagnosis in this particular highly inbred ethnic group.

Subjects, Material, and Methods

Subjects

Thirty-eight unrelated patients from several different Israeli ethnic groups (table 1) were classified as to apparent OCA type, principally on the basis of clinical criteria. Assays of skin or hairbulb tyrosinase activity (Witkop et al. 1989) were available only for patients 1, 9, 23, 25, 26, 34, and 35.

PCR Amplification of Human TYR Gene Segments

DNA was isolated from peripheral blood leukocytes by standard procedures. DNA fragments corresponding to the five TYR gene exons plus adjacent noncoding and flanking sequences (Giebel et al. 1991*b*) were am-

plified in duplicate from 0.1 μg genomic DNA of each proband by 35 cycles of the PCR (Saiki et al. 1988), as described elsewhere (Giebel et al. 1991*a*).

SSCP/Heteroduplex (HDX) and DNA Sequence Analyses

For patients 1–3, 26, and 32 the products of the duplicate PCR amplifications were pooled for each of the five TYR exons, purified in 4% polyacrylamide gels, eluted, and cloned in M13mp19. The nucleotide sequences of at least six independent clones per exon were determined (Sanger et al. 1977). For all other patients the duplicate PCR products for each exon were pooled and were screened by combined SSCP/HDX analysis using MDE gels (AT Biochemical) as described elsewhere (Spritz et al. 1992). For TYR exons 2–5 the PCR products were analyzed directly. For exon 1 the 1,060-bp PCR product was first cleaved by *Taq*I and *Pst*I to 520-, 385-, and 130-bp fragments, which were then analyzed as a mixture. In some cases exon 5 was also analyzed as two overlapping fragments, 422 and 433 bp in size, amplified separately as described elsewhere (Tripathi et al. 1993). TYR exon segments exhibiting aberrant SSCP/HDX patterns were independently reamplified in duplicate, purified, cloned, and sequenced as described above.

Restriction-Enzyme Cleavage Analysis

The G47D mutation abolishes a *Hae*III cleavage site (GGCC). To confirm SSCP/HDX and/or DNA sequence demonstration of the G47D mutation in patients 1–10, 17, and 27–29, a 293-bp fragment containing part of exon 1 was amplified by the PCR using as primers 5′-ATGCTCCTGGCTGTTTTGTACT-3′ and 5′-AGTTTCCACAGTTGAATCCC-3′. These fragments were then cleaved with *Hae*III and were analyzed by electrophoresis in either 6% polyacrylamide or 2% agarose gels.

Similarly, the E294K mutation both abolishes an *Ava*I site and creates a novel *Sty*I site. Both of these enzymes were used to confirm heterozygosity for this mutation in the exon 2 fragments amplified from DNA of patient 30 and his mother.

Polymorphism Haplotype Analysis

Three common DNA polymorphisms in or near the TYR gene were used for haplotype analysis of patients 1–8, 14, 15, 17, 22, 23, 28, and 30. A *Taq*I RFLP resulting from a C/A polymorphism in the promoter region (Oetting et al. 1991*b*) was analyzed by amplifying a 784-bp fragment by the PCR using as primers 5′-GCTCTTTAACGTGAGATATC-3′ and 5′-GGAGACACAGGCTCTAGGGA-3′ (Giebel et al. 1991*b*), digestion with *Taq*I, and electrophoresis in 2% agarose gels. An *Mbo*I RFLP resulting from a C/A polymorphism within codon 192 was analyzed as described elsewhere (Giebel and Spritz 1990). A *Bgl*II RFLP within the first intervening sequence (Spritz and Strunk 1990) was analyzed by cleavage of genomic DNAs by *Bgl*II and Southern blot hybridization (Southern 1975) using as probe cloned human TYR cDNA pMEL34 (Kwon et al. 1987). For patients 14 and 15 only, exon 4 was subjected to DNA sequence analyses to analyze an A/G polymorphism within codon 402 (Tripathi et al. 1991).

