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MUTATIONS UPDATE

Mutations in PMM2 That Cause Congenital Disorders of Glycosylation, Type Ia (CDG-Ia)

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The PMM2 gene, which is defective in CDG-Ia, was cloned three years ago [Matthijs et al., 1997b]. Several publications list PMM2 mutations [Matthijs et al., 1997b, 1998; Kjaergaard et al., 1998, 1999; Bjursell et al., 1998, 2000; Imtiaz et al., 2000] and a few mutations have appeared in case reports or abstracts [Crosby et al., 1999; Kondo et al., 1999; Krasnewich et al., 1999; Mizugishi et al., 1999; Vuillaumier-Barrot et al., 1999, 2000b]. However, the number of molecularly characterized cases is steadily increasing and many new mutations may never make it to the literature. Therefore, we decided to collate data from six research and diagnostic laboratories that have committed themselves to a systematic search for PMM2 mutations. In total we list 58 different mutations found in 249 patients from 23 countries. We have also collected demographic data and registered the number of deceased patients. The documentation of the genotype–phenotype correlation is certainly valuable, but is out of the scope of this molecular update. The list of mutations will also be available online (URL: http://www.kuleuven.ac.be/med/cdg) and investigators are invited to submit new data to this PMM2 mutation database. Hum Mutat 16:386–394, 2000.

KEY WORDS: congenital disorders of glycosylation, type Ia; CDG-Ia; carbohydrate-deficient glycoprotein syndrome; CDGS; phosphomannomutase 2; PMM2, mutation analysis

DATABASES:
PMM2 – OMIM: 601785, 212065(CDG-Ia); GDB:438697; GenBank: AH008020, NM_000303, U85773, AF157790, AF157791, AF157792, AF157793, AF157794, AF157795, AF157796; HGMD: PMM2; http://www.kuleuven.ac.be/med/cdg/ (CDG Mutation and Patient Databases)

INTRODUCTION

The Congenital Disorders of Glycosylation (CDG; MIM# 212065), formerly called Carbohydrate-deficient Glycoprotein syndromes (CDG or CDGS), is a fast growing group of diseases caused by defects in N-glycosylation.

Up to this date, seven types have been delineated and characterized at the molecular level, but it is reasonable to expect that the number of types will further increase regarding the numerous amount of enzymes and transporters involved in glycosylation. The different types are divided into two groups, CDG-I and CDG-II (MIM# 212066), according to recent recommendations [Participants, First International Workshop on CDGS, 1999, 2000] (see also MIM# 212065).

CDG-Ia is the most frequent type, and is caused by a deficiency in phosphomannomutase (PMM).

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This PMM converts mannose-6-phosphate to mannose-1-phosphate and is a key enzyme in the generation of GDP-mannose, necessary for the synthesis of N-linked glycans and GPI-anchors. A PMM gene, *PMM2* (MIM# 601785), located on chromosome 16p13, encodes the responsible protein [Matthijs et al., 1997b]. A second PMM gene, *PMM1* (MIM# 601786), located on chromosome 22q13, is not involved in the disorder and has slightly different enzymatic characteristics [Matthijs et al., 1997a; Pirard et al., 1999a].

Patients with CDG-Ia typically present at birth with an encephalopathy with marked axial hypotonia, a cerebellar hypoplasia, abnormal eye movements, and internal strabismus. The patients have feeding problems and show a peculiar abnormal distribution of subcutaneous fat and nipple retraction. Other features include peripheral neuropathy, retinitis pigmentosa, and hypogonadism. There is a high lethality in the first years of life mainly due to severe infections, liver insufficiency, or cardiomyopathy. However, the clinical presentation may vary, and the fat pads and inverted nipples are not always seen [Jaeken et al., 1980; for review: Jaeken et al., 1997, 2000].

