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Relative Frequency, Heterogeneity and Geographic Clustering of PKU Mutations in Norway

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Abstract

We have analysed 236 Norwegian phenylketonuria (PKU) alleles by a combination of mutation scanning methods, restriction enzyme-based assays and DNA sequencing. Thirty-three different mutations constituted 99.6% of all mutant alleles (only 1 allele remains unidentified), 23 of these have been identified also in other European countries. Twenty were predicted missense mutations, 6 splice mutations, 4 nonsense mutations and 2 deletion mutations and 1 mutation disrupted the start codon. The 8 most common mutations represented 83.5% of the PKU alleles, with single allele frequencies ranging from 5.9 to 15.7%. Four of these mutations (R261Q, R408W, Y414C, and IVS12nt1) are commonly occurring also in PKU patients in other European countries, while the other 4 (G46S, G272X, F299C, and R408Q) have higher frequencies in Norway than in any other country studied. Six mutations (I65T, L249F, P281L, Y356X, R158Q, and R252W) have frequencies between 0.8% and 2.1%, and 19 mutations were encountered only once. The majority of PKU mutations were found on the same RFLP/VNTR haplotype backgrounds in Norway as in other European populations, suggesting that only a few of the mutations may represent recurrent mutations (<3.4%). Among 10 mutations only reported for our population, we detected 2 de novo mutations (0.8%) arisen in Norway. From the birthplaces of the probands' grandparents, each mutation seemed to have an individual geographic distribution within Norway, with patterns of local mutation clustering. Our observations are compatible with multiple founder effects and genetic drift for the distribution of PKU mutations within Norway.

Introduction

The population distribution of genes for an increasing number of autosomal recessive disorders can now be studied directly on the mutational level. New techniques based on the polymerase chain reaction (PCR) have greatly improved the speed and simplicity of mutation detection. Population genetic studies are prolific in particular for diseases like phenylketonuria (PKU), with extensive mutation heterogeneity [1] and many normal markers in the phenylalanine hydroxylase (PAH) gene to provide highly informative RFLP haplotypes [2, 3]. The distribution of 5 prevalent PKU mutations (R158Q, R261Q, IVS10nt546, R408W, and IVS12nt1) throughout Europe has recently been investigated [4]. The strong associations between specific RFLP haplotypes and these PAH muta-

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tions suggest that the present distribution in Europe is largely due to founder effects and random genetic drift. However, a more detailed study of the distribution of PKU mutations within a single country might extend this hypothesis. In this respect Norway (4.3 million inhabitants, 324,000 km^2, 1,752 km measured by its long axis) may be particularly well suited for studies because of its relatively stable population structure for centuries and short average marital distance [5]. Previous observations have suggested that Norwegian PKU mutations might have originated from Celtic populations [6]. Treacy et al. [7] have proposed that PKU mutations I65T on haplotype 9.8 and R408W on haplotype 1.8 are markers for 'Celtic' PKU genes. The latter mutation has its peak incidence in the Irish population, but is also common in several other northwestern European populations, including the Norwegian. This distribution may be compatible with the hypothesis of a 'Celtic' PKU allele [8]. We have previously reported the identification and characterization of the 'Norwegian' PKU alleles G272X [9, 10], F299C [11], R408Q [11], and G46S [12] as well as a number of singly occurring alleles [13–16]. The mutation analysis is here extended to 236 PKU alleles. We have combined results from the mutation analysis with those from PAH haplotype analyses, and looked at the geographic backgrounds of mutations and haplotypes two generations back in order to map the distribution of each PKU mutation in Norway.

Materials and Methods

Patients

Blood samples from 118 Norwegian PKU families were collected as described previously [9, 11]. In these 118 families there were found a total of 149 registered PKU patients. This sample comprised all patients in the period 1966–1994 (134 patients), and 15 patients born before 1966. The incidence of PKU in Norway was 1:13,250 in the period from 1966–1994 [17]. Apart from families with more than one affected sibling (31 siblings), none of the families had knowledge of PKU occurring in other parts of their families.

Haplotypes

Haplotypes were determined as described [3, 9], but supplemented with PCR-based RFLP typing [18–21]. The laboratory protocol and the PCR primers for VNTR analysis were as described by Goltsov et al. [22].

