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Origins of Hyperphenylalaninemia in Israel

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Abstract

Mutations and polymorphisms at the phenylalanine hydroxylase (PAH) gene were used to study the genetic diversity of the Jewish and Palestinian Arab populations in Israel. PAH mutations are responsible for a large variety of hyperphenylalaninemias (HPAs), ranging from the autosomal recessive disease phenylketonuria to various degrees of nonclinical HPA. Seventy-two Jewish and 36 Palestinian Arab families with various HPAs, containing 115 affected genotypes, were studied by haplotype analysis, screening for previously known PAH lesions and a search for novel mutations. Forty-one PAH haplotypes were observed in this sample. Four mutations previously identified in Europe (IVS10nt546, R261Q, R408W and R158Q) were found, and were associated with the same haplotypes as in Europe, indicating possible gene flow from European populations into the Jewish and Palestinian gene pools. Of particular interest is a PAH allele with the IVS10nt546 mutation and haplotype 6, that might have originated in Italy more than 3,000 years ago and spread during the expansion of the Roman Empire. These results, together with previous identification of three PAH mutations unique to Palestinian Arabs [IVSnt2, Edel(197–205) and R270S], indicate that the relatively high genetic diversity of the Jewish and Palestinian populations reflects, in addition to genetic events unique to these communities, some gene flow from neighboring and conquering populations.

Key Words
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Introduction

Characterization of mutant genes associated with hereditary diseases is aimed primarily at understanding the molecular basis of these disorders. However, identification of sequence variations associated with normal and defective alleles can also contribute to our understanding of the mechanisms underlying the dynamics of gene flow among human populations. Alleles marked by mutations or polymorphic haplotypes or a combination of both may serve as useful markers to study the interrelationships between various ethnic groups and the origins of their genetic diversity.

The gene encoding the hepatic enzyme phenylalanine hydroxylase (PAH) has recently been a focus of such investigations. PAH catalyzes the irreversible hydroxylation of phenylalanine to tyrosine, thereby opening the catabolic pathway of this essential amino acid. Mutations in this gene cause a wide spectrum of disorders collectively called hyperphenylalaninemias (HPAs) [1]. Extreme HPA is expressed as the autosomal recessive disease phenylketonuria (PKU), which causes severe mental retardation. Early diagnosis of PKU and subsequent treatment with a low-phenylalanine diet can considerably reduce the brain damage, making newborn screening for HPA a common practice in many countries. Milder HPAs have no clinical consequences and are usually identified by biochemical tests.

Extensive studies in European, Mediterranean and Asian communities recently disclosed a wide variety of PAH mutations underlying these conditions [see ref. 2, 3 for reviews]. Mutations which affect PAH activity severely result in PKU in homozygotes or compound heterozygotes, while non-PKU HPA is usually caused by compound heterozygosity for a mutation of the severe type and a mutation with a milder effect on the protein [4–7]. PAH mutations show strong linkage disequilibrium with specific polymorphic haplotypes at the PAH locus [1–3]. Thus, mutant PAH genes can be 'tagged' with specific mutation-haplotype combinations, which enable one to follow their spread in different populations with great specificity [8].

More than 2 million Israeli newborns have been screened for HPA since 1960. PAH deficiencies were identified in some 450 infants, 30% of them showing PKU and the rest non-PKU HPA [9–11; unpubl. data]. Both types of HPA were identified among Jews and Palestinian Arabs. These two populations differ from the European and Asian communities studied thus far, and from each other. The Jewish population is composed mostly of immigrants from a variety of countries, and their descendents, while the Palestinian Arab population reflects the migrations and foreign conquests that have swept this country since the time of the Roman Empire. Both ethnic groups are therefore expected to show considerable genetic diversity for their small size. We are tracing the origins of this diversity, using as a probe the mutations and polymorphisms of the PAH gene.