From six centers dedicated to CDG, we collected the molecular data of 249 CDG-Ia patients. Three important conclusions arise. First, there is a plethora of missense mutations causing CDG-Ia, and one mutation, R141H, is particularly common. Second, the disease is largely underdiagnosed, or at least, molecular analysis is not widely offered. Third, a new figure for the number of deceased patients among CDG-Ia cases is 12% in the first year of life and 21% below the age of six.

The *PMM2* Gene

The cDNA of *PMM2* has an open reading frame of 738 bp and encodes a protein of 246 amino acids. With yeast SEC53 [Kepes and Schekman, 1988], the identity is 58% at the amino acid level. An alignment of PMM1, PMM2, SEC53, and the PMM enzyme of *D. Melanogaster* and *B. Bovis* is shown in Figure 1 [see also Matthijs et al., 1997a]. The *PMM2 gene* consists of 8 exons and spans at least 17kb of genomic DNA [Schollen et al., 1998]. It is important to mention the existence of the paralogous gene, *PMM1* on chromosome 22q13, and of a processed pseudogene *PMM2p* on chromosome 18 [Schollen et al., 1998]. The presence of these genes in the genome might interfere with certain mutation detection strategies.

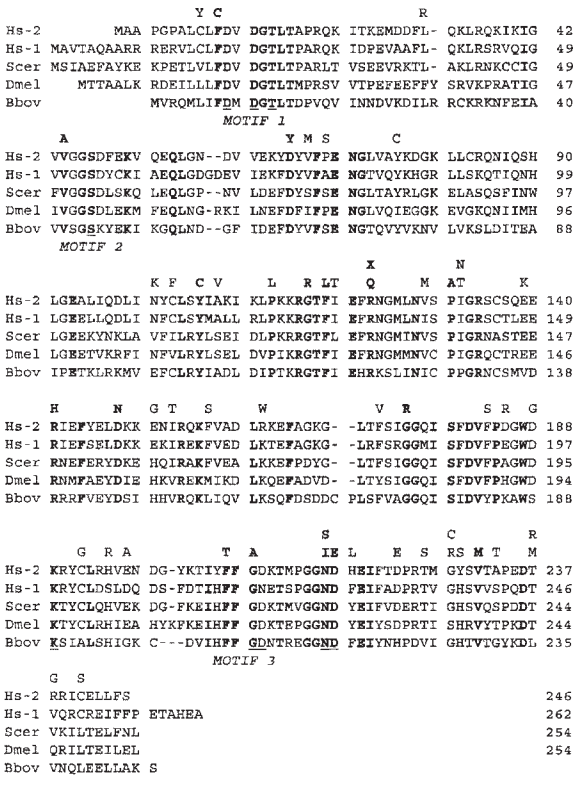

FIGURE 1. **Alignment of PMM2 and PMM1 from *Homo sapiens* (Hs-2 and Hs-1) with the enzymes from *Saccharomyces cerevisiae*, *Drosophila melanogaster,* and *Babesia bovis*. Strictly conserved residues (n = 64) are in bold and three motifs are underlined. The missense mutations and one nonsense mutation of human PMM2 are shown above the alignment.**

MATERIALS AND METHODS

Collection of Data

The mutation data were contributed by six laboratories (see list of authors). The results were anonymous but included the year of birth of the patients, the results of enzymatic measurements, and if available, the year of death of the deceased patients, and information about (affected) siblings. Also, the ethnic background and the country of origin of the patient were given, if known. Several techniques were used to screen for mutations [Matthijs et al., 1997b, 1998; Kjaergaard et al., 1998; Bjursell et al., 1998, 2000; Uller et al., 1999; Imtiaz et al., 2000], ranging from mutation-specific restriction-digestion to direct sequencing. However, given that mutations were detected on 404 of 410 disease alleles (in 205 families), it is concluded that sufficient scrutiny was applied in the contributing laboratories.