Results

Patients with Type IA OCA

As shown in table 1, patients 1–10 were all Moroccan Jews with clinical type IA OCA, with no apparent pigmentation of the skin, hair, or eyes and with low vision and nystagmus. L-DOPA incubation assay performed on the skin of patients 1 and 9 showed no apparent tyrosinase enzymatic activity. SSCP/HDX and DNA sequence and/or *Hae*III cleavage analyses showed that patients 1–9 were all homozygous for the same missense substitution, codon 47 GGC (Gly)→GAC (Asp) (G47D) (fig. 1*A*). Patient 10 was heterozygous for this mutation; no abnormality was detected on the other allele. The G47D mutation has been identified previously, in a single Caucasian patient (Oetting et al. 1991*a*) and in Hispanic patients from Puerto Rico and the Canary Islands (Oetting et al. 1993).

Patient 11 was a Moroccan/Tunisian Jew with type IA OCA. SSCP/HDX and DNA sequence analyses showed that he was heterozygous for a novel nonsense mutation, codon 278 CGA (Arg)→TGA (TER) (R278X) (fig. 1*B*). No abnormality was detected on the other allele. SSCP/HDX analysis of DNA of his mother, a Moroccan Jew, showed that she was also heterozygous for this substitution. Thus, the paternal (Tunisian) allele of patient 11 apparently contained either an occult mutation or a TYR gene deletion that was invisible to our PCR-based analyses.

Patients 12 and 13 were both Tunisian Jews with type IA OCA. SSCP/HDX analysis detected no abnormalities in either of these patients.

Patients 14–16 were Libyan Jews with type IA OCA. SSCP/HDX and DNA sequence analyses demonstrated that patients 14 and 15 were heterozygous for the same