Subjects and Methods

Sample Population

The population of individuals affected with HPAs in Israel, and their ascertainment, have been described previously [4, 9–11]. The great majority of HPA cases in the Jewish population was identified among Sephardic and Oriental Jews, rather than in Ashkenazi communities [9, 10]. Over a period of 5 years, we collected peripheral blood samples from 72 Jewish and 36 Palestinian families with HPAs. Since our primary interest was directed towards PKU, in most of these families (a total of 88) the probands had PKU. In 13 families non-PKU HPA was identified, and in 7 families both conditions were segregating [see ref. 4 for a detailed description of these families]. Altogether, 115 affected genotypes were studied (each of the 7 families with

Table 1. Polymorphic haplotypes identified in normal and mutant PAH genes in 108 studied families

Haplotypes[1]	Jews		Palestinian Arabs	
	normal genes	mutant genes	normal genes	mutant genes
1	32	29[2]	14	39[2]
2	7	3	1	3
3	2	–	3	–
4	34	16	14	4
5	4	3	3	4
6	2	21	2	12
7	6	6	7	8
8	2	–	–	–
9	–	–	2	–
10	4	2	–	–
11	1	–	1	–
12	7	1	4	–
14	1	–	–	–
15	–	–	1	–
18	1	1	–	–
19	1	–	–	–
27	3	1	2	–
28	10	4	2	–
29	–	–	1	–
32	1	–	–	–
33	2	–	–	–
36	1	2	–	–
37	–	–	2	–
41	1	2	–	–
42	1	–	2	4
43	1	–	–	–
44	1	–	–	–
47	1	–	–	–
50	1	–	–	–
52	2	–	–	1
56	1	–	–	–
57	–	–	1	–
58	1	–	–	–
59	1	–	–	–
61	–	–	1	–
62	1	–	–	–
63	1	–	–	–
64	–	1	–	–
65	1	–	–	–
66	–	–	–	1
6del[3]	–	52	–	–
Total	135	144	63	76

[1] Haplotype designation is according to Woo [18].
[2] Significantly different between Jews and Palestinian Arabs ($p < 0.001$).
[3] A derivative of haplotype 6 containing a deletion that eliminates the *Pvu*II (b) RFLP [19].

both PKU and non-PKU HPA contributed 2 affected genotypes). Among the Jewish families, 65 were of Sephardic or Oriental origin, 5 were Ashkenazi and 2 represented 'mixed marriages'. The parents were consanguineous in 34 of the 36 Palestinian families and in 6 of the 72 Jewish families.

Molecular Methods

Extraction of genomic DNA from peripheral blood samples, haplotype analysis, identification of previously known mutations and the search for new mutations have been previously described in detail [4, 12–16].

Results

Polymorphic Haplotypes at the PAH Locus

Eight RFLPs conveniently detected by the full cDNA of PAH [17, 18] were used to construct polymorphic PAH haplotypes in all the families studied. Forty-one haplotypes were observed in the sample population. Their distribution among normal and mutant PAH genes is shown in table 1. Complete haplotype analysis was not possible in some families, since not all parents were available. In other families, heterozygosity of all family members for one or two RFLPs and unavailability of additional family members precluded haplotype construction. Hence, the final number of haplotyped chromosomes is slightly smaller than expected on the basis of the sample size. Except for haplotype 6del which is unique to Yemenite Jewish PKU patients [19], all of the haplotypes identified in this study have also been observed in European and Mediterranean populations [2, 3, 8, 18, 20].

Although a considerable variety of haplotypes was found among Jews and Palestinian Arabs, in both groups most of the haplotypes occurred on a single or small number of chromosomes, with three or four haplotypes predominant. Haplotypes 1, 4 and 6 were abundant among both Jews and Palestinians; haplotype 28 was also frequent among Jews, as

Table 2. Geographic origin of mutant PAH genes among Jews

Country of origin	Haplotypes														
	1	2	4	5	6del	6	7	10	12	18	27	28	36	41	64
Algeria			1									1			
Tunisia			2									1			
Morroco	4		4			16	1			1			2		
Egypt								1							
Yemen	3		1		52		1				1			2	
Iraq	8					1	1	1							
Iran	2		1			3	1								
Syria	1														
Turkey	5		1				1					2			1
Afghanistan			2			1									
Azerbaijan			3				1								
Kurdistan	1														
Poland		2		1											
Lithuania	1														
Yugoslavia		1													
Germany			1						1						
Russia	4														
Rumania				2											
Total	29	3	16	3	52	21	6	2	1	1	1	4	2	2	1

was the 'Yemenite' PKU haplotype, 6del. For haplotypes other than 6del, χ^2 analysis indicated significant difference between frequencies among Jews and Palestinian Arabs only for mutant haplotype 1.

