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Characterization of Phenylalanine Hydroxylase Alleles in Untreated Phenylketonuria Patients from Victoria, Australia: Origin of Alleles and Haplotypes

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Summary

Mutations in the phenylalanine hydroxylase (PAH) gene were identified in a group of untreated phenylketonuria patients from Victoria, Australia. Ninety-eight percent of the alleles were identified, and a total of 26 different mutations were detected on 83 independent chromosomes. The three most prevalent mutations—R408W, I65T, and IVS12nt1—together accounted for 54% of the alleles. A number of alleles were demonstrated, by genealogical studies, to be of Irish or Scottish origin, including a newly described mutation 1197/1198 del A. The distribution and relative frequencies of the more common alleles in this population parallel observed frequencies in the British Isles and are consistent with the known history of Caucasian settlement of this region of Australia. We have analyzed the haplotype and polymorphic short tandem-repeat allele of the mutant chromosomes and describe a number of new associations.

Introduction

Phenylketonuria (PKU) is an autosomal recessive disorder that, if untreated, causes mental retardation due to a deficiency of the enzyme phenylalanine hydroxylase (PAH). More than 150 mutations have been described in the PAH gene (PAH Mutation Analysis Consortium, unpublished data), with multiple geographically distinct origins for PKU mutations and haplotype associations in the European population (Eisensmith et al. 1992).

This study examines the frequencies of mutations in a group of untreated Australian PKU patients from Victoria. The results are related to the history of Caucasian immigration to Australia. The analysis of a short tandem repeat (STR) identified by Goltsov et al. (1993), in addition to the classical haplotype-and-VNTR analysis, provides further insight into both possible haplotype divergence and the association of R408W and I65T mutations with several unusual haplotypes identified in this population. A preliminary report of this work was presented previously at the 1993 meeting of The American Society of Human Genetics (Ramus and Cotton 1993).

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Subjects, Material, and Methods

Caucasian probands in the State of Victoria, Australia, who had untreated PKU previously ascertained and classified (Pitt 1971*a*, 1971*b*; Pitt and Danks 1991) were studied. These untreated PKU patients were born between the years 1917 and 1965, before the introduction of newborn screening for PKU in Australia. Mutations in many of these probands have been described elsewhere (Ramus et al. 1993). Fifty-five patients from 42 apparently unrelated families were studied, resulting in a total of 84 chromosomes being analyzed. Two of the patients were later identified as being first cousins, and therefore calculations of allele frequencies are based on 83 independent chromosomes.

Genomic DNA from patients was extracted from whole blood (Miller et al. 1988), and blood was collected on Guthrie cards, from relevant family members. Appropriate regions of genomic DNA were amplified by PCR and were used for mutation analysis. PCR amplification from dried blood spots was performed using the method of Nelson et al. (1990).

Screening for mutations of exons 7 and 12 and the R158Q mutation has been reported elsewhere (Ramus et al. 1993). Several other mutations (F39L, I65T, L348V, S349P, and IVS10nt546) were screened for by enzymatic digestion using natural or created restriction-enzyme sites (Eiken et al. 1991). The F39L and L348V mutations were confirmed by sequencing, to exclude the possibility that a nearby mutation caused loss of the restriction site. PCR amplification of a 55/54-bp fragment from genomic DNA was used to screen for a new mutation, 1197/1198 del A, a deletion of 1 bp in exon 11 (S. J. Ramus, unpublished data). The remaining alleles were detected by amplification of cDNA and screening with

the chemical cleavage of mismatch (CCM) method (Ramus and Cotton, in press).

Relatives of the patients with either the 1197/1198 del A mutation or the F39L mutation supplied blood spots for carrier testing and partial haplotyping. Family-history information provided was used as a basis for a genealogical search. The Victorian index of births, deaths, and marriages was used to trace all ancestors of these probands, back to one generation before arrival in Australia, when possible. Information concerning marriages and baptisms in County Tipperary, Ireland, in the early 1800s was obtained from the Tipperary Heritage Unit–Family History Research Centre.

RFLP haplotype analysis was performed by PCR at five sites— *Pvu*II(a), *Bgl*II, *Msp*I, *Xmn*I, and *Pvu*II(b) (Dworniczak et al. 1992*a,* 1992*b;* Goltsov et al. 1992*b;* Wedemeyer et al. 1992; Scriver et al. 1994). The *Hin*dIII VNTR analysis by PCR was as described by Goltsov et al. (1992*a*). Full haplotyping was performed on genomic DNA from patients by Southern analysis of the remaining polymorphic sites, *Eco*RI and *Eco*RV (Lidsky et al. 1985). PKU haplotypes in this study are identified by specifying the *Hin*dIII VNTR allele as in the list by Eisensmith and Woo (1992). The highly polymorphic STR region (Goltsov et al. 1993) was also amplified by PCR and analyzed.

