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Time and Space Clusters of the French-Canadian M1V Phenylketonuria Mutation in France

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Summary

We performed mutation analysis and RFLP haplotype analysis of chromosomes associated with classical phenylketonuria (PKU) in contemporary French families. We also did genealogical reconstructions for seven obligate carriers in five contemporary French-Canadian families living in eastern Quebec, who carry the M1V mutation causing PKU. The M1V mutation, heretofore considered to be associated exclusively with French-Canadians, was found on 4 of 152 independent French chromosomes. The French and Quebec M1V mutations all occurred on RFLP haplotype 2. The contemporary mutant French chromosomes clustered in southern Brittany (Finistère Sud). Genealogical reconstructions of the Quebec families identified 53 shared ancestors and a center of diffusion in the Perche region in 17th century France. The two clusters in France, one historical and the other contemporary, are not incompatible, if one assumes the possibilities that settlers returned from Nouvelle France or moved from Perche to southern Brittany. The M1V mutation is serving as a useful marker for historical demography.

Introduction

Classical phenylketonuria (PKU) and variant forms of hyperphenylalaninemia (HP) are caused by deficient activity of human hepatic phenylalanine hydroxylase (PAH; E.C.1.14.16.1). Among Caucasians, about 1 in 10,000 live births has a persistent hyperphenylalaninemic phenotype (Scriver et al. 1989). Isolation of the full-length cDNA and the gene for PAH (Kwok et al. 1985; DiLella et al. 1986*a*) has enabled mutation analysis at the PAH locus (Levy 1989; John et al. 1990; Rey and Rey 1990). By means of at least eight RFLPs at the PAH locus, informative RFLP haplotypes have also been identified on normal and mutant chromosomes (Chakraborty et al. 1987), and combined mutation-haplotype analysis of mutant PAH chromosomes has already advanced our understanding of the population and evolutionary genetics of PKU (Kidd 1987; Avigdad et al. 1990).

John et al. (1989) identified a novel PAH mutation in French-Canadian PKU families living in eastern regions of Quebec Province in North America. This mutation, an A-to-G transition affecting the translation-initiation codon, here designated "M1V" to indicate the codon and the amino-acid substitution, results in a typical PKU phenotype in the homozygote. In the present paper, we report the identification of the M1V mutation in French PKU patients as well. It has been found in only three families living in southern Brittany (Finistère Sud). We also report the results of genealogical reconstructions on five French-Canadian families carrying the M1V mutation; they indicate a geographic origin in the Perche region in 17th-century

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France. The two geographic clusters are not necessarily incompatible.

Patients and Methods

French Patients

French patients with persistent postnatal HP were identified through referral centers in Brest, Vannes, Rennes, Saint-Brieuc, Angers, and Paris (Hôpital des Enfants-Malades). The patients represented 152 independent mutant PAH genes. Among them, 41 originated from Brittany, 40 from contiguous regions (9 from Normandy, 10 from Perche and Maine, 17 from Anjou, and four from Poitou), and 71 from the rest of France.

Mutation and Haplotype Analysis

Forty-eight of the 152 chromosomes were haplotyped according to the nomenclature of Woo (1988). To identify the M1V mutation, a 175-bp genomic fragment spanning the coding sequence of exon 1 of the PAH gene was submitted to PCR amplification (5′ primer, 5′-GAGGCCCTAAAAAGCCAGAGACCT-3′; and 3′ primer, 5′-TGGAGGCCCAAATTCCCCTAACTG-3′). PCR products were either digested with the restriction enzyme *Nla*III (fig. 1) or hybridized with allele-specific oligonucleotide (ASO) probes (John et al. 1989).

Genealogical Reconstructions

Information on dates and places of birth and marriage in Quebec for the seven obligate carriers of the M1V allele and their parents were obtained from the families. The genealogies of these seven carriers were reconstructed using the population register of Saguenay-Lac Saint-Jean, numerous marriage repositories, and genealogical dictionaries, to an average depth of 13 generations, allowing recognition of the French-Canadian population's founders in Europe—mainly in France—in the 17th century. They were then recorded in a computerized data base developed at SOREP. The genealogies were analyzed using software (also developed at SOREP) that, based on an algorithm determining the closest relationship between individuals at each generation, identifies the most likely founders in a set of families with a given disorder or mutation. The methodology of genealogical reconstruction and analysis has been described elsewhere (De Braekeleer 1991; De Braekeleer et al. 1991).