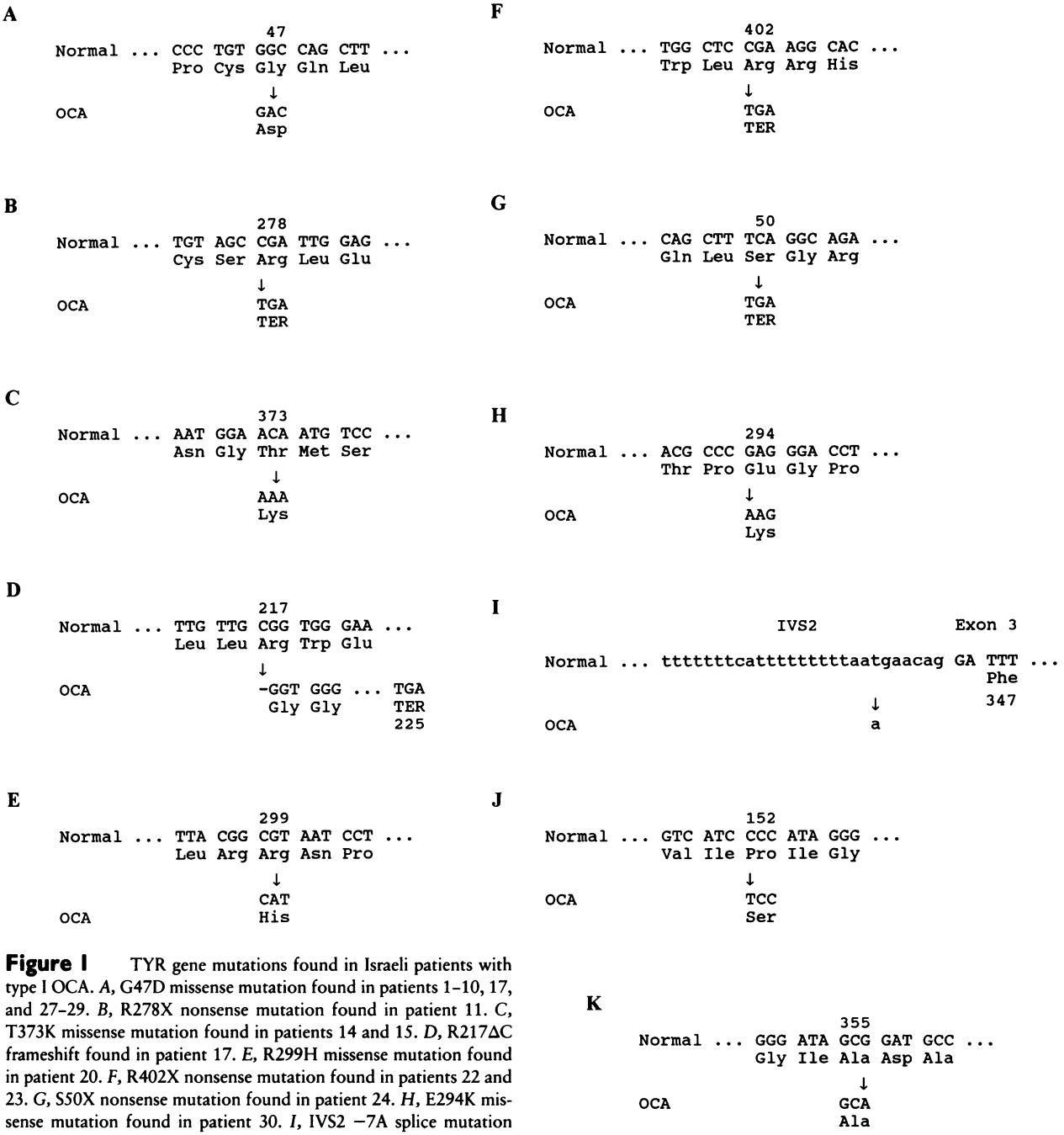

Figure 1 TYR gene mutations found in Israeli patients with type I OCA. *A*, G47D missense mutation found in patients 1–10, 17, and 27–29. *B*, R278X nonsense mutation found in patient 11. *C*, T373K missense mutation found in patients 14 and 15. *D*, R217ΔC frameshift found in patient 17. *E*, R299H missense mutation found in patient 20. *F*, R402X nonsense mutation found in patients 22 and 23. *G*, S50X nonsense mutation found in patient 24. *H*, E294K missense mutation found in patient 30. *I*, IVS2 −7A splice mutation found in patient 30. *J*, P152S missense mutation found in patient 32. *K*, A355A polymorphism found in patient 32.

missense mutation, codon 373, ACA (Thr)→AAA (Lys) (T373K) (fig. 1C). This mutation has previously been observed in numerous Caucasian individuals (Spritz et al. 1990; Spritz 1993). No abnormalities were detected on their other alleles, and no abnormality was detected in patient 16.

Patient 17, also with type IA OCA, was of mixed Ashkenazi, Sephardic, and Tunisian Jewish origin.

SSCP/HDX and DNA sequence analyses demonstrated that he was an apparent compound heterozygote for the G47D mutation described above and a novel frameshift at codon 217, CGG (Arg)→GG (217ΔC) (fig. 1*D*). His family was not available for study.

Patient 18 was an Ashkenazi Jew, and patient 19 was a Moroccan/Iraqi Jew; both had type IA OCA. SSCP/HDX analysis detected no abnormalities in either case.

Patient 20 was an inbred Christian Arab with type IA OCA. SSCP/HDX and DNA sequence analyses demonstrated that he was homozygous for a missense mutation at codon 299, CGT (Arg)→CAT (His) (R299H) (fig. 1*E*). This substitution has been observed previously, in a single Caucasian patient with type IA OCA (Tripathi et al. 1992*b*).

Patient 21 was a Christian Arab with type IA OCA. SSCP/HDX analysis detected no abnormalities.

Patients 22 and 23 were both inbred Christian Arabs with type IA OCA. Tyrosinase assay of hairbulbs from patient 23 showed no detectable activity. SSCP/HDX and DNA sequence analyses demonstrated that both of these patients were homozygous for a novel nonsense mutation, codon 402 CGA (Arg)→TGA (TER) (R402X) (fig. 1*F*). It is interesting that this mutation occurs at a residue that is polymorphic (R402Q) among normal Caucasians (Tripathi et al. 1991), and the R402X and R402Q substitutions appear to result from reciprocal deamination events at a CpG within the common form of the codon, CGA.