It was not always possible to obtain access to all relatives needed for full haplotyping; however, a number of haplotypes could be inferred by using the known haplotype associations of the second allele (Eisensmith et al. 1992; Scriver et al. 1994). Associations between particular mutations and the STR alleles were possible either when a patient was homozygous for a particular STR allele or if a patient was homozygous for a particular mutation. It was observed that all patients with the IVS12nt1 mutation (which was always associated with haplotype 3) had the 244 STR allele. Therefore, in patients heterozygous for this mutation, an association between the second mutation and the other STR allele can be inferred.

Results

Characterization of Alleles

Sequencing of exons 7 and 12 and the screening of genomic DNA for six known mutations identified 87% of the mutant alleles. Several other mutations were detected by using CCM and illegitimate transcripts (Ramus and Cotton, in press), resulting in 98% of alleles being identified. The remaining two alleles were sequenced, and no mutations could be detected in the coding region or splice sites. Twenty-six mutations were identified in the 83 independent chromosomes. Three alleles—R408W, I65T, and IVS12nt1—accounted for 54% of mutant chromosomes. The relative frequencies of the alleles are given in table 1. The haplotype (including VNTR) and the STR allele associated with each mutation are also given, where possible.

The R408W allele was the allele shared by the cousins in the study, and therefore only 16 of the 17 identified R408W alleles were independent chromosomes. Ten were associated with haplotype 1 (VNTR 8), and all of these were associated with an STR of 244 bp. Three of the R408W alleles were associated with haplotype 2 (VNTR 3). Two of these were associated with an STR of 240 bp, and one was associated with an STR of 248 bp. One R408W allele was found on a previously undefined haplotype (X in table 1), which is possibly derived from haplotype 2 (fig. 1). This allele was associated with an STR of 248.

On the basis of classical haplotyping it was not possible to determine whether the two remaining R408W alleles were associated with haplotype 1 or haplotype 2, since both haplotypes were possible and the second allele was unknown. One of the patients was homozygous for the 244 STR, which strongly suggests that the R408W allele is associated with haplotype 1. The haplotype of the R408W allele in the other patient could not be determined, since the patient was heterozygous for the 244 and 240 STR.

The 15 I65T alleles were found on several different haplotypes (fig. 2). Eleven of the I65T alleles were associated with haplotype 9 (VNTR 8). Eight of these were associated with the 248 STR, and three were associated with the 252 STR. One allele was on haplotype 5 (VNTR 9) and had an STR of 244 bp. Two I65T alleles were on haplotype 21 (VNTR 12) and had an STR of 240 bp. One of the alleles was on haplotype B (VNTR 3), a haplotype not included in the list of known haplotypes (Eisensmith and Woo 1992) but defined by Tyfield et al. (1993). This allele was found to be associated with an STR of 236 bp.

The R408Q mutation (Ramus et al. 1993) was associated with a novel haplotype (Y in table 1). This haplotype is the same as haplotype 12, found to be associated with R408Q in Sweden (Svensson et al. 1992), except that it was positive for the *Eco*RV polymorphism.

Origin of Alleles, as Determined by Genealogical Studies

One patient, from family A, was found to be heterozygous for a previously undescribed mutation—1197/1198 del A (authors' unpublished data)—and the I65T mutation. The remaining patients were screened for this deletion, and a second proband, from family B, was found to be heterozygous for this mutation and the F39L mutation.

The 1197/1198 del A mutation was found to be associated with haplotype 1 (VNTR 8) and the 248 STR, in both families. Since this mutation was on the same haplotype in both families and had not been previously

