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Per Guldberg[a]
Johannes Zschocke[b]
Atli Dagbjartsson[c]
Karen Friis Henriksen[a]
Flemming Güttler[a]

[a] The John F. Kennedy Institute, Glostrup, Denmark;
[b] Department of Paediatrics, Philipps University, Marburg, Germany;
[c] Department of Paediatrics, University Hospital, Reykjavik, Iceland

A Molecular Survey of Phenylketonuria in Iceland: Identification of a Founding Mutation and Evidence of Predominant Norse Settlement

Key Words
Phenylketonuria
Phenylalanine hydroxylase
Founder effect
Founder mutation
Iceland
Norway

Abstract
Iceland was settled during the late 9th and early 10th centuries AD by Vikings who arrived from Norway and the British Isles. Although it is generally acknowledged that the Vikings brought with them Celtic slaves, the relative contribution of these peoples to the modern Icelandic gene pool has been a matter of considerable discussion. Most population genetic studies using classical markers have indicated a large Irish genetic contribution. We have investigated the molecular basis of phenylketonuria (PKU) in 17 Icelandic patients and found 9 different mutations in the phenylalanine hydroxylase gene. One novel mutation, Y377fsdelT, accounts for more than 40% of the mutant chromosomes. Haplotype data support a common ancestral origin of the mutation, and genealogical examination extending back more than 5 generations shows that this mutation has probably arisen in an isolated part of southern Iceland and was enriched by a founder effect. At least 7 PKU mutations have originated outside iceland. The almost exclusively Scandinavian background of these mutations and the complete absence of common Irish PKU mutations strongly support historical and linguistic evidence of a predominant Scandinavian heritage of the Icelandic people.

Introduction

Phenylketonuria (PKU; McKusick MIM 261600) is one of the most prevalent inborn errors of amino acid metabolism with an overall frequency of 1 in 10,000 among Caucasian populations [1]. This autosomal recessive disorder is caused by transmission of mutations in the phenylalanine hydroxylase (PAH) gene and, in the untreated state, results in the development of mental retardation. Recent world-wide progress in defining the molecular nature of PKU has led to the identification of nearly 300 different PAH gene mutations which have been systematically recorded by the PAH Mutation Analysis Consortium (http://www.mcgill.ca/pahdb). For some of the more prevalent mutations, the study of their relative frequencies, their geographic distributions, and their associations with intragenic polymorphic markers has identified multiple founding populations for PKU [re-

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Flemming Güttler, MD, PhD
The John F. Kennedy Institute
Gl. Landevej 7
DK–2600 Glostrup (Denmark)
Tel. +45 4326 0100, Fax +45 4343 1130

viewed in ref. 2]. For a number of mutations with a narrower distribution, genealogical examination has traced their origin back to well-defined geographical areas [3–6] and even to single individuals [7]. These contributions demonstrate the usefulness of molecular studies of PKU in addressing various historical and anthropological issues.

Iceland is situated in the North Atlantic Ocean between Greenland and Norway and was settled in AD 870–930, chiefly by Vikings from western Scandinavia and the British Isles [8, 9]. Although several lines of evidence suggest that Norse Viking raiders leaving for Iceland from the British Isles brought with them freemen, wives and slaves of possible Irish origin, the Irish contribution to the parental Icelandic population has remained uncertain. Historical evidence indicates a vast majority of Norsemen among the original settlers, whereas several genetic studies have suggested that the Celtic contribution to the Icelandic gene pool is considerably higher than the Norse contribution [ref. 10, and references therein].

Except for a wave of emigration during the second half of the 19th century, the Icelandic population has experienced very little migration in the millennium since its settlement and must be considered a genetic isolate. The population seems to have increased steadily during the first centuries after its foundation but was reduced over the next 500 years as a result of famine, volcanic eruptions, and small-pox and plague epidemics, reaching a minimum of 34,000 in 1709 [8, 9]. The contemporary population is 260,000, and the incidence of PKU, as ascertained by newborn screening over 25 years, is 1 in 10,000. In the present study, we have defined the repertoire of PAH gene mutations associated with PKU in the 17 Icelandic patients, and we have established the associations between mutations and RFLP haplotypes [11], a short tandem repeat (STR) [12], and a variable number of tandem repeats (VNTR) [13]. Our findings demonstrate the total absence of common Irish mutations in Iceland and include the identification of a 1-bp frameshift deletion that accounts for 42% of the mutant chromosomes and is unique to Iceland.