Patient 24 was an inbred Christian Arab with type IA OCA. SSCP/HDX and DNA sequence analyses demonstrated that he was homozygous for another novel nonsense mutation, codon 50 TCA (Ser)→TGA (TER) (S50X) (fig. 1*G*). Both parents were heterozygous for this mutation.

Patients 25 and 26 were both Moslem Arabs with type IA OCA. L-DOPA incubation assay performed on the skin of both patients showed no detectable tyrosinase activity. SSCP/HDX analysis detected no abnormalities in patient 25, and complete DNA sequence analysis detected no abnormalities in patient 26.

Patients with Type IB OCA

Patients 27–29 were Moroccan Jews with type IB OCA. SSCP/HDX and DNA sequence and/or *Hae*III cleavage analyses demonstrated that all three were heterozygous for the G47D substitution, described above. We detected no abnormality of the other allele in any of these three patients.

Patient 30 was a Moroccan/Sephardic Jew with type IB OCA. SSCP/HDX and DNA sequence analyses demonstrated two different novel mutations. A missense mutation at codon 294, GAG→AAG, results in a Glu→Lys substitution (E294K) (fig. 1*H*) and was inherited from his mother, a Moroccan Jew. The other mutation is near the 3′ end of the second intervening sequence (IVS2), a t→a transition 7 bp upstream of the 3′ splice site (IVS2 −7A) (fig. 1*I*). This mutation creates a novel ag dinucleotide that most likely acts as a novel 3′ splice site for IVS2, interfering with normal splicing of tyrosinase pre-mRNA and resulting in an mRNA containing a frameshift. This is very similar to several mutations known to cause β^+-thalassemia (Spritz et al. 1981; Weatherall et al. 1989) and is the first apparent RNA splicing mutation described in association with albinism. We have also observed the IVS2 −7A mutation in four other patients of northern European origin, all with type IB OCA (authors' unpublished data).

Patients 31 and 32 were European Ashkenazi Jews with clinically apparent type IB OCA. SSCP/HDX analysis detected no abnormalities in patient 31. However, SSCP/HDX and DNA sequence analyses in patient 32 demonstrated two novel mutations. A missense substitution at codon 152, CCC→TCC, results in a Pro→Ser substitution at this site (P152S) (fig. 1*J*). A substitution at codon Ala355, GCG→GCA, is silent (A355A) (fig. 1*K*), and we detected no additional abnormalities. The family of patient 32 was not available for study; therefore, we could not determine whether these two mutations were in *cis* or in *trans*. Amino acid 152 is distant from the two segments of the tyrosinase polypeptide in which amino acid substitutions typically result in type IA OCA, possibly accounting for the relatively mild phenotype apparently associated with the P152S substitution. It will thus be important to test the effect of this substitution on tyrosinase stability and catalytic activity, by use of in vitro functional assays (Tripathi et al. 1992*a*).

Patients 33 and 34 were Moslem Arabs with type IB OCA. Assay of hairbulb tyrosinase in patient 34 showed no enzymatic activity. SSCP/HDX analyses detected no abnormalities in either of these patients.

Patients with Type II (Tyrosinase-positive) OCA

Patients 35 and 36 were Ashkenazi Jews, and patient 37 was a Moslem Arab; all three had clinically apparent type II OCA. Assay of hairbulb tyrosinase in patient 35 showed normal activity. SSCP/HDX analyses detected no abnormalities of the TYR gene in any of these three patients, consistent with our observation of mutations