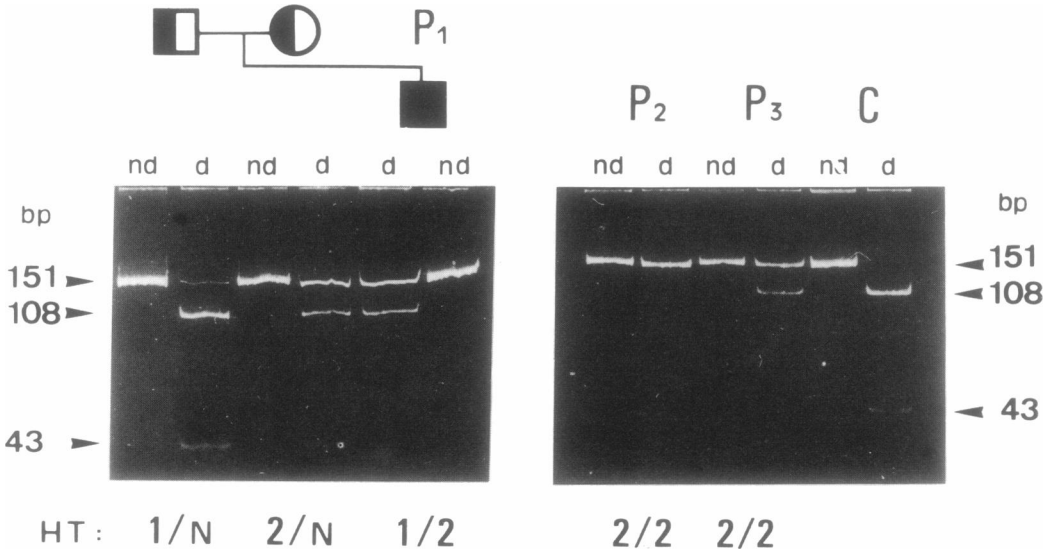

Figure 1 Detection of the M1V mutation (ATG→GTG) by *Nla*III digestion of PCR-amplified exon 1 of the PAH gene. Acrylamide gel electrophoresis of amplified exon 1 (151 bp) either digested (d) or not digested (nd) with *Nla*III. *Nla*III digestion normally generates two fragments, one of 43 bp and one of 108 bp. P_1, P_2, and P_3 are three PKU patients with the M1V genotype. F = father of patient P1; M = mother of patient P_1; and C = control. RFLP haplotypes (HT) at the PAH locus for each individual are indicated below (N = normal chromosome).

Results

French Chromosomes

A total of 152 mutant chromosomes from 76 unrelated French families were screened for the M1V mutation. It was only found on four chromosomes, in one homozygote and in two compound heterozygotes (fig. 1). The three patients had typical PKU phenotype. One of the compound heterozygotes also carried the R408W mutation on a mutant haplotype 2 chromosome (fig. 1, patient P_3).

The French M1V mutations were found in families living in Brittany (fig. 2). All three families can trace their origins in southern Brittany (Golfe du Morbihan) for at least four generations. The M1V mutation was not found on any of the 40 chromosomes from nearby geographic regions ($P < .2$ for a significant association between the M1V mutation and Brittany), nor on 71 chromosomes from elsewhere in France ($P < .05$). Accordingly, the M1V mutation has a strong association with the Finistère Sud region in contemporary French populations ($P < .001$).

As was the case in French Canada (John et al. 1989, 1990), in France the M1V mutation was found only on haplotype 2. This haplotype accounted for only 6 of the 48 chromosomes analyzed for the full PAH haplotype. Accordingly, there is a significant association between the M1V mutation and haplotype 2 in France ($\chi^2 = 28.04$, $P < .001$, with Yates' correction).

French-Canadian Genealogical Reconstructions

Affected families were all living in eastern Quebec, in the region of Beauce and Bellechasse or in the region of Saguenay-Lac Saint-Jean. The Saint Lawrence River lies between these regions. The eastern part of Quebec (Nouvelle France), lying east of the Saint Maurice River, has a demographic history different from that of the modern province's western half.