Most haplotypes were shared by wild-type and mutant alleles, and only two haplotypes in Jews and two in Palestinian Arabs were unique to mutant alleles (table 1). Haplotype 6 was found almost exclusively on mutant chromosomes in both groups. The major haplotypes on mutant alleles among Jews were 6del, 1, 4, 6 and 7 (table 1). While more than half of the mutations associated with haplotypes 4 and 6 originated in North African communities (table 2), mutant haplotype 1 was found mainly in Jewish patients from Middle Eastern countries.

Point Mutations in the PAH Gene

Tables 3 and 4 summarize PAH molecular lesions found to date in the Israeli population, their haplotype association and ethnic origins. The entire patient population – excluding the Yemenite Jewish PKU patients who are all homozygous for a deletion of PAH exon 3 [19] – was screened for the presence of eleven point mutations previously identified in European and Mediterranean populations: R158Q, R252W, R261Q, G272X, S273F, E280K, P281L, L311P, IVS10nt546, R408W and Y414C [2, 3]. Four of these mutations were identified on 50 of the 168 mutant alleles tested; 35 of the 50 alleles contained the splicing defect IVS10nt546 (table 3). Notably, the haplotype association of these mutations (table 4) was similar in the Israeli and European populations, with the exception of two alleles

Table 3. PAH mutations identified in the Israeli population

Mutation[1]	Location[2]	Consequence	Method of detection[3]	Number of positive PAH genes in Israel[4]	Haplotype association in European and Mediterranean populations	Haplotype association in Israel	References
Mutations previously identified in other populations							
IVS10nt546	I10	cryptic splice	RED	35	6, 10, 16, 24, 36, 43	5, 6, 36	31, 32, 36–41
R261Q	E7	Arg → Gln	RED	9	1, 2, 4, 28	1	25, 27, 32, 36, 42–48
R408W	E12	Arg → Trp	RED	4	1, 2, 5, 34	2	5, 28, 30, 32–34, 36, 40, 47, 49, 50
R158Q	E5	Arg → Gln	ASO	2	4, 16, 28	4	32, 35, 36, 47, 48, 51, 52
Mutations identified and characterized in Israel							
delE3	E3	deletion	SB	52	–	6del	19
IVS2nt1	I2	defective splicing	RED	12	–	7, 42	14
E6del(197–205)	E6	deletion	GE	2	–	4	13
R270S	E7	Arg → Ser	ASP	4	–	1	16
S349P[5]	E10	Ser → Pro	ASO	6	1	4	12, 21, 22

[1] The numbers denote positions of PAH residues and following letters denote the original and substituting residues, respectively.
[2] E = Exon; I = intron.
[3] ASO = Allel-specific oligonucleotide; RED = restriction enzyme digestion; SB = Southern blotting; GE = gel electrophoresis; ASP = allele-specific PCR.
[4] Out of 220 mutant PAH genes.
[5] This mutation was discovered independently by Forrest et al. [21] and John et al. [22]

Table 4. Association between PAH lesions, polymorphic haplotypes and ethnic origin in the Israeli population

Mutations:	IVS10nt546				R261Q		R408W		R158Q		delE3	
Haplotypes:	6	5	36	others	1	others	2	others	4	others	6del	others
Palestinians	12/12	2/4		0/60	6/39	0/37	1/3	0/73	2/4	0/72	0/0	0/76
Jews												
Tunisia												
Morroco	15/16		1/2									
Yemen											52/52	
Iraq	1/1				2/8							
Iran	3/3				1/2							
Afghanistan	1/1											
Poland							2/2					
Yugoslavia							1/1					
Total	20/21	0/3	1/2	0/118	3/29	0/116	3/3	0/141	0/144		52/52	0/92

carrying the IVS10nt546 mutation on the background of haplotype 5, found in a Palestinian Arab patient. This association suggests that several mutant PAH alleles among Jews and Palestinians share common origins with similar alleles in these countries.

A search for novel point mutations in the sample population has yielded thus far four mutations: E6del(197–205) [13] IVS2nt1 [14], R270S [16] and S349P [12]. The first three mutations are unique to Palestinian Arabs and were not reported in any other population, while S349P was discovered independently in French-Canadian and European patients [21, 22]. Haplotype association of these mutations is shown in table 4.

Two mutations were identified in 20 of 28 mutant PAH genes among Moroccan Jewish patients: IVS10nt546, which was associated mostly with haplotype 6, and on one chromosome with haplotype 36; and S349P, all associated with haplotype 4 (table 4). The origin of these chromosomes in this community was studied by screening 17 Moslem Moroccan patients for the same mutations. The IVS10nt546 mutation was found in one patient, in association with haplotypes 6 and 36, and S349P was identified in another one, with haplotype 4.