Table 2

Haplotype Analysis of TYR Gene Mutations in Israeli Patients with Type I OCA

Patient[a]	Ethnic Group	Mutation	Polymorphism[b]			Haplotype[c]
			−199	192	*Bgl*II	
I1	Moroccan Jew	G47D/G47D	+/+	+/+	+/+	1,9/1,9
I2	Moroccan Jew	G47D/G47D	+/+	+/+	+/+	1,9/1,9
I3	Moroccan Jew	G47D/G47D	+/+	+/+	+/+	1,9/1,9
I4	Moroccan Jew	G47D/G47D	+/+	+/+	+/+	1,9/1,9
I5	Moroccan Jew	G47D/G47D	+/+	+/+	+/+	1,9/1,9
I6	Moroccan Jew	G47D/G47D	+/+	+/+	+/+	1,9/1,9
I7	Moroccan Jew	G47D/G47D	+/+	+/+	+/+	1,9/1,9
I8	Moroccan Jew	G47D/G47D	+/+	+/+	+/+	1,9/1,9
I17	Mixed Jewish	G47D/217ΔC	+/+	+/+	+/−	1,9/1,3
I28	Moroccan Jew	G47D/?		+/−	+/+	Indeterminate
I14	Libyan Jew	T373K/?	+/+	+/−	+/−	Indeterminate
I15	Libyan Jew	T373K/?	+/+	+/−	+/−	Indeterminate
US1	Northern European	T373K/D383N	+/+	−/−	−/−	4,12/4,12
US2	Northern European	T373K/N382K	+/+	−/−	−/−	4,12/4,12
US3	Northern European	T373K/R422Q	+/+	−/−	−/−	4,12/4,12
US4	Northern European	T373K/G446S	+/+	+/−	+/−	Indeterminate
US5	Northern European	T373K/G446S	+/+	+/−	+/−	Indeterminate
US6	Northern European	T373K/?	+/+	+/−	+/−	Indeterminate
I22	Arab Christian	R402X/R402X	−/−	+/+	−/−	7,15/7,15
I23	Arab Christian	R402X/R402X	−/−	+/+	−/−	7,15/7,15
US7	Northern European	R402X/R402X	−/−	+/+	−/−	7,15/7,15
I11	Mixed Jewish	R278X/?	+/+	+/+	+/+	1,9,17/1,9,17
G1	Guyanan	R278X/?	+/+	+/−	+/−	
I30	Mixed Jewish	IVS2 −7A/E294K	+/−	+/+	−/−	3,11/7,15
US8	Northern European	IVS2 −7A/V275F	+/−	+/+	+/−	Indeterminate
US9	Northern European	IVS2 −7A/S380P	+/+	+/+	+/−	3,11/1,9,17

[a] I = Israeli; US = United States; and G = Guyanan.

[b] A plus sign (+) indicates the presence of the polymorphic *Taq*I (−199), *Mbo*I (192), and *Bgl*II sites; − indicates absence of the site; and a question mark (?) indicates that no mutation was identified on one allele in some patients.

[c] Assigned according to the method of Oetting et al. (1993); because one polymorphism used by those authors was not analyzed, multiple alternative haplotypes are indicated.

of the P gene in many patients with type II OCA (Lee et al., in press).

Patient with Ocular Albinism

Patient 38 was an Ashkenazi Jew with clinically apparent ocular albinism; the family history was negative. SSCP/HDX analysis detected no apparent abnormalities of the TYR gene.

Haplotype Analysis

Patients 1–8 were all Moroccan Jews homozygous for the G47D substitution. As shown in table 2, all of these patients (I1–I8) were homozygous for a +++ TYR polymorphism haplotype. Patient 17, of mixed Jewish ancestry, was heterozygous for the G47D mutation and was also an obligate heterozygote for the +++ haplotype. In patient 28, a Moroccan Jew heterozygous for the G47D mutation, haplotype analysis was indeterminate but was consistent with this assignment. Thus, in all patients analyzed, the G47D mutation was apparently associated with a +++ haplotype.

Israeli patient 11, of mixed Jewish origin, was heterozygous for the R278X nonsense mutation. We have also observed this substitution in a patient of Guyanan origin (Tripathi et al. 1993). As shown in table 2, Israeli patient I11 was homozygous for the +++ haplotype. Haplotype analysis of the Guyanan patient G1 was indeterminate; however, haplotype analysis of his mother demonstrated that the R278X mutation was associated with the +++ haplotype. Therefore, the R278X muta-

tion appears to be associated with the same haplotype in the Guyanan and Israeli patients.