Table 1

Frequencies of Mutations with Haplotype and STR Associations

Mutation	Haplotype + VNTR	STR	No. of Alleles	Frequency (%)	Total (%)
R408W	1.8	244	11	13.2	
	X.8	248	1	1.2	
	2.3	240	2	2.4	19.3
	2.3	248	1	1.2	
	1.8/2.3	244/240	1	1.2	
I65T	9.8	252	3	3.6	
	9.8	248	8	9.6	
	5.9	244	1	1.2	18.1
	B.3	236	1	1.2	
	21.12	240	2	2.4	
IVS12nt1	3.8	244	13	15.7	15.7
IVS10nt546	6.7	252	4	4.8	6.0
	6.7	248	1	1.2	
R261Q	4.3	248	2	2.4	4.8
	28.3	248	1	1.2	
	1.8	240	1	1.2	
F39L	1.8	240	3	3.6	3.6
F299C	8.9	236	2	2.4	2.4
L348V	9.8	244	2	2.4	2.4
R243X	4.3	236	2	2.4	2.4
1197/1198delA	1.8	248	2	2.4	2.4
IVS7nt1	4.3	236	1	1.2	2.4
	4.3	240	1	1.2	
G148S	2.3	244	1	1.2	2.4
	7.8	236	1	1.2	
R252W	7.8	236	1	1.2	2.4
	1.8	244	1	1.2	
R408Q	Y.12	232	1	1.2	1.2
G272X	7.8	228	1	1.2	1.2
E280K	1.8	240	1	1.2	1.2
P281L	1.8	240	1	1.2	1.2
S349P	1.7	240	1	1.2	1.2
R158Q	4.3	236	1	1.2	1.2
A395P	1.7	240	1	1.2	1.2
L48S	4.3	236	1	1.2	1.2
ΔI94	7.8	232	1	1.2	1.2
47delCT	1.8	244	1	1.2	1.2
IVS4nt1	1.8	244	1	1.2	1.2
Unknown	4.3	244	1	1.2	1.2
Unknown	1.8/2.3	240/244	1	1.2	1.2

NOTE.—Where two haplotypes are given for one allele, e.g., (1.8/2.3), the mutation/haplotype association could not be distinguished. In five patients there was not enough DNA available for complete haplotyping using Southern analysis, and therefore 10 alleles are missing one or more polymorphic sites used for haplotyping. One allele each of Ivs12nt1(3.8-244), I65T(9.8-248), I65T(9.8-252), and R252W(7.8-236) are missing the *Eco*RV polymorphic site; I65T(9.8-248) and R408W(X.8-248) are missing the *Eco*RI polymorphic site; and Ivs12nt1(3.8-244) and G272X(7.8-228) are missing both *Eco*RV and *Eco*RI. For the patient with the two R408W(2.3-240) alleles, only enough DNA was available for analysis of the VNTR and STR. The remaining polymorphic sites were consistent with the haplotype given. The mutation/haplotype associations in these five patients were found in several of our other patients (I65T and Ivs12nt1) and in other studies.

identified, a genealogical investigation was performed to determine whether the two probands had a common ancestor (fig. 3). For each of these patients, an ancestor of the carrier parent was found who shared the same surname (X and Y in table 1), and both of these individuals had been born in Ireland. An individual, most likely X (same name and age), was baptized in 1812 in the same church, in a small parish in County Tipperary, in which Y was married in 1819. We suggest that X and Y were related, perhaps brother and sister. Since the

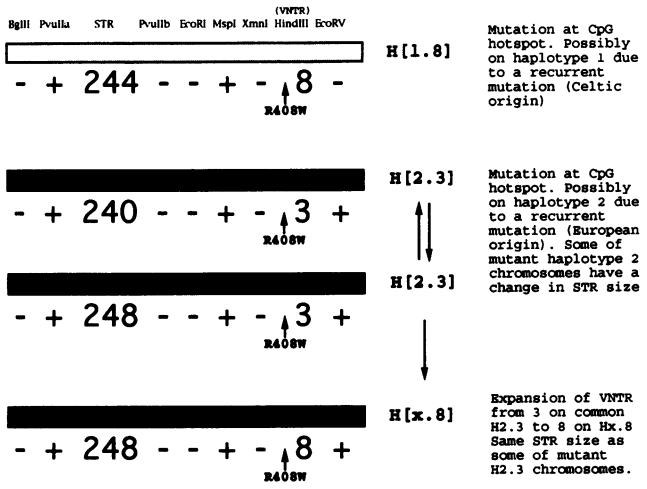

Figure 1 Diagram of the different haplotypes found to be associated with the R408W allele in our population. Arrows indicate the possible sequence of events, and the possible changes that would result in these haplotypes are given. The *Eco*RI site on allele X could not be determined, because of the insufficient amount of DNA available.

Irish Catholic Church records in this region go back only to 1804, this relationship could not be confirmed.