Discussion

The variety of polymorphisms and mutations at the PAH locus makes it a useful tool in molecular population genetics. Due to the strong linkage disequilibrium between PAH mutations and specific haplotypes, it is highly probable that alleles carrying the same mutation-haplotype combination are identical in descent. This notion allows one to trace the origin of such alleles back in time and geography, and to uncover mechanisms underlying the genetic diversity or genetic similarity of different communities. Typical examples are the identification of a single PAH deletion

IVS2nt1			E6del(197–205)		S349P		R270S	
7	42	others	5	others	4	others	1	others
8/8	4/4	0/64	2/4	0/72	0/4	0/72	4/39	0/37
					2/2			
					4/4			
	0/144			0/144	6/16	0/127		0/144

responsible for all PKU cases among Yemenite Jews, which was traced back to specific families several hundred years ago [19], and the recent localization of the origins of several PAH mutations to specific European ethnic subgroups [8].

The two populations investigated in this study are very different from each other: The Jewish population of Israel is composed mostly of immigrants who have come from numerous countries since the beginning of the 20th century, and their descendents. These people have ancient roots in this region that date back to the Jewish Kingdom of the first and second millenia BC. The spread of the Jewish people around the world following the destruction of the Kingdom and deportation by the Romans in the first century AD should have resulted in increased genetic diversity despite their relative genetic seclusion in the diaspora. The Palestinian Arab population originated in Arab tribes that migrated to Palestine from the Arabian Peninsula at the time of the Roman-Byzantine Empire, and expanded during the Arab-Islamic conquest of the country. The Palestinian Arabs have been residing in this country since that time, living under different rulers and assimilating various waves of immigrants, including the crusaders and the Turks.

Haplotype analysis in this study was based on eight RFLPs [17] which served to establish a commonly used haplotype nomenclature [18]. Additional polymorphisms are being discovered at the PAH locus [23–25], and their incorporation into the existing haplotype system should refine this nomenclature [20]. It should be noted that the subjects studied do not constitute random samples of their communities, since only families with HPAs were selected for analysis. A relatively large number of different haplotypes was found in the Jewish and Palestinian Arab populations in relation to their size, reflecting considerable genetic variability. But, a small number of haplotypes account for the majority of PAH alleles identified. The geographic origins of the mutant PAH haplotypes in the Israeli Jewish population summarized in table 2 show that the majority of HPA cases among Jews in Israel are in families of north African, Additional Eastern and Asian origin. It is noteworthy that in some cases the same haplotype appears on mutant alleles from countries in different parts of the world, e.g., haplotypes 1, 4, 6 and 7.

However, haplotypes alone, particularly the more abundant ones, give only a weak indication of genetic similarity and can be used to pinpoint genetic relationships between different communities only when the associated mutations are identified [26]. Haplotype 1, for example, was found to harbor at least 14 different mutations in Europe [2]. It is noteworthy in this regard that mutations common to the Israeli and European populations are associated in most cases with the same haplotypes (table 3), indicating possible common origins. Three of these mutations – R158Q, R261Q and R408W – are associated with the dinucleotide CpG, a known hot spot for mutations [27], and therefore recurrence of these mutations cannot be ruled out [28, 29]. However, it seems to us that the similar haplotype association favors the possibility of common origin, particularly in the case of the R408W/haplotype 2 allele which is rare in the Israeli population (table 4). It is not surprising to see this allele in Jews from eastern Europe (table 4), since it has a Balto-Slavic origin and accounts for a high proportion of PKU alleles in eastern Europe and Germany [2, 8, 30–35]. It is interesting to note the appearance of this allele and the R158Q/haplotype 4 combination in Palestinians (table 4). Gene flow from Europe into the Palestinian gene pool could have occurred at various periods in history: during the waves of migration from western