Patients 14 and 15 were both Libyan Jews heterozygous for the T373K mutation. This mutation is relatively common among patients of northern European origin (Spritz et al. 1990; Spritz 1993). As shown in table 2, the T373K mutation was associated with the +−− haplotype in Caucasian patients US1, US2, and US3. The haplotype could not be determined in Israeli patients I14 and I15, but it is consistent with this assignment. DNA sequence analysis of PCR-amplified exon 4 demonstrated that Israeli patients 14 and 15 were both heterozygous at polymorphic codon 402 (Tripathi et al. 1991); elsewhere we have shown that the T373K mutation is associated with 402CGA (Spritz et al. 1990; Tripathi et al. 1992*b*) in Caucasians.

Patients 22 and 23 were both inbred Arab Christians homozygous for a novel nonsense mutation, R402X. As shown in table 2, both patients were homozygous for the −+− haplotype. We have also observed the R402X mutation in two Italian patients with type IA OCA. As shown in table 2, one of these individuals, patient US7, who was homozygous, by descent, for this mutant allele (authors' unpublished data), was also homozygous for the −+− haplotype. Thus, the R402X nonsense mutation appears to be associated with the same haplotype in the Italian and Arab Christian patients.

Israeli patient 30 was a compound heterozygote for two novel mutations, E294K and IVS2 −7A. We have also observed the IVS2 −7A mutation in four individuals of northern European origin. Haplotype analysis in Israeli patient I30 and his (Moroccan Jewish) mother, who carried the E294K mutation, and in two of the American patients (US8 and US9) was indeterminate. However, the data are consistent with the three patients sharing a common haplotype (++−).

Discussion

We have screened for mutations of the TYR gene in 38 Israeli patients with OCA. As shown in table 1, these patients derived from a variety of different ethnic groups, including Moroccan, Tunisian, Libyan, Sephardic (Spanish), and Ashkenazi (European) Jews, as well as Arab Christians and Moslems. The majority of patients were classified on the basis of clinical criteria, as assays of tyrosinase activity were not available for most.

Of the 34 patients with apparent type I OCA, we detected mutations of the TYR gene in 23 (68%). This is comparable to the frequencies of TYR gene mutations detected in patients of Caucasian and of Indo-Pakistani ethnic origins (Spritz 1993). Seven of the mutations that we detected among these Israeli patients have not been described previously, and some may thus be unique to these groups. However, some of the mutations that we detected have also been identified in other populations. Our observation of different prevalent mutant alleles in each Israeli subpopulation bears interpretation in light of the different histories of those groups.

Among Israeli patients of Moroccan Jewish origin, the G47D mutant allele (fig. 1*A*) is highly predominant. Of the 18 independent obligate type IA OCA alleles sampled among the Moroccan Jewish patients studied here, 15 (83%) contained the G47D mutation. In addition, patients 27–29, who were also of Moroccan Jewish origin but had type IB OCA, all were heterozygous for the G47D mutation. The G47D mutation is extremely rare among patients of northern European Caucasian origin (Spritz 1993); indeed, it has been observed in only one family (Oetting et al. 1991*a*). Recently, however, Oetting et al. (1993) identified the G47D mutation in a large number of Hispanic patients from Puerto Rico and in a patient from the Canary Islands, a territory of Spain.

The history of the Sephardic (Spanish) and Moroccan Jewish populations may provide clues to the origin and spread of the G47D mutation. The majority of the Moroccan Jewish population was indigenous, having arrived during antiquity. Some migration of Sephardic Jews to northern Africa occurred by the 7th century (Beinart 1992)—and again in 1391 and in 1492, when the Sephardic Jews were expelled from Spain to various other countries around the Mediterranean (Morocco, Libya, Tunisia, Portugal, Italy, Turkey, and the Balkans) (Gilbert 1993). In addition, during the 16th and 17th centuries Morocco became a haven for conversos (Jews converted to Christianity) fleeing Spain, Portugal, Madeira, and the Canary Islands. The close geographic proximity and political relationship of Spain to Morocco and the Canary Islands, the exploration of Puerto Rico by Spanish sailors, and the historical population migration from the Canary Islands to Puerto Rico strongly suggest that the G47D mutations in all of these populations derive from a common origin. This is supported by our observation of the G47D substitution in a patient of mixed Sephardic Jewish origin (patient 17) and by the fact that the G47D mutation appears to be associated with the same RFLP haplotype in Moroccan Jews (table 2) as in patients from the Canary Islands,

Puerto Rico, and the United States (Oetting et al. 1993). The G47D mutant allele may have existed in the indigenous Moroccan Jewish population, or it may have been introduced subsequently, most likely by Sephardic Jews or Spanish sailors.