Even without the evidence for a common ancestor, it is likely that this mutation has an origin in Ireland. Six of the eight great-grandparents of the carrier parent in family A, as well as three of four grandparents of the carrier parent in family B, were Irish. The I65T allele, the second allele in family A, was also found to be of Irish origin, since all ancestors of the carrier parent were Irish (fig. 3).

Individuals from three families were identified as being heterozygous for the F39L mutation. This allele had previously been reported in one other patient, also from Victoria, Australia (Forrest et al. 1991). One of the F39L mutations (in family B) was shown to be of Irish origin, since the carrier father was Irish born (fig. 3). In another family (family C), the father was found to be the carrier of the F39L allele; and his ancestors were traced to determine the countries of origin (fig. 4). No family information was available for the other patient (in family D), but families C and D had the same surname (with slight variation in spelling), suggesting a common paternal ancestor and, therefore, Scottish origin of the mutation. The F39L mutation was found to be associated with haplotype 1 (VNTR 8) in families B–D. In all three families the F39L mutation was found to be associated with the 240 STR. In addition to the genealogical studies performed for these families, we have documented the place of birth of the 22 parents who were known to be born in a country other than Australia (table 2).

Discussion

The analysis of a cohort of untreated PKU patients in Victoria, Australia, has identified 98% of the mutant alleles. Several of these alleles are in linkage disequilibrium with more than one haplotype and STR allele (table 1). For a number of alleles—i.e., 1197/1198 del A, F39L, and I65T—genealogical studies have established the origin as Ireland and Scotland (figs. 3 and 4). For parents known to be born overseas, the country of origin is listed in table 2 (22 alleles). For the 40 Australian-born parents, the years of birth were between 1900 and 1948 (median 1921), a period when the majority of Caucasian Australians were of Anglo-Celtic (i.e., English, Irish, Scottish, and Welsh) origin (fig. 5). The remaining 11 patients with unknown family histories are also likely to be of Anglo-Celtic origin. Of these 61 independent alleles of Australian or undetermined origin, the I65T mutation constitutes 20% of alleles, and the R408W/haplotype 1 allele constitutes a further 16%. The Ivs12nt1 allele, the most prevalent mutation currently detected in England, accounts for another 20% of alleles.

The I65T mutation segregated with a number of different haplotypes in our cohort (table 1). The association with haplotypes 9 (VNTR 8), 5 (VNTR 9), and B (VNTR 3) was consistent with previous reports (Treacy et al. 1993; Tyfield et al. 1993). The association of two of the I65T alleles with haplotype 21 (VNTR 12) was novel. The information required to determine whether there was a common ancestor of the two I65T haplotype 21 patients was not available.

This mutation is not at a CpG hotspot, and the DNA sequence in this region does not suggest hypermutability at this position (Cooper and Krawczak 1993). The different haplotype associations are therefore unlikely to

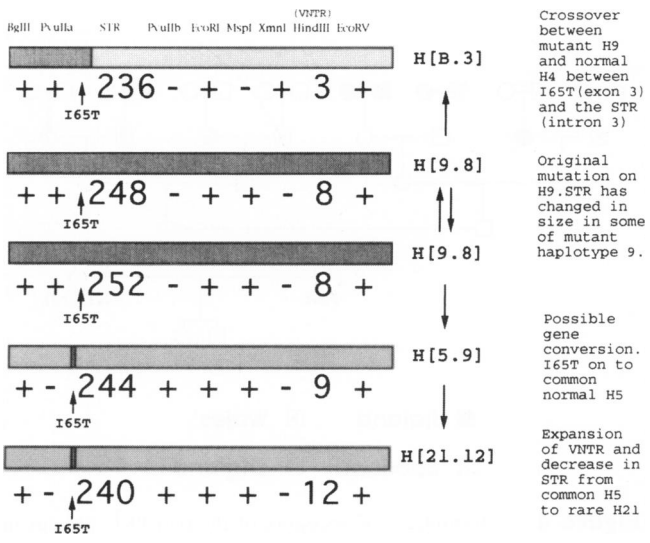

Figure 2 Diagram of the different haplotypes found to be associated with the I65T allele in our population. Arrows indicate the possible sequence of events, and the possible changes that would result in these haplotypes are given.