Materials and Methods

Patients

PKU mutations were investigated in all known patients with PKU born in Iceland in the period 1947–1996. All patients were of well-documented Icelandic ancestry. There were 17 patients, including one pair of siblings and one father and son who both had PKU, resulting in a total of 31 apparently independent PKU alleles. Criteria for diagnosis and classification of PKU were as previously described [14]. Genealogical examination was performed by the Genetic Committee, University of Reykjavik, where systematic, computerized registration of family histories extends back more than 4 generations. Whole blood was collected in EDTA anticoagulant, and DNA was extracted by a salt precipitation method [15].

Molecular Analysis

Detection of mutations in the PAH gene was essentially as described [16]. Briefly, genomic DNA was PCR-amplified and GC-clamped using 13 sets of primers that cover all 13 PAH gene exons and the flanking consensus sequences required for RNA splicing. Primer sets are given in previous reports [17, 18]. PCR was carried out in final volumes of 15 µl, containing 10 m*M* Tris-HCl (pH 8.3), 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.02% gelatin, 0.2 m*M* cresol red, 12% sucrose, 200 µ*M* each dNTP, 0.8 µ*M* each primer, 500 ng of DNA, and 0.8 U of Taq DNA polymerase. The inclusion of cresol red and sucrose in the reaction mixture enables direct gel loading of the PCR product with no need for further addition of loading buffer [19]. The amplification protocol consisted of an initial denaturation step of 94 °C for 5 min, followed by forty rounds of thermal cycling (94 °C for 20 s, 55 °C for 20 s, and 72 °C for 20 s), and a final incuabtion at 72 °C for 5 min. The PCR product was loaded onto a composite, formamide-free denaturing gradient gel consisting of 6% polyacrylamide with a bottom gel containing a uniform concentration of 9.5 *M* urea, and a top gel containing a 0–9.5 *M* urea gradient. Electrophoresis was performed at 160 V for 4.5 h in 1 × TAE buffer kept at a constant temperature of 60 °C. Sequencing of the double-stranded PCR products was performed with the Thermo Sequenase Cycle Sequencing Kit (Amersham) using ^{33}P-end-labelled, non-GC clamped primers as sequencing primers. RFLP haplotypes were determined by PCR and Southern blotting [for references, see ref. 4]. The VNTR and STR systems were analysed by previously described PCR-based assays [12, 13]. Numbering of STR sizes was as described by Zschocke et al. [20].

Results

Mutation analysis in the 17 Icelandic PKU patients was accomplished by PCR amplification and GC-clamping of specific PAH gene fragments, scanning of all PCR products for mutations by 'broad-range' denaturing gradient gel electrophoresis (DGGE) [16], and direct sequencing of DGGE-positive samples. This approach led to the identification of a potentially causative PAH mutation on all 34 mutant chromosomes. There were 9 different mutations (table 1). The most common PKU mutation in the Icelandic population is a previously undescribed deletion of one of two successive thymidine residues in codons 376 and 377 in exon 11 of the PAH gene (fig. 1). Y377fsdelT was found on 13 apparently independent alleles in four homozygous patients and 5 genetic compounds, and accounts for 42% of mutant chromosomes in Iceland. The deletion results in a frameshift and the introduction of a termination codon at residue 399. It

Table 1. Frequencies and haplotype associations of PAH gene mutations in Icelandic PKU patients

Mutation name	Nucleotide change	RFLP haplotype	VNTR	STR[a]	Alleles[b]	Possible origin
Y377fsdelT	1128/29 del T	4	3	234	13 (42)	? Iceland
P281L	CCg→CTg	1	8	246 (5) 242 (1)	6 (19)	? Iceland
F299C	TTT→TGT	8	9	234	4 (13)	NW-Norway
Y414C	TAC→TGC	4	3	238	2 (7)	Scandinavia
IVS12nt1	g→a	3	8	246	2 (7)	Denmark
R68S	AGA→AGT	1	8	246	1 (3)	?
R111X	CGA→TGA	4	3	234	1 (3)	?
S273F	TCC→TTC	7	3	238	1 (3)	W. Europe
R408W	CGG→TGG	2	3	238	1 (3)	E. Europe
Total number of apparently independent chromosomes					31 (100)	

[a] The numbering of STR allelles is as defined by Zschocke et al. [20].
[b] Relative frequencies are shown in parentheses (%).

causes a severe PKU phenotype in homozygous patients and in genetic compounds carrying this mutation in combination with a known severe mutation. Because the Y377fsdelT mutation does not affect the thermostability of the amplified fragment and therefore cannot be visualized as a mobility shift on denaturing gradient gels, DGGE-based detection of this mutation in the homozygous constellation requires that patient DNA be mixed with normal control DNA to allow formation of heteroduplexes.