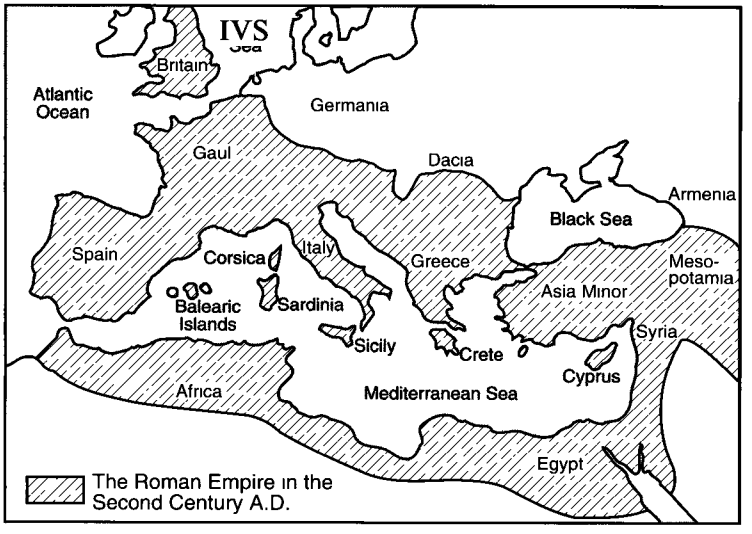

Fig. 1. Boundaries of the Roman Empire at its height during the 2nd century A.D.

and central Europe (including the Balkan region) in the 1st century BC, during the Roman conquest of the region up to the Arab-Islamic conquest in the 7th century AD, and during the Crusades from 1099 AD through the 13th century. Additionally, Christian pilgrimages to the Holy Land were continuous throughout the last 20 centuries.

Most prominent on the list of mutant PAH alleles common to Israeli and other populations is IVS10nt546/haplotype 6 (tables 3, 4). It shows a unique and widespread geographic distribution, with particular prevalence in eastern Europe and Turkey, and has also been reported in Italy, Spain and western Europe [31, 36–41]. Romano et al. [41] detected this allele in the eastern part of Sicily and concluded that the origin was probably Italic, before the 10th century BC. A compilation of the geographic locations of the IVS10nt546/haplotype 6 allele based on this and previous studies leads us to suggest that this allele may indeed have originated in Italy before the first millenium BC, and spread through the Mediterranean littoral countries and eastern Europe during the expansion of the Roman Empire in the first century BC. The regions included in that Empire at its height in the 2nd century AD (fig. 1) roughly correlate with the current prevalence map of this allele. The appearance of the mutation in Palestinian Arabs may reflect the Roman and subsequent Byzantine centuries-long rule in Palestine. The local Christian population assumed the Islamic religion during the Arab conquest in the 7th century, and was totally assimilated into the Arab population.

The predominance of the IVS10nt546/haplotype 6 chromosome among Moroccan Jews is intriguing. The frequency of this chromosome in our Moslem sample population is low (1/34) but, interestingly, the only other IVS10nt546 chromosome in the Moslem sample carries haplotype 36 found with this mutation in one Jewish patient. Another PAH allele found in one Moslem patient and among the Jewish patients in Morocco is S349P/haplotype 4. Jewish-Moslem intermarriages were extremely rare in this country, being strictly forbidden by the Jewish religion.

A possible source of the mutation in the Jewish gene pool might be the conversion to Judaism of local populations, including the Berbers, that took place during the last centuries of Roman and Byzantine rule in the area, before the Arab-Islamic conquest at the end of the 7th century.

The few IVS10nt546/haplotype 6 alleles found in Jews from Asian countries (table 4) can be attributed to occasional migrations eastward of Jews fleeing religious persecution under Roman/Byzantine rule. A similar conclusion may be drawn for the R261Q/haplotype 1 allele which, however, does not penetrate the Moroccan-Jewish gene pool.

A clear example of a recurrent mutation in our population is S349P. This mutation was identified independently in an Australian PKU patient for whom the haplotype association was not reported [21], and in French-Canadian patients in association with haplotype 1 [22]. The same mutation was discovered independently in our laboratory in Jewish patients of north African extraction, on the background of haplotype 4 [12]. The recurrence of this mutation is particularly interesting because it does not involve a CpG dinucleotide.

A search for novel mutations among Palestinian PKU patients has led to the identification of 3 'private' mutations not shared by other populations (table 3). It is still possible that these mutations did not occur within the Palestinian community, and might be common to other Arab communities in the region – a question that can be resolved by screening PKU patients in neighboring countries.

The usefulness of PAH genotypes as population genetic markers should increase considerably once the entire spectrum of HPA mutations is revealed and haplotype nomenclature further refined by additional polymorphisms. We expect these sequence variations to disclose additional, unknown origins accounting for the genetic diversity of the peoples of our region.

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