SPECTRUM OF PMM2 MUTATIONS

A Preponderance of Missense Mutations

Of the 58 different mutations found in 205 CDG-Ia families (Table 1), 53 are of the missense type. Homozygosity or compound heterozygosity for truncating mutations is not compatible with life (see below). As a result, each patient has at least one (missense) mutation that retains residual activity. This is also supported by the observation that the three single base pair deletions, the splice site mutation (IVS 3+2 C>T), and the one nonsense mutation (R123X) observed to date, are never combined with the most frequent mutation R141H. The latter is a severe mutation with virtually no residual activity [Pirard et al., 1999b].

In the case of F11C and L32R, two nucleotides are mutated (32TC>GT and 95TA>GC). In three patients from two families, A233T was found on the same allele as T237R. The alanine at position 233 is not conserved between PMM2, PMM1, and the enzyme of *S. cerevisiae*, *D. melanogaster*, and *B. bovis* (Fig. 1). It has not been confirmed whether A233T is a mutation rather than a (rare) polymorphism.

In four instances, two mutations affecting the same nucleotide result in d.ifferent amino acid substitutions: 395T > C or 395T > A (I132T or I132N); 647A > T or 647A > G (N216I or N216S); 682G > C or 682G > T (G228R or G228C); 710C > G or 710C >T (T237R or T237M).

Founder Effects and No Mutation Hotspots

Figure 2 illustrates the frequency and the distribution of the mutations in the *PMM2* gene. The two most common mutations are R141H and F119L, accounting for, respectively, 37% and 16% of all mutant chromosomes. Bjursell et al. [1997, 1998] previously documented a clear founder effect for F119L in the Scandinavian population. R141H is also associated with a specific haplotype, and most likely is the result of one mutational event [Schollen et al., 2000]. Only four other mutations are found in more than 10 families. The mutations are scattered over the gene (Fig. 2) with a predominance for exon 8 and exon 5 (15 and 13 mutations, respectively). Only three mutations were found in exon 1 and 2 in exon 2. This has important consequences on the practical approach for mutation analysis: only screening methods like DHPLC, SSCP, DGGE, or direct sequencing can effectively identify the plethora of mutations found in most populations. In contrast, in the Danish population, a specific test for F119L and R141H would pick up 90% of the CDG-Ia patients.

The susceptibility of CpG dinucleotides to mutation seems to vary along the gene. From 25 CpG dinucleotides in the coding region, only 9 are affected by mutations. Seven of these 9 are located in the middle of the gene (mainly in exon 5). The only instance in which the same CpG dinucleotide is mutated on the coding and the non-coding strand is in the 367C>T and 368G>A mutations, resulting in the R123X and R123Q mutations, respectively. Remarkably, the R123Q mutation has arisen independently on two chromosomes, as we can deduce from its association with a polymorphism (324 g>a) in one but not the other instance [Matthijs et al., 1998]. It is intriguing that a R141C mutation has not yet been observed. This mutation would result from a C to T transition in the same CpG dinucleotide, which is mutated in R141H [see Schollen et al., 1998].

Based on the structural data obtained with P-type ATPases, Aravind et al. [1998] have delineated three motifs in the phosphomannomutases that may be directly involved in the catalytic activity (underlined residues in Fig. 1). Pirard et al. [1999a] have pinpointed the phosphorylation site to the aspartate residue at position 12 in PMM2, in motif 1. Mutations at this position would probably result in zero residual activity, and have not (yet) been identified. On the other hand, a (conservative) substitution (aspartic acid to glutamic acid at position 217) in motif 3 does not fully impair the enzyme. Some mutations, occurring distal to motif 3, have a significant residual activity: the D223E, T226S, V231M, and C241S mutant proteins retain 25–50% activity [Kjaergaard et al., 1999; Pirard et al., 1999b; Vuillaumier-Barrot et al., 2000b]. From this, we infer that mutations in the C-terminal part of the protein are less severe. However, T237R mutation virtually inactivates the protein [Kjaergaard et al., 1999], while A233T may be a polymorphic variant [Matthijs et al., 1998]. A more detailed structural analysis of the phsophomannomutases is required to predict the impact of a novel mutation on the enzymatic activity of the mutant protein.

