Am. J. Hum. Genet. 46:970–974, 1990

Recurrent Mutation, Gene Conversion, or Recombination at the Human Phenylalanine Hydroxylase Locus: Evidence in French-Canadians and a Catalog of Mutations

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Summary

The codon 408 mutation (CGG→TGG, Arg→Trp) in exon 12 of the phenylalanine hydroxylase (PAH) gene occurs on haplotype 1 in French-Canadians; elsewhere this mutation (R408W) occurs on haplotype 2. A CpG dinucleotide is involved. The finding is compatible with a recurrent mutation, gene conversion, or a single recombination between haplotypes 2 and 1. A tabulation of 20 known mutations at the PAH locus reveals three instances of putative recurrent mutation.

Introduction

Mutations at the phenylalanine hydroxylase (PAH) locus cause hyperphenylalaninemia. In several populations their frequency, in aggregate, approaches that of genetic polymorphisms (Scriver et al. 1988). Genetic drift, selection, hypermutability, and reproductive compensation are conventional explanations for high allele frequencies at a locus. French-Canadians show extensive genetic variation at the PAH locus (John et al. 1989), and on 18 mutant chromosomes in nine families we now have evidence for as many as seven different mutant alleles. Here we give evidence compatible with a recurrent mutation, crossing-over, or gene conversion between RFLP haplotypes in these families; a particular mutation in codon 408 (R408W) is usually associated with haplotype 2 in European populations, but it is found on haplotype 1 in the French-Canadian families. We also summarize present knowledge of PAH mutations and associations with haplotypes and populations. Recurrent mutation, gene conversion, or crossing-over within the PAH gene has apparently occurred at least three times in Western populations.

Received November 7, 1989; revision received December 21, 1989.

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0002-9297/90/4605-0016$02.00

Subjects and Methods

We analyzed the PAH gene in nine French-Canadian families (18 mutant chromosomes) in whom there is a proband with phenylketonuria (PKU) or non-PKU hyperphenylalaninemia (for details on families, see John et al. 1989). PKU is defined as a plasma phenylalanine value maintained above 1.2 mM on a normal diet and a dietary tolerance for phenylalanine below 500 mg/d; non-PKU hyperphenylalaninemia is a milder variant. We used RFLP haplotyping, polymerase chain reaction (PCR) amplification, dot-blotting, allele-specific oligonucleotides, and direct sequencing according to methods described elsewhere (John et al. 1989).

Results

Dot-blot analysis with allele-specific oligonucleotides (DiLella et al. 1988) indicated that the R408W PKU mutation (codon 408, CGG→TGG, Arg→Trp) (DiLella et al. 1987) is present on haplotype 1 chromosomes in our French-Canadian families (fig. 1). This finding was confirmed by sequence analysis (data not shown). A CpG dinucleotide is involved in the mutation. The R408W allele was found on three of the seven mutant

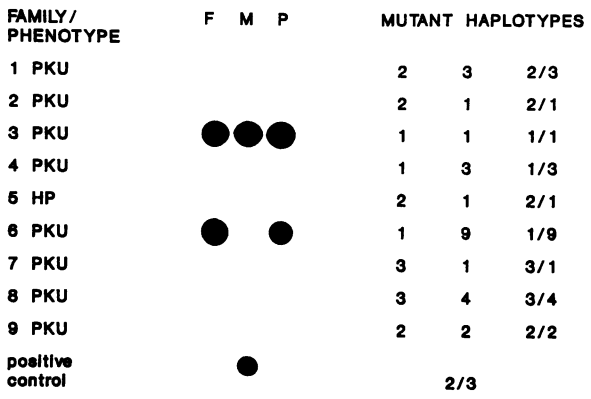

Figure 1 Dot blot analysis with the probe for the R408W mutation (CGG→TGG) in nine French-Canadian families (numbering system is the same as that reported by John et al. [1989]). The mutation is associated with three of the seven haplotype 1 chromosomes; it does not occur on haplotype 2 in this set. F = father; M = mother; P = proband (patient); order of haplotypes is F, M, P in the rows. HP = non-PKU hyperphenylalaninemia (PAH variant).

haplotype 1 chromosomes in French-Canadians; it did not occur on any other mutant haplotype (haplotypes 2, 3, 4, and 9) present in these families. It has been found previously only on haplotype 2 in European populations (Chakraborty et al. 1987) (see table 1).