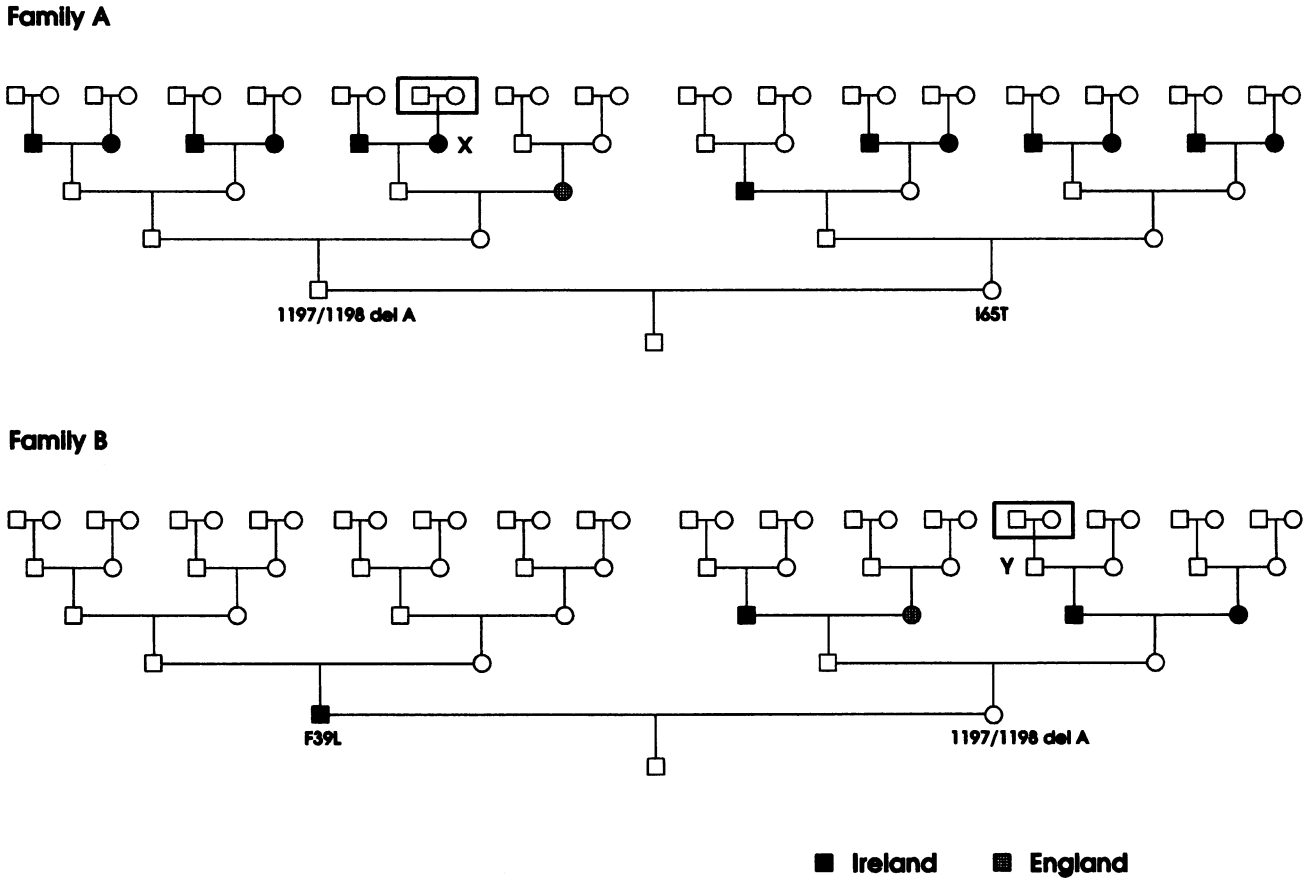

Figure 3 Birthplaces of individuals emigrating to Australia (see key to shading, in fig.). Ancestors of the patient in family A and of the patient in family B were traced to one generation before arrival in Australia. The father in family A is the carrier of 1197/1198 del A, and the mother is the carrier of I65T. The father in family B is the carrier of F39L, and the mother is the carrier of 1197/1198 del A. The individuals in the box (parents of X and Y) are the proposed common ancestors of both patients, one of whom would be the carrier of the 1197/1198 del A mutation.