Mutation Detection

Screening for mutations was performed by single-strand conformation polymorphism (SSCP) analysis (both ^{32}P and fluorescence detection) [11, 12, 23] and denaturing gradient gel electrophoresis (DGGE) [24, 16]. Details and results of the mutation scanning applying DGGE are given elsewhere [16]. PCR primers used to amplify exons with flanking sequences for SSCP analyses were as follows: exon 1: 1A2/1B [13], exon 2: 2A/2B [12], exon 3: 3A/3B [25], exon 4: 4A1/4B2 [14], exon 5: 5A/5B [26], exon 6: 6A/6B [27], exon 7: 7A1/7B1 [26], exon 8: 8A/8B [11], exon 9: 9A/9B (5'-TCTGGCCACCCATCACCTTT-3'/5'-CTATAGCACTCCACCATCCA-3', exon 10: 10A/10B [28], exon 11: 11A/11B [28], exon 12: 12A/12B [29] and for exon 13:13A/13B [12].

For restriction-enzyme-based mutation assays (table 1), we applied the same PCR primers as for SSCP analyses. In addition, the following primers were used (for ACRS [30] primers are the mismatch nucleotides in bold face): 1B2 [13], 2B46Sa [12], 3B65Ta (5'-AACGAGAAGGTCTAGATTC**G**-3'), 4A2 (5'-CCTTCTCTGTGTTTCAGT-3'), 5A158Ms [30], 6B218Ha (5'-TGGGGAATGTTATCTTCATGGA**G**G-3'), 6BEXON6nt-96Ma (5'-AGTGGAAAAATGTGATTGTA**G**TCA-3'), 7A2/7B2 [A and B in ref. 9], 7B243Ms (5'-GAAAGCAGGCCAGCCACAGG**C**C-3'), 7B280Ms (5'-AGCTGGAGGACAGTACTCACGGT**C**-3'), 7A281Sa (5'-ATCCAAGCCCATGTATACC**T**CCG**G**AC-3'), 8A299Hi [11], 8B300Hh (5'-CCTTACCTGGGAAAACTG**C**G-3'), 12A414Ra [31] and 12BIVS12Ra [30]. The methods for exon amplification and restriction-enzyme-based mutation assays have been previously described [30, 31]. The details and modifications of PCR reactions used in this study are summarized in table 1. DNA sequencing was performed as described previously [9, 12, 13]. Restriction-enzyme-based assays (table 1) were used to identify and verify mutations as well as to document mendelian transmission for the mutant alleles.

Geographic Data

Information on birthplaces was available from 445 (94%) of the 472 grandparents of the PKU patients (data collected by questionnaire). To compare the number of gene sources (pairs of PKU grandparents) with the regional population density (fig. 1k), Norway was subdivided into 5 regions ([32] and fig. 1). Southern Norway includes the 15 counties in the south, northern Norway includes the 3 northernmost counties. The number of presumed gene sources was compared to the resident population in 1930 (2.8 million inhabitants).

Results

Heterogeneity and Relative Frequencies of PKU Mutations

Twenty-six mutations were detected by SSCP screening of all 13 exons of the PAH gene, and were verified by DNA sequencing. Seven additional mutations (F55fs, IVS2nt1, IVS3nt-6, V177L, G218V, R297C and delV399/R400fs) were detected by DGGE as reported elsewhere [16]. These 33 mutations (table 2) predicted 1 mutation disrupting the start codon, 20 missense mutations, 6 splice mutations, 4 nonsense mutations and 2 deletion mutations. Twenty-three of the mutations were also found in other European populations [33], while 10 mutations (M1I, IVS2nt1, IVS3nt-6, D143G, H170R, EXON6nt-96, V177L, A259T, R297C and delV399/R400fs) were only observed in Norway. Restriction-