Two mutations, P281L and F299C were found to be moderately common in the Icelandic population, with relative allele frequencies of 19% (6/31) and 13% (4/31), respectively. Six other mutations were each found on two (Y414C and IVS12nt1) or one (R68S, R111X, S273F, and R408W) PKU chromosomes. Patients who were genetic compounds for Y414C or R68S and a known severe PKU mutation displayed a mild PKU phenotype, whilst all other patients suffered from severe PKU.

Analysis of polymorphic restriction sites and determination of VNTR and STR sizes were performed in all patient samples. Complete RLFP, VNTR and STR haplotyping was feasible in the 4 patients who are homozygous for the Y377fsdelT mutation, in 1 patient who is the son of a Y377fsdelT homozygote, and in 2 additional patients where DNA from both parents was available. All 10 fully characterized chromosomes carrying the Y377fsdelT mutation were associated with RFLP haplotype 4, the 3-copy VNTR allele, and the 234-bp STR allele. By assuming that the 3 remaining Y377fsdelT-alleles also had this chromosomal background, RFLP haplotype/VNTR/STR associations could be inferred for all 9 different mutations (table 1). The P281L mutation was consistently found on RFLP haplotype 1 in association with the 8-copy VNTR allele; however, one of the chromosomes carried the 242-bp STR allele whereas the remaining 5 chromosomes carried the 246-bp STR allele.

For all patients carrying the Y377fsdelT mutation, genealogical reconstructions were established, extending back at least five generations. The family of some patients could be traced to the beginning of the 18th century when the first general census in Iceland was held. No cases of consanguinity were identified, only weak genealogical links could be established, and direct proof of kinship between the affected individuals could not be provided. Geographically, however, 129 out of 208 ancestors of parents carrying the Y377fsdelT mutation were born in the southern part of Iceland covered by the three counties Vestur-Skaftafells, Austur-Skaftafells, and Rangarvalla (fig. 2). Seventy of these ancestors lived in Vestur-Skaftafells, south of the Vatnajökull glacier.

Discussion

There has been considerable discussion on the proportion of Irish genes in the Icelanders. Available historical and linguistic evidence points to a predominant Norwe-

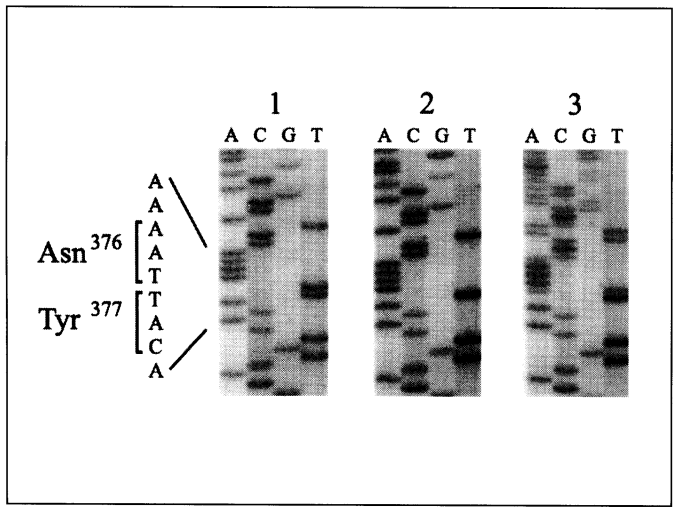

Fig. 1. Sequence analysis of PAH exon 11. Lane 1: normal control; lane 2 = PKU patient homoallelic for the Y377fsdelT mutation; lane 3 = PKU patient heteroallelic for the Y377fsdelT mutation.

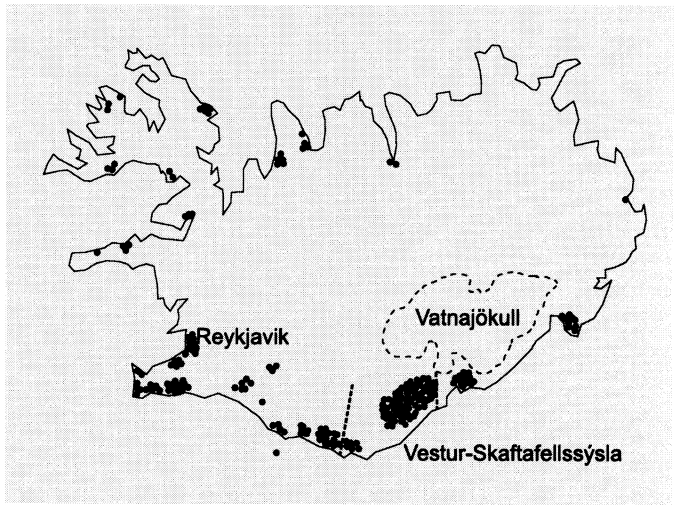

Fig. 2. Map of Iceland showing birth places of 208 acestors of parents carrying the Y377fsdelT mutation. The borders of the county Vestur-Skaftafells are indicated by dashed lines.