In contrast, we did not detect the G47D mutation among any of the Israeli patients from other Jewish subgroups of the Mediterranean basin. However, two of the three Libyan Jewish patients with type IA OCA whom we studied were heterozygous for a different missense mutation, T373K (fig. 1C). This mutation accounts for ~14% of type I OCA alleles among northern European Caucasian patients (Spritz et al. 1990; Spritz 1993). Although not conclusive, the haplotype data were consistent with origination of the T373K mutation in the Libyan Jewish and northern European populations from a common ancestor. Here, history is less helpful; principal Jewish settlement of Libya occurred during antiquity, and subsequent influx was relatively minor. The T373K mutant allele thus may have existed in the Jewish population during antiquity, or it may have been introduced to Libya by European seafarers.

We did not observe either the G47D or T373K mutant alleles among the small number of Tunisian Jews studied; in fact, we were not able to identify any abnormalities in these patients. Principal settlement of Jews in Tunisia occurred during antiquity. There was additional settlement during the 1391 emigration of Sephardic Jews from Spain, but very little following the Spanish expulsion of 1492. A minor Jewish influx occurred during the 17th century, principally from Italy (Livorno). Thus, the Jewish settlement of Tunisia was largely independent of those of Morocco or Libya.

Together, our data indicate that the principal type I OCA mutant alleles in the Moroccan, Libyan, and Tunisian Jewish populations were probably introduced independently. It is surprising that there appears to have been little genetic admixture of OCA alleles among these groups, despite considerable recorded contact. The very high prevalence of the G47D mutant allele among Moroccan Jewish and Puerto Rican patients with type IA OCA should facilitate carrier detection and prenatal diagnosis of type IA OCA in high-risk families derived from these ethnic groups.

Acknowledgments

We thank Dr. T. Falik-Borenstein for the sample from patient 24 and Lerer Israela for laboratory assistance. This work was supported by March of Dimes Birth Defects Foundation clinical research grant 6-408 and by National Institutes of Health grant AR 39892 (both to R.A.S.). This is paper 3383 from the Laboratory of Genetics, University of Wisconsin.

References

Barton DE, Kwon BS, Francke U (1988) Human tyrosinase gene mapped to chromosome 11 (q14-q21), defines second region of homology with mouse chromosome 7. Genomics 3:17–24

Beinart H (1992) The Jews in Spain: their origin to their expulsion. In: Porter R, Harrel-Hoshen S (eds) The Sephardic Jews 1492–1992. Beit Hatfuzot, pp 21–41

Giebel LB, Musarella MA, Spritz RA (1991*a*) A nonsense mutation in the tyrosinase gene of Afghan patients with tyrosinase negative (type IA) oculocutaneous albinism. J Med Genet 28:464–467

Giebel LB, Spritz RA (1990) RFLP for Mbo I in the human tyrosinase (TYR) gene detected by PCR. Nucleic Acids Res 18:3103

Giebel LB, Strunk KM, Spritz RA (1991*b*) Organization and nucleotide sequence of the human tyrosinase gene and a truncated tyrosinase related segment. Genomics 9:435–445

Gilbert M (1969) Atlas of Jewish history. Weidenfied & Nicholson, London

Kwon BS, Haq AK, Pomerantz SH, Halaban R (1987) Isolation and sequence of a cDNA clone for human tyrosinase that maps at the mouse *c*-albino locus. Proc Natl Acad Sci USA 84:7473–7477

Lee S-T, Nicholls RD, Bundey S, Laxova R, Musarella M, Spritz R (1994) Mutations of the *P* gene in oculocutaneous albinism, ocular albinism, and Prader-Willi syndrome plus albinism. N Engl J Med (in press)