Table 1. Restriction-enzyme-based mutation assays

Mutation	nt N/M	Loc.	RE	RE origin	Primers	PCR product	NA, bp/MA, bp
M1I	ATG/ATA	E1	*Nsp*I	NA	1A2/1B2[1,3]	109	57+52/109
G46S	GGT/AGT	E2	*Sau*96I	AC	2A/2B46Sa	188	169+19/188
F55fs[7]	TTT del T	E2	–	–	2A/2B	297	(S)
IVS2nt1	gt/at	I2	*Mae*III	AC	2A/2BIVS2nt1Ma	211	211/190+21
I65T	ATT/ACT	E3	*Taq*I	AC	3A/3B65Ta[4]	132	112+20/132
IVS3nt-6	t/a	I3	*Hinf*I	MA	4A1/4B2	165[6]	115+50/60+55+50
D143G	GAT/GGT	E4	*Mbo*I	AC	4A2/4B143Mb[1,4]	111	91+20/111
R158Q	CGG/CAG	E5	*Msp*I	AC	5A158Ms/5B	157	137+20/157
H170R	CAT/CGT	E5	*Msp*I	MA	5A/5B	260	260/159+101
EXON6nt-96[8]	A/g	E6	*Mae*III	AC	6A/6BEXON6nt-96Ma	223	223/198+25
V177L	GTG/CTG	E6	*Xho*I	NA	6A/6B	357	245+112/357
G218V	GGC/GTC	E6	*Hae*III	AC	6A/6B218Ha	269	269/244+25
L249F[7]	CTT/TTT	E7	–	–	7A1/7B1	291	(S)
R243X	CGA/TGA	E7	*Msp*I	AC	7A1/7B243Ms	104	81+23/104
R252W	CGG/TGG	E7	*Ava*I	NA	7A1/7B1[2,4]	291	184+107/291
A259T	GCC/ACC	E7	*Bst*NI	NA	7A2/7B2[2,4]	156	76+80/156
R261Q	CGA/CAA	E7	*Hinf*I	NA	7A1/7B1[2,4]	291	154+137/291
R261X	CGA/TGA	E7	*Dde*I	MA	7A1/7B1[2,4]	291	291/135+156
G272X	GGA/TGA	E7	*Bam*HI	NA	7A1/7B1[2,4]	291	169+122/291
E280K	GAA/AAA	E7	*Msp*I	AC	7A1/7B280Ms	217	191+26/217
P281L	CCg/CTg	E7	*Sau*96I	AC	7A281Sa/7B1	121	98+23/121
IVS7nt1	gt/at	I7	*Nla*III	MA	7A1/7B1	291	22+147+122/22+147+31+91
R297C	CGC/TGC	E8	*Mbo*I	NA	8A/8B	300[6]	300/181+119
F299C	TTT/TGT	E8	*Hind*III	AC	8A299Hi/8B[4]	140[6]	120+20/140
A300S	GCC/TCC	E8	*Hha*I	AC	8A/8B300Hh[4]	152	134+18/152
S349P	TCA/CCA	E10	*Pfl*mI	MA	10A/10B[3,4,5]	250[6]	250/140+110
IVS10nt-11	g/a	I10	*Dde*I	MA	11A/11B	319	319/240+79
Y356X	TAC/TAG	E11	*Rsa*I	NA	11A/11B	319	232+87/319
delV399/R400fs[7]	del GTAA or TAAG	E11	–	–	11A/11B	319	(S)
R408W	CGG/TGG	E12	*Sty*I	MA	12A/12B	245	245/148+97
R408Q	CGG/CAG	E12	*Hae*III	NA	12A/12B	245	115+103+27/218+27
Y414C	TAC/TGC	E12	*Rsa*I	AC	12A414Ra/12B	148	127+21/148
IVS12nt1	gt/at	I12	*Rsa*I	AC	12A/12BIVS12nt1Ra	216	195+21/216

E = exon; I = intron; N = normal; M = mutant; NA = normal allele; MA = mutant allele;
AC = amplification created; S = DNA sequencing; RE = restriction enzyme; nt = nucleotide; bp = basepairs.

1 PCR annealing temperature 58 °C instead of 55 °C.
2 PCR annealing temperature 65 °C instead of 55 °C.
3 'Hot start PCR' [37].
4 40 PCR cycles instead of 30 cycles.
5 $[Mg^{2+}]$ = 1.25 m*M*.
6 PCR product size estimated from agarose gel electrophoresis.
7 Restriction enzyme-based assay was not successful, and direct DNA sequencing was used.
8 Y204C [33] has recently been shown to be a splice mutation [15].

enzyme-based assays were established for the majority of the mutations to screen for mutations as well as detect their parental transmission. The mutation assays are summarized in table 1. Two mutations (0.8%), M1I [13] and IVS3nt-6 [16], were de novo mutations, the others were inherited. The 8 most frequent mutations comprised 83.5% of the PKU alleles, with relative frequencies ranging from 5.9 to 15.7% (table 2). The I65T, L249F, P281L, Y356X, R158Q and R252W were present at low frequencies (0.8–2.1%), while 19 other mutations were repre-