gian Viking colonisation, and Icelandic language and culture reveal very little Irish influence. The first historical accounts of the early settlers of Iceland date back to the 12th century. The most important and detailed source is the Book of the Settlement *(Landnámabók)* which mentions more than 400 settlers by name, origin, and land claims [8, 9]. On the basis of this account, it has been estimated that about 85% of the settlers came from Norway and that about 12% came from the British Isles. However, considering that the population at the end of the 11th century has been estimated to exceed 60,000, it is clear that the *Landnámabók* covers only a small fraction of the settlers, probably less than 10%. The lack of information in the early written sources about the remaining settlers has led to several research approaches to clarify their origin.

Physical anthropological investigations have demonstrated much more pronounced similarities between the peoples of Iceland and Ireland than between the peoples of Iceland and Norway, pointing to a considerable admixture of Irish stock in the Icelanders. These findings were reinforced by the observation that the proportion of blood group O in Iceland is similar to that found in Ireland and Scotland, but significantly higher than that found in Norway [21]. Although the validity of ABO blood group gene frequencies for estimating the Icelandic admixture has since been questioned [22, 23], most genetic studies up to now have indicated a considerable Irish component of the Icelandic gene pool [10 and references therein].

More direct links between an ancient founder population and a contemporary people may be established by the identification of highly specific genetic markers, such as recessive disease mutations [24]. In this study, we have identified and characterized 9 different mutations responsible for PKU in Iceland. Seven of these mutations have been described on the same haplotype backgrounds in other European populations, and origins outside Iceland appear likely (table 1). Two factors are of particular interest in this respect; the likely Scandinavian background of these mutations and the total absence of Irish mutations. At least 3 of the 7 mutations and 8 of the 12 mutant alleles that did not originate in Iceland can be traced to areas historically inhabited by Viking peoples. The most informative mutation is probably F299C, the third most common mutation in Iceland. This mutation is particularly common on the northwestern coast of Norway where it accounts for 17% of PKU alleles [6, 24]. It is the commonest mutation in this region and has probably originated there. Similarly, the mutations Y414C, IVS12nt1, and R408W on RFLP haplotype 2 are prevalent in Norway and other Scandinavian regions that were historically inhabited by Vikings, and R68S and R111X are also encountered in these populations [25].

In contrast, none of the mutations that are frequent in Ireland or Scotland (R408W on RFLP haplotype 1, I65T, F39L, and L348V) [26, 27] was identified in the Icelandic PKU patients. This makes the immigration of a large number of Irish people during the colonisation of Iceland

unlikely. The current incidence of PKU and the calculated frequency of PKU carriers in Ireland (1:30) are among the highest in the world [28], and mutations of Irish origin have been identified as the main PKU alleles in Canadian [4], US American [18] and Australian [5] peoples of Irish descent. Even considering that the frequency of PKU alleles in Ireland at the end of the first millennium AD could have been somewhat lower than it is today, Irish PKU mutations should be found in Icelandic patients if Irish genes had provided a major part of the Icelandic gene pool.

The finding of a PKU mutation, Y377fsdelT, that is very common in Iceland and has not yet been identified elsewhere was not surprising. Genetic drift would be expected in the populations of an isolated island, and previous evidence of a founder effect in Iceland includes common mutations in adenine phosphoribosyltransferase (APRT) deficiency [29] and breast cancer [30]. All families harbouring the Y377fsdelT mutation are likely to be descendants of a common progenitor who lived in Iceland at least 7 generations ago. Tracing the families at least four generations back revealed two clusterings of ancestors; one in the southern part of Iceland and one in the western part (fig. 2). In contrast to the present situation, the area around the capital Reykjavik was sparsely populated during the 18th and 19th centuries [9], and the ancestors clustering in this region represent mainly young generations. The most likely origin of the Y377fsdelT mutation is therefore the south of Iceland. In particular, a clustering of ancestors was identified in the county Vestur-Skaftafells which has been relatively isolated from other parts of Iceland until the middle of this century when a road to this area was constructed. The high frequency of the Y377fsdelT mutation is probably due to genetic drift and may partly explain the relatively high incidence of PKU in Iceland.

In summary, we have used PKU mutations as specific genetic markers to suggest a predominant Norse heritage of the Icelandic people. The increasing knowledge about origins and frequencies of mutations causing PKU and other common autosomal recessive disorders may serve as an important tool to trace ancient and recent human migration and settlement.

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