Polymorphisms

A small number of coding and non-coding polymorphisms has been reported. A non-coding polymorphism 324G>A has been detected in association with R123Q, in only one population, and has been detected on non-disease chro-

TABLE 1. Overview of the Mutations in the PMM2 Gene

Exon	Base change	AA-change	Number of patients	Number of families	Country	Reference center
Exon 1	24-2 delC	Frameshift	3	2	CAN, F	3,6
	26 G>A	C9Y	9	7	F, S, USA (French/German)	1, 2, 3
	32 TC>GT	F11C	2	1	S	2
Exon 2	95 TA>GC	L32R	5	3	CAN, F, I	3, 6
	131 T>C	V44A	3	3	EC, ES	1
Exon 3	193 G>T	D65Y	8	6	ES, PT, F (Portuguese)	1
	199 G>A	V67M	2	1	D	2
	205 C>T	P69S	1	1	AUS	1
	227 A>G	Y76C	1	1	ES, PT, F (Portuguese)	1
	IVS 3+2 C>T	Splice variant	2	2	D, ES	1
Exon 4	303 C>G	N101K	1	1	D	1
	309 T>G	C103F	1	1	F	6
	317 A>G	Y106C	1	1	B	1
	323 C>T	A108V	2	2	F	1, 6
	324-325 delG	Frameshift	1	1	D	1
	338 C>T	P113L	26	21	AU, B, D, ES, F, NL, PL, PT, S, USA	1, 2, 3, 6
Exon 5	349 G>C	G117R	1	1	DK	5
	357 C>A	F119L	82	68	AU, B, CH, D, DK, F, N, NL, S, UK, USA	1, 2, 3, 4, 5
	359 T>C	I120T	1	1	USA	1
	367 C>T	R123X	1	1	ES	1
	368 G>A	R123Q	17	15	AU, ES, F, I, NL, PT, S, USA	1, 2, 3, 6
	385 G>A	V129M	6	5	ES, F, I, USA	1, 6
	391 C>G	P131A	1	1	F	1
	395 T>C	I132T	4	4	F, I	1, 6
	395 T>A	I132N	1	1	UK	4
	398-399 del C	Frameshift	1	1	USA	1
	415 G>A	E139K	6	4	F	6
	422 G>A	R141H	186	152	AR, AU, AUS, B, CAN, CH, D, DK, EC, ES, F, FL, I, IR, N, NL, PE, PT, S, UK, USA	1, 2, 3, 4, 5, 6
	442 G>A	D148N	2	2	UK, USA	3, 4
Exon 6	452 A>G	E151G	1	1	NL	1
	458 T>C	I153T	3	3	ES, F, PT, USA	1, 3, 6
	470 T>C	F157S	8	5	ES, F, I, PL, USA	1, 3
	484 C>T	R162W	2	2	NL, UK	1
	514 T>G	F172V	1	1	PL	2
	523 G>C	G175R	1	1	B	1
Exon 7	548 T>C	F183S	12	10	D, S, UK	2, 4
	554 A>G	D185G	2	1	S	2
	563 A>G	D188G	5	5	B, NL	1
	574 T>G	C192G	1	1	DK	5
	584 A>T	H195R	1	1	D	1
	590 A>C	E197A	1	1	ES	1
	617 T>C	F206T	1	1	ES	1
	623 G>C	G208A	2	2	UK, USA	1, 4
Exon 8	647 A>T	N216I	4	3	I, T	1
	647 A>G	N216S	1	1	D	2
	651 C>A	D217E	1	1	S	2
	653 A>T	H218L	1	1	D	1
	669 C>G	D223E	1	1	DK	5
	677 C>G	T226S	4	2	ES, F	1, 6
	682 G>C	G228R	2	1	D	2
	682 G>T	G228C	1	1	AU	1
	686 A>C	Y229S	1	1	J	1
	691 G>A	V231M	29	24	AR, AU, B, D, F, I, PE, S, UK, USA	1, 2, 4, 6
	697 G>A*	A233T*	3	2	D, F	1
	710 C>G	T237R	8	5	D, DK, F, S	1, 2, 5
	710 C>T	T237M	11	9	ES, F, I, IR, UK, USA	1, 4
	712 C>G	R238G	1	1	FL	2
	722 G>C	C241S	8	6	B, ES, F, USA	1, 3, 6
	ND		6	6	CAN, D, F, PL, UK	2, 3, 4, 6
Total			501	411		