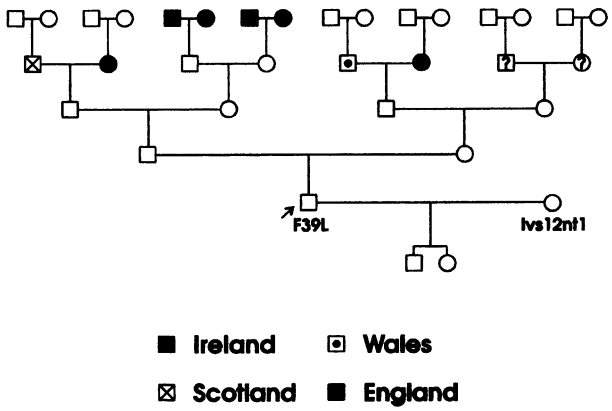

Figure 4 Birthplaces of ancestors of the two PKU siblings in family C (see key to symbols, in fig.). Since the father was found to be the carrier of the F39L mutation, all his ancestors were traced to those individuals arriving in Australia, where possible. A question mark (?) indicates that the birthplace of an individual could not be determined.

be due to a recurrent mutation and may have resulted from evolution of the background haplotype, because of recombination. In our population the I65T mutation was most commonly found on a haplotype 9 (VNTR 8) STR 248 background, determined to be of Irish origin in family A and possibly of English origin in another family (table 2). One I65T allele was found on a haplotype 5 background, and another was found on a haplotype B background, as seen in southwestern England.

Tyfield et al. (1993) proposed that the I65T mutation arose on haplotype 9 (VNTR 8) and that it may have become associated with haplotype 5 (VNTR 9) by recombination, since this haplotype is common in the normal population in southwestern England. It was also suggested that the B haplotype arose from a crossover between a mutant haplotype 9 and a normal haplotype 4 chromosome. In support of this hypothesis, we observed that the STR associated with this B haplotype was 236, which was observed only on haplotypes 4 and 7 (more commonly on haplotype 4) in our study. Figure 2 illustrates the possible divergence of haplotypes from the

Table 2

Origin of 22 Alleles (Mutation and Haplotype) from the Country of Birth of the Parents

Family and Mutation	Haplotype	STR	Country of Origin[a]
10:			
L48S	4.3	236	Latvia
47delCT	1.8	244	Latvia
22:			
Ivs12nt1	3.8	244	Poland
R243X	4.3	236	Poland
33:			
R243X	4.3	236	Yugoslavia
A395P	1.7	240	Yugoslavia
6:			
Ivs10nt546	6.7	252	Italy
Ivs10nt546	6.7	252	Italy
41:			
R408W	1.8/2.3	244/240	Austria
Unknown	1.8/2.3	244/240	Austria
29:			
R408W	1.8	244	Ireland
Unknown	4.3	244	Ireland
32:			
Ivs10nt546	6.7	252	Poland
R408W	2.3	248	Czechoslovakia
19:			
Ivs7nt1	4.3	236	Holland
Ivs4nt1	1.8	244	Germany
40:			
R252W	7.8	236	Germany
I65T	9.8	252	United States
4:			
I65T	9.8	248	England
I65T	9.8	252	India
16:[b]			
F39L	1.8	240	Ireland
2:			
R408W	2.3	240	England

[a] In six families, both parents were born in the same country; in four families, the parents were born in different countries (in these cases [i.e., families 32, 19, 40, and 4], the origin of each allele could not be determined); and, in two families, one parent was born in Australia and the other parent was born overseas.

[b] Family B in text and fig. 3.

more frequent haplotype 9 (VNTR 8), with suggested mechanisms. The only difference between the haplotype 5 (VNTR 9) and the unique I65T/haplotype 21 (VNTR 12) association observed in our population is in (*a*) the length of the *Hin*dIII VNTR (9 vs. 12 repeats, respectively) and (*b*) the length of the STR (244 vs. 240, respectively).

The evolution from one haplotype to another by a difference in size, in one or both of the polymorphic repeats in the PAH gene, can be seen, from the data in table 1 and figures 1 and 2, to be quite common. Several mutations (i.e., I65T H9.8, R408W H2.3, IVS10nt546 H6.7, and IVS7nt1 H4.3) have more than one STR allele associated with one haplotype. The STR and, to a lesser extent, the VNTR seem to have a much higher mutation rate than the other polymorphic sites in the PAH gene. This relatively high mutation rate is expected, since tandem-repetitive hypervariable DNA has been shown to have a very high spontaneous mutation rate (Jeffreys et al. 1988).

The R408W chromosomes were observed on four distinct haplotypes. All R408W haplotype 1 (VNTR 8) chromosomes were in linkage disequilibrium with the 244-bp STR, of Irish origin in one family (table 2). The R408W haplotype 2 (VNTR 3) chromosomes differed in the size of the STR. The R408W mutations on haplotypes 1 (VNTR 8) and 2 (VNTR 3) have been shown to be due to recurrent mutations (Byck et al. 1994). We report an apparently undescribed haplotype (i.e., X) segregating with the R408W allele. This haplotype may have evolved from haplotype 1, with expansion of the STR and recombination changing the *Eco*RV site; however, since the STR size is the same as haplotype 2, which is also positive for *Eco*RV, it seems more likely that only the VNTR has changed in size. Figure 1 illustrates proposed mechanisms for these observations.