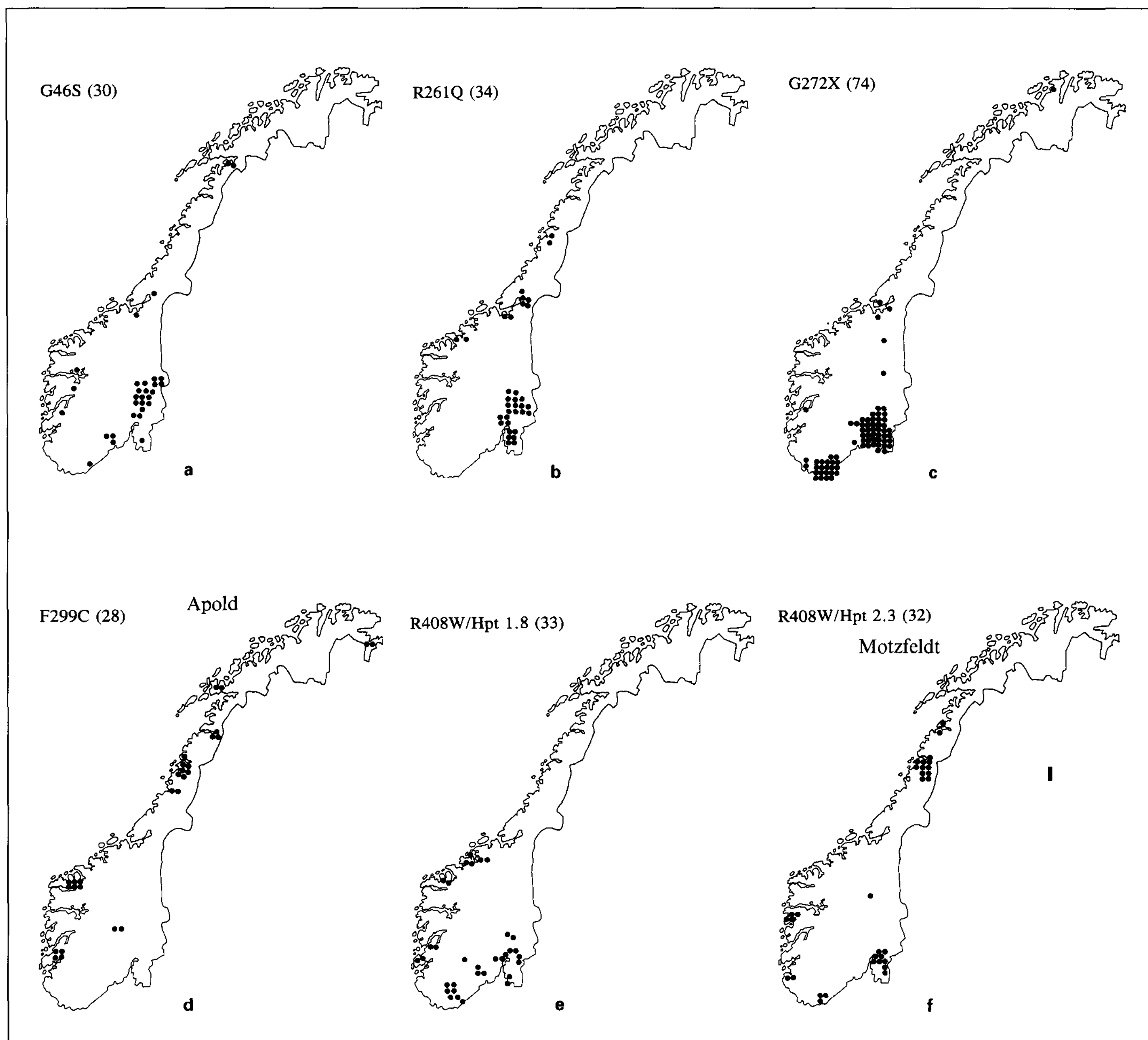
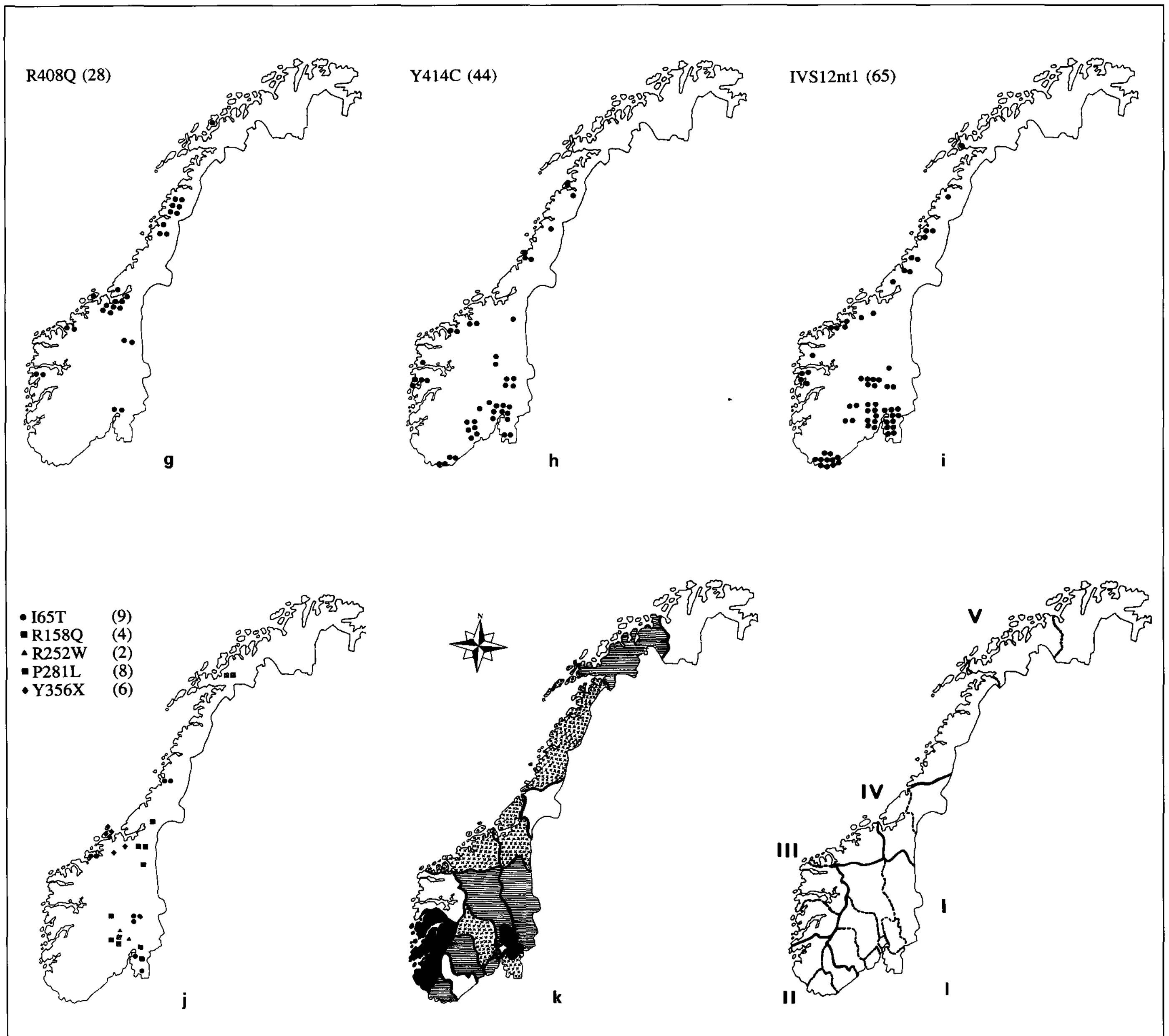