Numbering is based on cDNA sequence and is started at the ATG codon (reference sequence: Genbank AH008020).
The reference centers are numbered as in the authors list.
*A233T is found on the same allele as T237R.
The R123Q mutation was mislabeled as R123G in an earlier publication [Matthijs et al., 1997b, 1998].

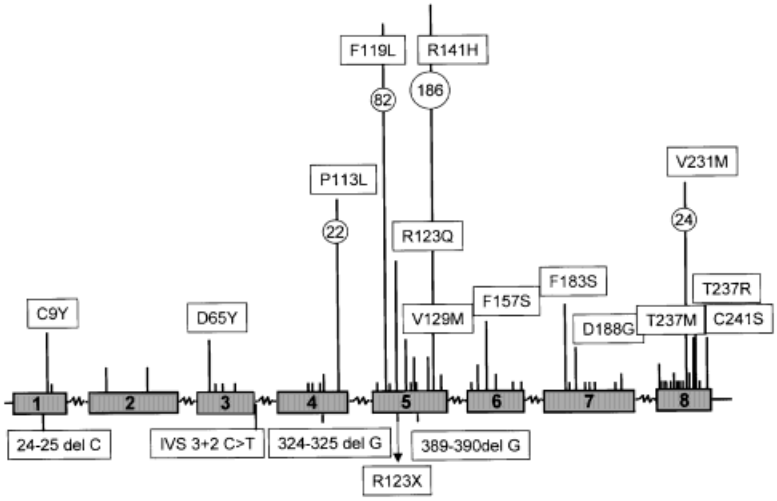

FIGURE 2. **Frequency and position of the mutations in the PMM2 gene. Missense mutations are indicated at the top and truncating mutations at the bottom. The length of the bars is relative to the frequency of the mutations.**

mosomes. In two Italian brothers, the silent mutation 699G>A was found on the same allele as the N216I mutation. Three intronic polymorphisms in introns 4 and 5 may interfere with mutation detection, and should be taken into account when designing primers for amplification of exon 5. The intronic polymorphism, IVS4-58del ATG, has a frequency of 10% in the French population [Vuillaumier-Barrot et al., 2000a], IVS5+19C/T and IVS5+22T/A are frequent polymorphisms [Bjursell et al., 2000]. An A is present at position IVS5+22 on the common R141H allele.

LACK OF HOMOZYGOTES FOR THE MOST FREQUENT MUTATION

When looking at the genotypes of the CDG-Ia patients, there is only one frequent combination: The F119L/R141H genotype was observed in 68 patients in 56 families (81%, 50%, 32%, and 30% of the genotypes observed in Denmark, the Netherlands, Sweden, and Germany, respectively). The most intriguing observation is the total lack of patients homozygous for R141H [Kjaergaard et al., 1998; Matthijs et al., 1998; Bjursell et al., 2000]. The R141H mutation is present in more than 73% of the Caucasian patients with CDG-Ia. On a mathematical basis, the frequency of homozygotes for this mutation should be 13%, or 32 patients in this series. The R141H/R141H genotype has not been observed to date and it is concluded that this combination is lethal [Schollen et al., 2000]. The extremely low enzymatic activity of the recombinant mutant protein supports this inference [Pirard et al., 1999b]. On the other hand, patients homozygous for F119L (three from Scandinavia and one from the Netherlands), D65Y (one from Portugal), N216I (one from Turkey), and F183S (one from Sweden) have been identified.