One French-Canadian proband of consanguineous parents (family 3; fig. 1) is homozygous for the R408W mutation on haplotype 1; the other (family 6; fig. 1), is a genetic compound for both the R408W mutation on haplotype 1 (inherited from the father) and an unidentified mutation on haplotype 9. Both probands have the PKU metabolic phenotype. The families live in close proximity in an isolated region of the Gaspé in eastern Quebec.

Discussion

Spontaneous deamination of 5-methylcytosine in CpG dinucleotides has been implicated as a mechanism for "hot spots" of point mutation at several loci (for review, see Cooper and Youssoufian 1988; also see Youssoufian et al. 1988; Vulliamy et al. 1988; Koeberl et al. 1989). Three laboratories have now independently found evidence for CpG dinucleotides as sites of putative recurrent mutation at the PAH locus. First, Abadie et al. (1989) described two "Mediterranean" French patients homozygous for both haplotype 4 and the E280K mutation (G→A transition). This allele, involving a CpG dinucleotide, was reported orginally on haplotype 38 (Lyonnet et al. 1989). Since haplotypes 4 and 38 differ at all eight RFLP sites (Woo 1988), it is unlikely that either recombination or gene conversion is the explanation for the presence of this mutation on both haplotypes; recurrent mutation is the likely explanation. Second, Okano et al. (1990) have identified the same mutation (E280K) on haplotype 1 chromosomes in Danes. Since a double recombination is required to convert haplotype 38 to haplotype 1, either a recurrent mutation or a gene conversion seems the likely explanation for this finding. Third, we found the R408W mutation (CGG→TGG) on haplotype 1 chromosomes in the French-Canadian population; hitherto this mutation has been reported in complete linkage disequilibrium with haplotype 2 in all other populations (see table 1). A single crossover in a region 3′ to the mutation and 5′ to the *Hin*dIII site (fig. 2), or gene conversion in this region, could have transferred the mutation from haplotype 2 to haplotype 1, but, again, recurrent mutation is likely because a CpG dinucleotide is involved.

Okano et al. (1989) surmize that a mutation should be associated with two haplotypes in the source population when a recombination event has occurred. We have not detected the R408W mutation on any haplotype 2 chromosome in French-Canadians so far (John et al. 1989). Although our sample number is still small, this finding coupled to hypermutability (42-fold increased) at CpG sites (Cooper and Youssoufian 1988) is compatible with recurrence of the R408W mutation on a second haplotype in French-Canadian ancestors.

Our present knowledge of mutations at the PAH locus is summarized in table 1. Of the 20 mutations listed, nine occur at CpG sites. The associated haplotypes have not been reported for several of the mutations, and how

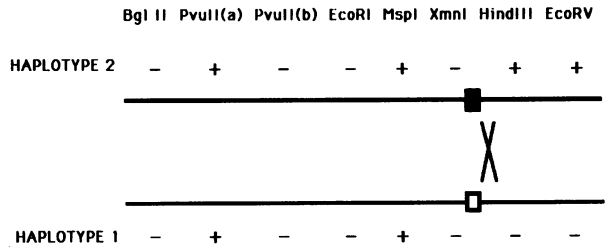

Figure 2 RFLP haplotypes 1 and 2 at the human PAH locus (Woo 1988). A single crossover is sufficient to transfer a codon 408 mutation in exon 12 from haplotype 2 to haplotype 1, if it is not a recurrent mutation or gene conversion. The filled and open boxes represent mutant and normal alleles, respectively, in exon 12. The mutation itself involves a CpG dinucleotide which is a hypermutable site.