Fig. 1a–i. Maps of Norway showing birthplaces of the grandparents of PKU patients for individual mutations. ● = Indicates the actual birthplace of a grandparent. **j** Five PKU mutations illustrated in one map with individual symbols showing birthplaces of the grandparents of PKU patients. PKU grandparents born outside Norway (n = 6) were from England (n = 2, R252W), Denmark (n = 2, R261Q), USA (n = 1, R408W/hpt 1.8), and Sweden (n = 1, IVSl2nt1). **k** The population density in 1930 (corresponding approximately to time of birth of grandparents) is shown. The population density is given as a percentage of the total population for each county in Norway in 1930 (Norwegian Official Statistics, Oslo 1932). Filled black areas: >7.00%; crossed areas: 5.00–7.00%; hatched areas: 3.00–4.99%; white areas: <3.00%. **l** The 18 counties (stippled lines) of Norway grouped in five regions (filled lines). Region I: the 7 counties in southeast Norway; region II: the 3 southern most counties; region III: the 3 counties in the west; region IV: the 2 counties in middle Norway; region V: the 3 northernmost counties.



sented by a single allele among Norwegian PKU patients. These 33 mutations constitute 235 alleles out of 236 investigated. Compound heterozygosity was found for 82.2% of the patients, and 21 homozygous patients were found (11 different mutations).