Other genotypes seem to be missing as well. For instance R123Q, F157S, and T237R, with frequencies of 15, five, and eight, respectively, in this series, have not been observed in combination with R141H, although their existence was statistically possible. Again, the recombinant T237R protein has no detectable enzymatic activity [Kjaergaard et al., 1999]. The effect of the two other mutations on PMM2 activity has not been measured, but we can infer from the genotype data that they must severely affect the protein.

These observations clearly support the hypothesis that residual PMM2 activity is required for survival and that genotypes with two mutations retaining no enzymatic activity are not compatible with life.

GEOGRAPHICAL ORIGIN AND SPREAD OF THE MUTATIONS

The series includes patients from 23 countries (see Table 2). When ordered according to number of confirmed cases per number of inhabitants, Denmark, Sweden, the Netherlands, and Belgium take the lead. This may partly be explained by a founder effect in Denmark and in Southern Sweden [Bjursell et al., 1997; Kjaergaard et al., 1998], and partly be due to a lack of awareness of the disease.

The distribution and frequency of the different mutations in the European countries varies greatly, except for the frequent R141H mutation (Fig. 3a and Table 1) which is found in all countries and accounts for 23% (in Spain) to 45% (in Belgium) of all mutant chromosomes. There is a clear founder effect for F119L in South Scandinavia. In

TABLE 2. Number of Confirmed CDG-Ia Families Per Country

Country	Number of families
Argentina (AR)	1
Australia (AUS)	1
Austria (AU)	5
Belgium (B)	10
Canada (CAN)	2
Denmark (DK)	22
Ecuador (EC)	1
Germany (D)	26
Finland (FL)	1
France (F)	29
Ireland (IR)	1
Italy (I)	7
Japan (J)	1
Netherlands (NL)	16
Norway (N)	2
Peru (PE)	1
Poland (PL)	2
Portugal (PT)	5
Spain (ES)	13
Sweden (S)	28
Switzerland (CH)	1
Turkey (T)	1
United Kingdom (UK)	14
USA	16

Denmark, it accounts for 48% of the disease alleles. Moving southward, this proportion gradually decreases. In the neighboring countries, F119L still varies between 17% in the Netherlands and 11% in Germany. In the Spanish, Portuguese, and Italian population, this mutation has not been observed yet.

This founder effect has implications for the variety of other mutations found in the different regions. In Denmark only six different mutations are found in 22 families. In contrast, in Spain, 13 families have been diagnosed with 16 different mutations (Fig. 3b). Only a handful of mutations appear to be definitely regional. Up to now, D188G has only been observed in Flanders and the Netherlands, D65Y is a founder mutation of Portuguese origin (5/6 in Portugal and one in Spain), E139K has been observed only in four French families, and C9Y and F183S are largely restricted to Sweden (5/7 and 8/10 cases, respectively). The distribution of P113L is also particular: 10 of 22 alleles were found in German patients, the other 12 are scattered over the other countries.

CLINICAL OBSERVATIONS

Distribution of Patients According to Year of Birth

The year of birth of the patients in this series ranged from 1949 to 1999, with a strong bias towards young patients (see Fig. 4): 54% of the patients are below 10 years of age (born 1990 or