Table 1

PAH Mutations

Exon (X) or Intron (I)	Mutation[a]	DNA Change	Haplotype	Ancestry or Region[b]	Reference
1X	M1V	A→G	2	French-Canadian	John et al. 1989
2X	F39L	C→G	. . .	Australia	Forrest et al. 1989
1X–2X deletion (boundaries unspecified)			. . .	Scots	Sullivan et al. 1985
3X	R111ter	C→T[c]	4	Orientals	Wang et al. 1989*a*, 1989*b*
3X	Exon deletion		. . .	Yemenite Jews	Avigad et al. 1987
5X	R158Q	G→A[c]	4	Swiss, northern Europeans	Okano et al. 1989, 1990
				Federal Republic of Germany	Dworniczak et al. 1989
6X	Y204C	A→G	4	Chinese	Wang et al. 1989*b*
7X	R243ter	C→T[c]	. . .	Caucasians	Okano et al. 1989
7X	R252W	C→T[c]	. . .	Mediterranean	Abadie et al. 1989
				Caucasians	Okano et al. 1989
7X	L255S	T→C[d]	. . .	American blacks	Hofman et al. 1987; D. Valle, personal communication
7X	R261Q	G→A[c]	1	Swiss, Europeans	Okano et al. 1990
				French	Lyonnet et al. 1989
				Mediterranean	Abadie et al. 1989
7X	E280K	G→A[c]	38,4	Algeria	Abadie et al. 1989
			38	France	Lyonnet et al. 1988, 1989
			1	Danes	Okano et al. 1990
7X	P281L	C→T[c]	. . .	Caucasians	Okano et al. 1989
8X	F299C	T→G	. . .	Caucasians	Okano et al. 1989
9X	L311P	T→C[e]	11	German Democratic Republic	Lichter-Konecki et al. 1988*a*, 1988*b*
			10		Riess et al. 1988
			7	Greeks	Hofman et al. 1989
12X	R408W	C→T[c]	2	Danes	DiLella et al. 1987
				Swiss, Scots	Sullivan et al. 1989
				French	Rey et al. 1988
				Italians	Devoto et al. 1989
			1	French-Canadians	Present paper
12X	R413P	G→C[c]	4	Chinese	Wang et al. 1989*b*
12I	(splice donor junction	G→A	3	Danes	DiLella et al. 1986; Marvit et al. 1987
				France	Rey et al. 1988
				Italian	Devoto et al. 1989
				German Democratic Republic	Lichter-Konecki et al. 1988*a*
				Federal Republic of Germany	Aulehla-Scholz et al. 1988
				French-Canadians	John et al. 1989
				Scots	Sullivan et al. 1989
. . .	I deletion (codon?)	. . .	2	Portuguese, France	Lyonnet et al. 1988

NOTE.—All mutations cause hyperphenylalaninemia under normal dietary conditions. The distinction between PKU and non-PKU hyperphenylalaninemia phenotypes is unclear in many reports and is not cataloged here.

[a] First letter denotes the normal amino acid (one-letter code), number denotes the codon/residue, and second letter denotes the substituted amino acid; ter = termination (stop) codon.

[b] According to information available.

[c] At 5′ CpG 3′ dinucleotide in sense or antisense strand.

[d] A 13-kb *Msp*I RFLP replaces the normal 19-kb or 23-kb fragments. The putative parent haplotype is 37.

[e] Introduces *Msp*I RFLP allele.

many of them occur on multiple haplotypes is not yet known.

Allelic heterogeneity within a specific population or isolate is not unusual. It is seen, for example, at the β-globin locus in French-Canadians (Kaplan et al. 1990), at the ornithine δ-aminotransferase (gyrate atrophy) locus in Finns (Mitchell et al. 1989), and at the hexosaminidase α chain (Tay-Sachs) locus in Ashkenazi Jews (Myerowitz 1988). It is now evident that allelic heterogeneity at the PAH locus is also the rule, both within and between populations. This finding is relevant both in the search for explanations of expressed polymorphism at the PAH locus and in the design of DNA reagents for molecular diagnosis.

Acknowledgment

We thank Kenneth Morgan for advice and discussion. This work was supported by the Thomas and Elizabeth Williams Scholarship (to S.W.M.J.), The McGill University–Montreal Children's Hospital Research Institute, le Fonds de Recherche en Santé du Quebec, Le Programme d'Actions Structurantes (MESS gouvernement du Quebec), The Quebec Network of Genetic Medicine, The Medical Research Council of Canada, and the Howard Hughes Medical Institute. We thank Savio L. C. Woo for the full-length cDNA probe of the PAH gene, primer sequences, and the positive control.

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