Haplotype Associations

Each of the PKU mutations was strongly associated with a single RFLP/VNTR haplotype. One single allele of R261Q, P281L, R408Q and IVS12nt1, respectively, was found on an alternate haplotype (table 2). However, the R408W mutations were equally distributed between haplotypes 1.8 (n = 18) and 2.3 (n = 16). Preliminary data on 14 of these Norwegian R408W alleles were included in a recent collaborative European study [8].

Silent Polymorphisms

Through the mutation search we also identified 6 predicted silent polymorphisms within the PAH gene (table 3). Three of these polymorphisms are located within

Table 2. Norwegian PKU mutations

Mutation	Frequency		Haplotypes (RFLP Hpt VNTR)	Frequency in other populations, %		
	n	%		Denmark	Sweden	N-Ireland
G272X	37	15.7	7.8	1.6	2.3	–
IVS12nt1	35	14.7	3.8, 4.8[1]	37.3	15.9	1.6
R408W	34	14.4	1.8[2], 2.3[3]	18.2[4]	21.6[5]	39.1[6]
Y414C	26	11.0	4.3	10.0	18.8	3.1
R261Q	19	8.0	1.8, 5.8[1]	1.6	1.7	1.6
G46S	17	7.1	5.9	1.3	4.5	3.1
F299C	16	6.7	8.9	–	–	3.1
R408Q	14	5.9	12.12, 5.8[1]	0.3	1.7	4.7
I65T	5	2.1	9.8	0.7	–	23.4
P281L	4	1.7	1.7, 4.3[1]	1.3	2.3	–
Y356X	3	1.3	12.12	0.3	–	–
R158Q	2	0.8	4.3	2.9	1.7	1.6
L249F	2	0.8	1.7	–	–	1.6
R252W	2	0.8	2.3	–	1.7	–
M1I	1	0.4	7.8	–	–	–
F55fs	1	0.4	1.8	–	–	–
IVS2nt1	1	0.4	n.d.	–	–	–
IVS3nt-6	1	0.4	5.8	–	–	–
D143G	1	0.4	11.8	–	–	–
H170R	1	0.4	7.8	–	–	–
EXON6nt-96	1	0.4	4.3	–	–	–
V177L	1	0.4	6.8	–	–	–
G218V	1	0.4	1.8	0.3	–	–
R243X	1	0.4	7.8[7]	–	1.1	3.1
A259T	1	0.4	3.8	–	–	–
R261X	1	0.4	1.7	–	–	–
E280K	1	0.4	1.8	2.9	–	–
IVS7nt1	1	0.4	4.3	0.3	–	–
R297C	1	0.4	4.3	–	–	–
A300S	1	0.4	1.8	–	–	–
S349P	1	0.4	1.7	0.3	–	1.6
IVS10nt-11	1	0.4	n.d.	5.2	–	–
delV399/R400fs	1	0.4	7.8	–	–	–
Unknown	1	0.4	n.d.	1.0	22.7	0.0
Total	236	100.0				

The relative frequency of Norwegian PKU mutations (total number n=236) is compared to the relative frequency of PKU mutations in Denmark (n = 308) [24], Sweden (n = 176) [38, 12] and Northern Ireland (n = 64) [39]. Other references to the individual mutations are available in the PKU Mutation Analysis Consortium [33]. Hpt = Haplotype; n.d. = not determined; – = data not available/not found.

1 Only 1 case.

2 Relative frequency in Norway: 7.6%.

3 Relative frequency in Norway: 7.8%.

4 Denmark: Haplotype 1.8: 1.3%, haplotype 2.3: 16.9% (n = 308) [8].

5 Sweden: Haplotype 1.8: 1.1%, haplotype 2.3: 20.2 (n = 178) [8].

6 Northern Ireland: Both haplotype 1.8 and 2.3 detected (n = 64) [39].

7 This allele is associated with a unique intragenic *Msp*I polymorphism detected on Southern blotting (a 19-kb band replaced by bands of approximately 16 and 8 kb).