The haplotype on which the R408Q mutation was found in our patient was different than that on which it was found in another study (Svensson et al. 1992). Although this mutation is at a CpG hotspot, this new haplotype association is probably not due to a recurrent mutation, since both these haplotypes are identical, except for the *Eco*RV allele. Since both haplotypes have a VNTR of 12 repeats, which is relatively rare, the different haplotypes are possibly due to recombination with a crossover between the VNTR and the *Eco*RV polymorphism.

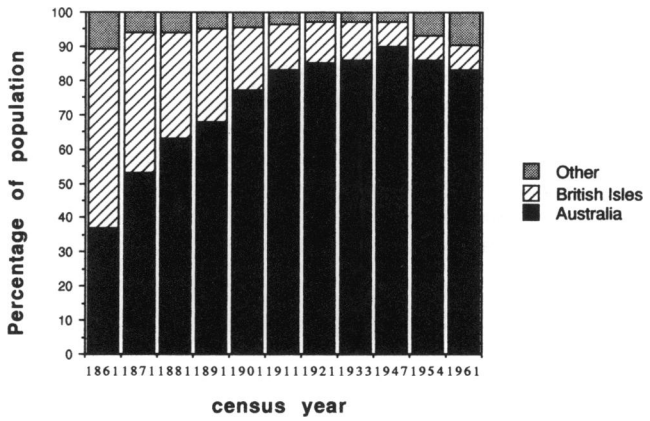

Figure 5 Birthplace of individuals in Australia, for census years 1861 to 1961 (see key to shading, in fig.). From the founding of the Caucasian population, the majority of individuals born in Australia have also been of Anglo-Celtic origin. The graph is based on statistics from the Australian census (Price 1987).

Both the high frequency of alleles common in England and Ireland and genealogical studies tracing the origin of several alleles to Ireland and Scotland are consistent with the historical data of Australian settlement. The early settlement of Australia by Caucasians began in 1788, with the transportation of convicts from England, Ireland, and Scotland. Social and economic problems in Ireland, such as overcrowding, poverty, and the potato famine, resulted in a high proportion of Irish people among the free settlers arriving in Australia during the 1800s. The census of 1846 showed that 25% of people in Victoria were Irish born (Coughlan 1965). The Australian gold rush also led to an increase in immigration to Australia. In 1938, 90% of Australians were of Anglo-Celtic descent, and this predominance lasted until the end of World War II (Price 1987).

In summary, 98% of the mutant alleles in our cohort of untreated PKU patients from Victoria, Australia, have been identified. Three mutations—IVS12nt1, I65T, and R408W—were found to account for 54% of the mutant chromosomes. The composition of the recent Australian population has now changed, so that it is one of the most ethnically mixed in the world. The frequencies of PKU alleles would be expected to be somewhat different and also to reflect Asian and southern European immigration. It is important to note, however, that the three common mutations are still prevalent in a recent sample of hyperphenylalaninemic patients (authors' unpublished data). These results are of historic interest and are of importance in any screening for mutations in patients of Anglo-Celtic descent.

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References

Byck S, Morgan K, Tyfield L, Dworniczak B, Scriver CR (1994) Evidence for origin, by recurrent mutation, of the phenylalanine hydroxylase R408W mutation on two haplotypes in European and Quebec populations. Hum Mol Genet 3:1675–1677

Cooper D, Krawczak M (1993) Single base-pair substitutions. In: Human gene mutation. BIOS Scientific, Oxford, pp 109–161

Coughlan N (1965) The coming of the Irish to Victoria. Hist Stud Aust N Z 12(45): 68–86

Dworniczak B, Wedemeyer N, Eigel A, Horst J (1992*a*) PCR detection of the Pvu II (Ea) RFLP at the human phenylalanine hydroxylase (PAH) locus. Nucleic Acids Res 19:1958

Dworniczak B, Wedemeyer N, Horst J (1992*b*) PCR detection of the Bgl II RFLP at the human phenylalanine hydroxylase (PAH) locus. Nucleic Acids Res 19:1958