Although there are many pitfalls in genealogical reconstruction (e.g., adoption, nonpaternity, and missing and false links), there is evidence of a founder effect for the Quebec M1V allele. Among a large number of potential founders, 53 were common to all seven obligate carriers of the M1V allele in present-day Quebec. All ancestors originated from Europe, 19 (35.8%) from the small historical region of France known as Mortagne-Perche, and all in the early 17th century. Furthermore, 39 of 43 ancestors of known origin came from Perche and adjacent counties, and, in this search, we identified no ancestors from Brittany. The Mortagne-Perche region (fig. 3) contributed

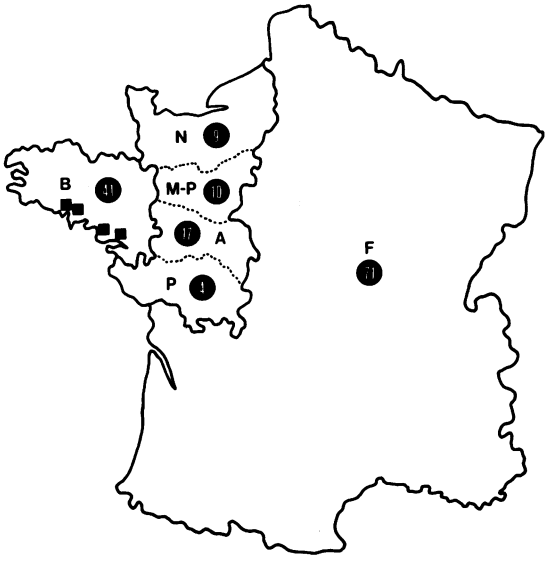

Figure 2 Map of France showing the geographic location of the four French M1V alleles identified (squares). The number of PKU chromosomes tested in each geographic region is given in circles: B = Britanny; N = Normandy; M-P = Maine-Perche; A = Anjou; P = Poitou; and F = the rest of France.

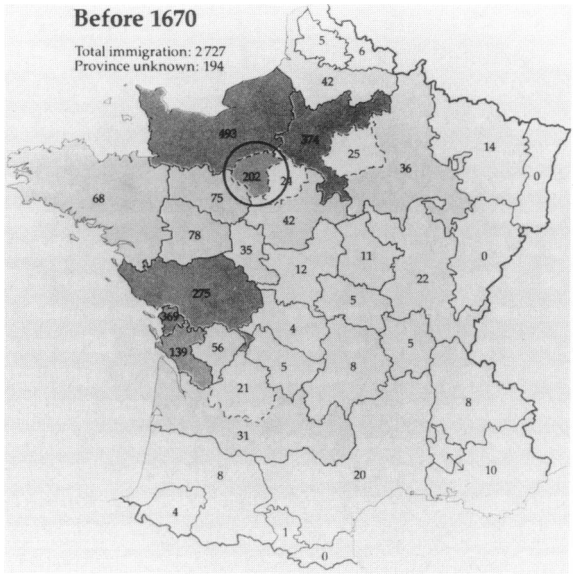

Figure 3 Historical map of France in the early 17th century, showing the center of diffusion for the French-Canadian M1V mutation. Data are based on genealogical reconstructions beginning with five contemporary French-Canadian families living in eastern Quebec (seven independent M1V chromosomes). Over one-third of the 52 putative ancestors of the Quebec families came from the circled region (Mortagne-Perche).

202 (7.4%) of the founders of the French-Canadian population at its early beginnings (before 1670) and only 15 persons between 1670 and 1760 (Charbonneau et al. 1987; Charbonneau and Robert 1987). Therefore, it appears that Mortagne-Perche is the most likely center of diffusion of the M1V allele. However, we cannot exclude other founders as sources of contemporary M1V alleles in Quebec.

Discussion

In 1989, John et al. described a new PKU mutation found in French Canadians living in eastern Quebec province. This mutation (an A→G transition) affects the translation-initiation codon of the PAH gene (M1V). It is associated with RFLP haplotype 2 in Quebec and has occurred on 7 of 24 mutant chromosomes (S. W. M. John, R. Rozen, and C. R. Scriver, unpublished data) that have been fully characterized for their associated RFLP haplotypes, metabolic phenotype, and ethnicity.

We screened 152 contemporary French mutant PAH chromosomes for the M1V mutation. It was found on only four (2.6%) of the PKU chromosomes, each time in association with haplotype 2. All four M1V alleles occurred in families who have lived in southern Brittany for at least four generations.