Table 3. Silent polymorphisms in the PAH gene found among Norwegians

Polymorphism	nt change	Exon-Intron	RFLP Hpt VNTR
168+19	t>c	I2	n.d.
353–22	c>t	I3	4.3
V245V	G>A	E7	4.3
Q232Q	A>G	E6	3.8, 7.8, 4.3
L385L	G>C	E11	3.8, 7.8
1316–35	c>t	I12	5.9

Hpt = Haplotype; nt = nucleotide; n.d. = not determined.

exons, and 3 in introns. These polymorphisms were also observed in other European populations [33], except for the 353-22c>t polymorphism. Five of the polymorphisms were associated with only one, or a few, specific RFLP haplotypes/VNTRs (table 3), while incomplete family data precluded the assignment of the 168+19t>c polymorphism.

Geographic Distributions of Mutations within Norway

Data on the birthplaces of 445 grandparents in the PKU families assigned 439 to a specific area in Norway (fig. 1), whereas 6 were Caucasian immigrants (see legend to figure 1). Only one individual in each pair of grandparents represents the true origin of the mutation (gene source), but in the gross majority of cases the 2 grandparents were born in the same district/county (80–90%, depending upon the regional borders chosen: data not shown). Individual maps with grandparental birthplaces are presented for the 8 most frequent Norwegian mutations (fig. 1a–i). The 2 R408W mutations on different haplotypes are illustrated on separate maps (fig. 1e, f), while 5 low-frequency mutations are presented in a single map (fig. 1j). Figure 1k shows the population density (for each county in Norway) approximately at the time of birth of these grandparents, for comparison.

Grandparental G46S alleles are clustered in the southeastern part of Norway (fig. 1a) with a few alleles that originate from other areas. R261Q alleles show a similar distribution as G46S, but in addition, a minor cluster also appears in the central inland of Norway (fig. 1b). G272X alleles appear in two separate clusters in southern and southeastern Norway, with almost no alleles encountered in other parts of the country (fig. 1c). F299C alleles form several small clusters along the western and northern cost of Norway (fig. 1d). Similarly, R408Q alleles are distributed in coastal areas, mainly in two clusters (one in middle Norway and one in northern Norway) (fig. 1g). The 2 R408W mutations are found in different areas (fig. 1e, f). R408W/Hpt 1.8 alleles are widely spread, but restricted to southern Norway. R408W/Hpt 2.3 alleles are found in two very distant main clusters (one area in southeastern and one in northern Norway). The distribution of these mutations seems to deviate from the general population density (with the exception of R408W/Hpt 1.8) (fig. 1k). However, the Y414C and IVS12nt1 alleles are found in all parts of Norway (fig. 1h, i), distributed grossly according to the population density. Also for the low-frequency mutations, there may be some clustering (fig. 1j, e.g. the Y356X alleles are only encountered in a small area on the coast of middle Norway).

A simplistic comparison between population density and the distribution of PKU grandparents' birthplaces in 1930 (from fig. 1) showed how the number of PKU mutation gene sources varied in different parts of Norway. We found approximately 7 mutated alleles per 100 000 inhabitants in the north (region V), 9 in middle Norway (region IV), 6 in the west (region III), 14 in the southeast (region I), and 9 in the south (region II). A further subdivision into all 18 counties increased the regional variation (ranging from 2 to 20 per 100,000 inhabitants for the different counties).

Discussion

Our results show that even within a small and presumed genetically stable population like the Norwegian, the mutational basis of PKU is highly heterogeneous, with more than 33 different mutant alleles. A mutation analysis with a detection rate close to 100% has recently been performed in the Danish PKU population, identifying 35 different mutations among 308 PKU alleles [24]. Only 16 of the mutations are found in both Scandinavian populations (table 2). The extensive mutation heterogeneity, as well as the differences in the spectrum of mutations between the two neighboring countries, indicate multiple origins for PKU alleles even in this small geographic region.