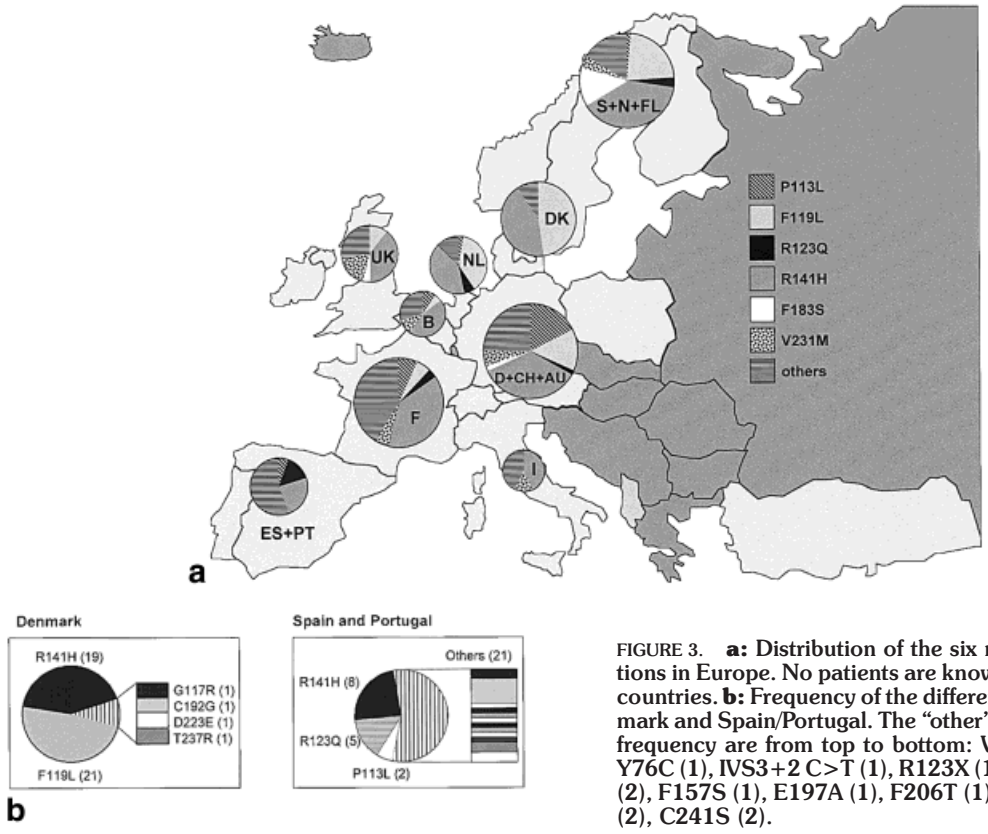

FIGURE 3. **a:** Distribution of the six most frequent mutations in Europe. No patients are known in the darker gray countries. **b:** Frequency of the different mutations in Denmark and Spain/Portugal. The "other" mutations and their frequency are from top to bottom: V44A (2), D65Y (5), Y76C (1), IVS3+2 C>T (1), R123X (1), V129M (1), I153T (2), F157S (1), E197A (1), F206T (1), T226S (1), T237M (2), C241S (2).

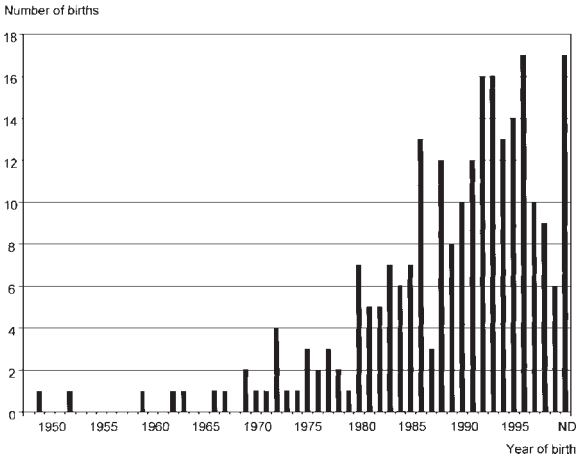

FIGURE 4. **Distribution of patients according to age. Identical twins were included once. ND: not documented.**

later). At the same time, it is obvious from Figure 4 that the diagnosis is pending in older children and adults: only 41 adult cases (>18 y, born before 1982) were included in this series. A few other adult cases, reported by Krasnewich et al. [1999], are not included in this study. There is no reason to believe that most patients born before 1980 would have died; on the contrary, the disease seems to stabilize with age. The number of patients in each year is less from 1997 to 1999 than from 1991 to 1996. It is tempting to infer from this observation that there is a lag-phase for diagnosis of a few years in young children.