The M1V mutation is consistently associated with RFLP haplotype 2 at the PAH locus, both in Quebec and in France. The allele does not involve a CpG dinucleotide. Accordingly, it is likely to be the result of a single mutational event that occurred before migration of settlers from France to Nouvelle France in Quebec.

Specific mutant genotypes tend to be linked to particular RFLP haplotypes at the PAH locus (DiLella et al. 1986*b*, 1987). The association between PAH mutation, RFLP haplotype, and population tends to be even more specific (Rey et al. 1988; John et al. 1990; C. R. Scriver, unpublished data). Accordingly, neither haplotype nor mutation can be considered predictive of a particular association, unless the population is taken into account. For example, haplotype 2 may associate with different mutations in different populations: the M1V mutation in contemporary French and French-Canadians (present article), the deletion I94 mutation in southwestern Europeans (Caillaud et al. 1990, 1991), the R261Q mutation in Portuguese (Caillaud et al. 1990; Okano et al. 1990*a*), and the R408W mutation in northern Europeans (DiLella et al. 1987) and in eastern Europeans (Jaruzelska et al. 1991; Kalaydjieva et al. 1991; Zygulska et al. 1991). On the other hand, the same mutation may associate with different haplotypes in different populations: the R408W mutation associates with haplotype 1 in French-Canadians (John et al. 1990) and in persons living in southwest England (Tyfield et al. 1991), with haplotype 2 in northern Europeans (DiLella et al. 1987), and with haplotype 44 in Chinese (Tsai et al. 1990). Similarly, the R261Q mutation is on haplotype 1 in French and Italians (Abadie et al. 1989) and on haplotype 2 in Portuguese (Okano et al. 1990*a*); and, again, the E280K mutation is on haplotypes 38, 4, and 28 in north Africans and French (Lyonnet et al. 1989; Berthelon et al. 1991; Labrune et al. 1991) and on haplotype 1 in Danes (Okano et al. 1990*b*). Whether the latter type of association reflects recurrent mutation or spread across haplotypes is not known in most of those examples.

PAH mutations other than M1V have contributed to the "founding" of the PKU phenotype in French-Canadians (John et al. 1990). One of these mutations is R408W. Our two groups found it associating with RFLP haplotype 1 on three French-Canadian chromosomes (John et al. 1990) and on one French chromosome (Lyonnet et al. 1990), and Tyfield et al. (1991) report this association in southwest England. We have reasons to believe that the R408W mutation on haplotype 1 is recurrent (John et al. 1990) and will be found primarily in the French settlers known as Acadians (C. R. Scriver, D. Cole, M. Ludman, C. Riddell, and R. Rosen, unpublished data). Acadian or Breton origins of the corresponding English families (Tyfield et al. 1991) are a point to reconsider.

Although there are two different geographic locations for the M1V allele in France—one in Mortagne-Perche, for the reconstructed historical center of diffusion for ancestors of French-Canadian M1V carriers, and another in Finistère Sud, for the contemporary cluster of M1V mutations in French families—the two clusters are not necessarily incompatible. The genealogical reconstructions merely indicate a possible geographic region (Perche) and a likely time horizon (early 17th century) for the origin of the M1V mutation in contemporary Quebec (De Braekeleer et al. 1990). Migration from Mortagne-Perche to Nouvelle France involved about 217 identified persons, of whom 202 migrated before 1670 (Charbonneau and Robert 1987). Accordingly, genetic drift is a reasonable explanation for the appearance of the M1V mutation in descendants of these settlers. Between 1608 and 1759, on the order of 10,000 persons from France (Charbonneau et al. 1987; Charbonneau and Robert

1987) formed the ancestral core of Nouvelle France in Québec. Of the 30,000 (approximate) original settlers and descendants, about half returned to France (Trudel 1983; Boleda 1984). Where they settled on return is not as well known as are the origins of the ancestors of present-Quebecois. Thus, the contemporary cluster of French M1V alleles may reflect either returned chromosomes or local historical migration within France, across 300 km from Perche to the coast. As it happens, genealogies are sometimes more difficult to reconstruct in France than in Québec, where a revolution did not disrupt archives. Work in progress may eventually better explain the two historical clusters of M1V mutations in France. In the meantime, the mutation serves as a useful marker for historical demography.

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