Eiken HG, Odland E, Boman H, Skjelkvale L, Engebretsen, Apold J (1991) Application of natural and amplification created restriction sites for the diagnosis of PKU mutations. Nucleic Acids Res 19:1427–1430

Eisensmith RC, Okano Y, Dasovich M, Wang T, Güttler F, Lou H, Guldberg P, et al (1992) Multiple origins for phenylketonuria in Europe. Am J Hum Genet 51:1355–1365

Eisensmith RC, Woo SLC (1992) Updated listing of haplotypes at the human phenylalanine hydroxylase (PAH) locus. Am J Hum Genet 51:1445–1448

Forrest SM, Dahl HH, Howells DW, Dianzani I, Cotton RGH (1991) Mutation detection in phenylketonuria by using chemical cleavage of mismatch: importance of using probes from both normal and patient samples. Am J Hum Genet 49:175–183

Goltsov AA, Eisensmith RC, Konecki DS, Lichter-Konecki U, Woo SLC (1992*a*) Associations between mutations and a VNTR in the human phenylalanine hydroxylase gene. Am J Hum Genet 51:627–636

Goltsov AA, Eisensmith RC, Naughton ER, Jin L, Chakraborty R, Woo SLC (1993) A single polymorphic STR system in the human phenylalanine hydroxylase gene permits rapid prenatal diagnosis and carrier screening for phenylketonuria. Hum Mol Genet 2:577–581

Goltsov AA, Eisensmith RC, Woo SLC (1992b) Detection of the Xmn I RFLP at the human PAH locus by PCR. Nucleic Acids Res 20:927

Jeffreys AJ, Royle NJ, Wilson V, Wong Z (1988) Spontaneous mutation rates to new length alleles at tandem-repetitive hypervariable loci in human DNA. Nature 332:278–281

Lidsky AS, Ledley FD, DiLella AG, Kwok SCM, Daiger SP, Robson KJH, Woo SLC (1985) Extensive restriction site polymorphism at the human phenylalanine hydroxylase locus and application in prenatal diagnosis of phenylketonuria. Am J Hum Genet 37:619–634

Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215

Nelson PV, Carey WF, Morris CP (1990) Gene amplification directly from Guthrie blood spots. Lancet 336:1451–1452

Pitt D (1971*a*) The natural history of untreated phenylketonuria. Med J Aust 1:378–383

——— (1971*b*) Phenylketonuria with normal intelligence: report of two cases. Aust J Ment Retard 1:160–163

Pitt D, Danks DM (1991) The natural history of untreated phenylketonuria over 20 years. J Paediatr Child Health 27:189–190

Price C (1987) Immigration and ethnic origin in Australian historical statistics. In: Vamplew W (ed) Australian historical statistics. Fairfax, Syme & Weldon, Sydney, pp 2–22

Ramus SJ, Cotton RGH. Mutations Ivs4nt1, 47 del CT and G148S identified in the phenylalanine hydroxylase gene by RT-PCR of illegitimate transcripts and chemical cleavage of mismatch. Hum Mutat (in press)

——— (1993) A new phenylketonuria (PKU) mutation detected by illegitimate transcription results in RNA mis-splicing: founder effect and PKU in Australia. Am J Hum Genet Suppl 53:1218

Ramus SJ, Forrest SM, Pitt DB, Saleeba JA, Cotton RGH (1993) Comparison of genotype and intellectual phenotype in untreated PKU patients. J Med Genet 30:401–405

——— (1992) CpG hotspot results in second mutation at codon 408 of the phenylalanine hydroxylase gene. Hum Genet 90:147–148

Scriver CR, Hoang L, Byck S, Prevost L (1994) PAH Mutation Analysis Consortium, 1994 release.

Svensson E, Eisensmith RC, Dworniczak B, Von Dobeln U, Hagenfeldt L, Horst J, Woo SLC (1992) Two missense mutations causing mild hyperphenylalaninemia associated with DNA haplotype 12. Hum Mutat 1:129–137

Treacy E, Byck S, Clow C, Scriver CR (1993) "Celtic" PKU chromosomes found? evidence in two regions of Quebec Province. Eur J Hum Genet 1:220–228

Tyfield LA, Osborn MJ, King SK, Jones MM, Holton JB (1993) Molecular basis of phenylketonuria in a English population. Dev Brain Dysfunct 6:60–67

Wedemeyer N, Dworniczak B, Horst J (1992) PCR detection of the Msp I (Aa) RFLP at the human phenylalanine hydroxylase (PAH) locus. Nucleic Acids Res 19:1959