The strong association of individual mutations with specific RFLP haplotypes/VNTRs points to single origins for most mutations. Almost all Norwegian mutations shared with other European populations were found on similar haplotypes in Norway. The R408W mutations are equally distributed between haplotypes 1.8 and 2.3 in Norway, suggesting recurrent mutations [8, 34]. In some parts of Europe, mutation I65T is found on several haplo-

types. In Norway, as in other north-western European populations, I65T is exclusively associated with haplotype 9.8 [33]. The European PKU mutations found also in Norway, in most cases seem to have derived from the same founder as PKU mutations encountered in other parts of Europe.

Recurrent mutation, gene conversion or recombination events do not seem to occur frequently. However, there are a few exceptions. We find the R243X (n = 1), R252W (n = 2) and the R261X (n = 1) mutation on other haplotypes than previously reported, and single R261Q, P281L, R408Q and IVS12nt1 mutations on new haplotypes for these mutations. These 8 obervations (3.4%) could be examples of either recurrent mutation or gene rearrangements. At least 5 of these mutations seem to be recurrent, as the polymorphic PAH gene markers differ both at 5′ and 3′ from the mutation, requiring more than one event or a gene conversion to explain the haplotype alterations by crossover. Six of these 8 mutations on a new haplotype background involve a CpG dinucleotide, usually considered a hotspot for new mutations [35]. We have previously suggested recurrent mutation for the S349P mutation in Europe [36], and for 2 new mutations (M1I and IVS3nt-6) we have documented de novo mutation events [13, 16]. Neither of the 3 latter mutations involved a CpG dinucleotide. Thus, new or recurrent mutations seem to be relatively common in PKU, while gene rearrangements seem to be rare events. The new or recurrent mutations on Norwegian PKU alleles are divided rather evenly between CpG dinucleotides on one hand and all other combinations of nucleotides on the other.

The frequencies of the individual PKU mutations in Norway are different from those found in Denmark, Sweden, and Northern Ireland (table 2). For the G46S, G272X, F299C and R408Q mutations, we find a higher frequency in Norway than in any other population studied so far. However, rare examples of at least 3 of these 4 typical 'Norwegian' mutations are also found in Denmark, Sweden and Northern Ireland. We have recently given details on frequencies of the G272X/haplotype 7 mutation in some European countries [10]. A similar pattern of a high relative frequency in Norway and a lower frequency in Northern Europe might also be predicted for the G46S mutation (table 2) [12].

G46S is a 'central inland mutation' of Norway, while G272X is mainly a 'coastal mutation' in the southeastern parts of Norway [9]. The F299C, R408Q and R408W/haplotype 2.3 mutations could be traced to the northwestern coast. Through the ages, the major means of transportation in Norway has been by ship. These restricted northwestern coastal mutations might therefore have been brought in by founders relatively recently, or might have arisen as new mutations, and may have been maintained in the slowly expanding population of the coastal area. The F299C, R408Q and R408W/haplotype 2.3 mutations are also found in Northern Ireland. They could be examples of mutations brought to the British Isles from Norway or in the opposite direction, and now form part of a postulated set of mutations shared by populations surrounding the North Sea.

The R261Q, the R408W/haplotype 1.8, the Y414C and the IVS12nt1 mutations have patterns of distribution quite different from those mentioned above. All are most frequently found in the southeastern parts of Norway.These mutations are common also in other Caucasian populations, and if their distributions also follow the population density in Norway, we might suggest very early or repeated ingress of these mutations into Norway. This is also supported by a high frequency of Y414C and IVS12nt1 in Denmark and Sweden.

A hypothesis that Celtic PKU genes were the major origin of PKU in Norway was based on a high incidence of PKU in Ireland and Scotland and a connection of birthplaces of PKU children's grandparents to sites with Viking graves containing Western imported objects [6]. Direct comparison between today's PKU mutations in Norway and Ireland indicates that hypothetical 'Celtic genes' probably contribute to only a minor fraction of Norwegian PKU genes. However, comparing the geographic distribution of the frequent 'Celtic' PKU mutations I65T and R408W/haplotype 1.8 with the same archeological data [fig. 2 in ref. 6] may suggest possible routes for these mutations across the North Sea.

The contribution of PKU alleles from Europe to Norway seems substantial, but also new mutations and founder populations within Norway are important. The regional clustering of mutations within Norway may be explained in part by the fjord- and mountain-rich topography of Norway that has preserved a stable resident population. The regional variations (in number of gene sources) of PKU mutations observed here also indicate substantial variation of carrier frequencies of PKU in different parts of Norway.

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