Lerner AB, Fitzpatrick TB (1950) Biochemistry of melanin formation. Physiol Rev 30:91–126

Oetting WS, Handoko HY, Mentink MM, Paller AS, White JG, King RA (1991*a*) Molecular analysis of an extended family with type IA (tyrosinase-negative) oculocutaneous albinism. J Invest Dermatol 97:15–19

Oetting WS, King RA (1993) Molecular basis of type I (tyrosinase-related) oculocutaneous albinism: mutations and polymorphisms of the human tyrosinase gene. Hum Mutat 2:1–6

Oetting WS, Roed CM, Mentink MM, Handoko HY, King RA (1991*b*) PCR detection of a *Taq*I polymorphism at the CCAATT box of the human tyrosinase (TYR) gene. Nucleic Acids Res 19:5800

Oetting WS, Witkop CJ Jr, Brown SA, Colomer R, Fryer JP, Bloom KE, King RA (1993) A frequent tyrosinase gene mutation associated with type I-A (tyrosinase-negative) oculocutaneous albinism in Puerto Rico. Am J Hum Genet 52:17–23

Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, et al (1988) Primer directed enzymatic am-

plification of DNA with a thermostable DNA polymerase. Science 239:487–491

Sanger F, Nicklen S, Coulson AR (1977) DNA sequencing with chain terminating inhibitors. Proc Natl Acad Sci USA 74:5463–5467

Shibahara S, Tomita Y, Tagami H, Muller RM (1988) Molecular basis for the heterogeneity of human tyrosinase. Tohoku J Exp Med 156:403–414

Southern EM (1975) Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 98:503–517

Spritz RA (1993) Molecular genetics of oculocutaneous albinism. Sem Dermatol 12:167–172

Spritz RA, Holmes SA, Ramesar R, Greenberg J, Curtis D, Beighton P (1992) Mutations of the *KIT* (mast/stem cell growth factor receptor) proto-oncogene account for a continuous range of phenotypes in human piebaldism. Am J Hum Genet 51:1058–1065

Spritz RA, Jagadeeswaran P, Choudary PV, Biro PA, Elder JT, DeRiel JK, Manley JL, et al (1981) Base substitution in an intervening sequence of a β^+-thalassemic human globin gene. Proc Natl Acad Sci USA 78:2455–2459

Spritz RA, Strunk KM (1990) RFLP for BglII at the human tyrosinase (TYR) locus. Nucleic Acids Res 18:3672

Spritz RA, Strunk KM, Giebel LB, King RA (1990) Detection of mutations in the tyrosinase gene on a patient with type IA oculocutaneous albinism. N Engl J Med 322:1724–1728

Tripathi RK, Bundey S, Musarella MA, Droetto S, Strunk KM, Holmes SA, Spritz RA (1993) Mutations of the tyrosinase gene in Indo-Pakistani patients with type I (tyrosinase-deficient) oculocutaneous albinism (OCA). Am J Hum Genet 53:1173–1179

Tripathi RK, Giebel LB, Strunk KM, Spritz RA (1991) A polymorphism of the human tyrosinase gene is associated with temperature-sensitive enzymatic activity. Gene Expr 1:103–110

Tripathi RK, Hearing VJ, Urabe K, Aroca P, Spritz RA (1992*a*) Mutational mapping of the catalytic activities of human tyrosinase. J Biol Chem 267:23707–23712

Tripathi RK, Strunk KM, Giebel LB, Weleber RG, Spritz RA (1992*b*) Tyrosinase gene mutations in type I (tyrosinase-deficient) oculocutaneous albinism define two clusters of missense substitutions. Am J Med Genet 43:865–871

Weatherall DJ, Clegg JB, Higgs DR, Wood WG (1989) The hemoglobinopathies. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic basis of inherited disease. McGraw-Hill, New York, pp 2281–2339

Witkop CJ Jr, Quevedo WC Jr, Fitzpatrick TB, King RA (1989) Albinism. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) The metabolic basis of inherited disease. McGraw-Hill, New York, pp 2905–2947