Genotype–Phenotype Correlation

An extensive phenotype–genotype correlation has not been published to date and is far beyond the scope of this paper. Actually, the wide variety of genotypes hampers the study of this correlation.

From 132 patients for which we could ascertain the information, 38 had died: 16 of these 38 patients (42%) died in their first year of life and 11 died between the age of one and six (29%). Two children died at 10 and 13 years due to the disease, while one teenage patient died at 16 in a car accident. From eight deceased patients we have no information about the age of death. In total, 28% of the diagnosed patients died of a disease-related cause, and at least 70% of all deaths occurred before the age of six years.

Forty-three of the 132 patients had the F119L/R141H genotype, of which 13 (30%) died (14, including the patient who died in an accident). Combinations of D65Y with R141H, R123Q, or F157S result in severe phenotypes with a death rate of four in five. This is surprising because D65Y must be a mild mutation, having been observed in homozygous state [Matthijs et al., 1998], and the specific activity of the recombinant enzyme is 50% (but with a reduced stability) [Pirard et al., 1999b]. From five patients with the D188G/R141H genotype, four have died while the fifth patient is severely affected [Matthijs et al., 1998]. The specific activity of the D188G mutant enzyme is reduced < 2% of the normal enzyme, whereas R141H is virtually inactive [Pirard et al., 1999b]. From 16 patients with the V231M/R141H genotype, nine have died. V231M mutant protein still has 38.5% of activity but is extremely unstable [Pirard et al., 1999b].

From the nine mutations affecting amino acids which are not conserved between PMM1, PMM2, and PMM of different species (see Fig. 1), at least four are associated with milder phenotypes. C241S was found in four families from a group of mildly affected patients with relatively high residual PMM2 activity (Grünewald et al., in preparation). One patient with a H218L/R141H genotype also has a partial phosphomannomutase deficiency. A P69S/R141H genotype was found in a typical CDG-Ia patient, in whom phosphomannomutase was deficient but the transferrin isoelectric focusing (IEF) pattern was normal [Fletcher et al., 2000]. The latter finding is no longer unique, and points towards a pitfall in the use of the transferrin IEF assay for diagnosis of CDG (N. Seta, H. Freeze, unpublished observations).

An Estimated Frequency of the Disease and Implications for Counseling

A determination of the carrier frequency for the frequent R141H mutation in Danish and Flemish/Dutch patients has allowed us to estimate the frequency of the disease (Schollen et al., 2000]. It may be as high as 1/20,000. This is at least twice as high as the estimation made by Kristiansson et al. [1998] and others on the basis of the observed number of cases. A lack of homozygotes for some of the rare mutations may result in an overestimation. A conservative estimate of the number of patients born with CDG-Ia every year is 200 in Europe and in the United States. Probably less than half of these are being diagnosed at the moment.

The fact that R141H is relatively frequent in most populations (1/72 in the Flemish/Dutch and Danish population) has a particular implication

for counseling. A carrier of a mutation different from R141H has an a priori risk of 1 in 300 for a child with the disease. The frequency of the other "rare" mutations has been estimated to be between 1/300 and 1/400. So, if R141H is excluded in the partner, the risk is reduced to 1/1200 to 1/1600 [Schollen et al., 2000].

FUTURE PROSPECTS

In less than three years, the mutation spectrum of *PMM2* in CDG-Ia patients comprises 58 mutations. This is an impressive series, but the list is probably not exhaustive. If the number of mutations is calculated relative to the size of the protein (246 amino acids), it has not yet reached the mutation density observed in the CFTR gene or other well known genes. Other mutations will still be found in more CDG-Ia patients. In analogy to cystic fibrosis and CFTR, a small number of frequent mutations makes up a huge percentage of the mutant chromosomes in most populations.

Since the identification of the gene and the further delineation of CDG-Ia, the number of diagnosed cases has almost doubled. Still, on the basis of our calculations, many cases remain undiagnosed. We hope that our efforts will contribute to a better awareness of CDG.

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