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Molecular Genetics and Metabolism 86 (2005) 353–359
Molecular Genetics and Metabolism
www.elsevier.com/locate/ymgme

Mutations c.459+1G>A and p.P426L in the ARSA gene: Prevalence in metachromatic leukodystrophy patients from European countries

Agnieszka Lugowska [a,*], Olga Amaral [b], Johannes Berger [c], Linda Berna [d], Nils U. Bosshard [e], Amparo Chabas [f], Anthony Fensom [g], Volkmar Gieselmann [h], Natalia G. Gorovenko [i], Willy Lissens [j], Jan-Eric Mansson [k], Ana Marcao [b], Helen Michelakakis [l], Hanno Bernheimer [m], Natalia V. Ol'khovych [i], Stefano Regis [n], Richard Sinke [o], Anna Tylki-Szymanska [p], Barbara Czartoryska [a]

[a] *Institute of Psychiatry and Neurology, Department of Genetics, Warsaw, Poland*
[b] *Institute of Medical Genetics Jacinto Magalhaes, Porto, Portugal*
[c] *Center for Brain Research, Medical University Vienna, Vienna, Austria*
[d] *Institute of Inherited Metabolic Diseases, Charles University, Prague, Czech Republic*
[e] *Division of Metabolism and Molecular Pediatrics, University Children's Hospital, Zurich, Switzerland*
[f] *Department of Inborn Errors of Metabolism, Institut Bioquimica Clinica, Mejia Lequerica, Barcelona, Spain*
[g] *Genetics Centre, Guy's Hospital, London, Great Britain, UK*
[h] *Institut für Physiologische Chemie, Rheinische Friedrich Wilhelms Universität, Bonn, Germany*
[i] *Kyiv Medical Academy for Postgraduate Education, Kyiv, Ukraine*
[j] *Center for Medical Genetics, University Hospital VUB, Brussels, Belgium*
[k] *Neurochemistry Laboratory Medicine/Clinical Chemistry, Sahlgren's University Hospital, Molndal, Sweden*
[l] *Department of Enzymology and Cellular Function, Institute of Child Health, Athens, Greece*
[m] *Clinical Institute of Neurology, Medical University of Vienna, Vienna, Austria*
[n] *Department of Metabolic Diseases, Institute G. Gaslini, Genoa, Italy*
[o] *Department of Medical Genetics, University Medical Center, Utrecht, The Netherlands*
[p] *Department of Metabolic Diseases, Memorial Institute Child Health Centre, Warsaw, Poland*

Received 31 May 2005; received in revised form 18 July 2005; accepted 18 July 2005
Available online 2 September 2005

Abstract

In this multicentre study, we examined the prevalence of two mutations in the arylsulfatase A (ARSA) gene, i.e., c.459+1G>A and p.P426L, in 384 unrelated European patients presenting with different types of metachromatic leukodystrophy (MLD). In total, c.459+1G>A was found 194 times among the 768 investigated ARSA alleles (25%), whereas p.P426L was identified 143 times (18.6%). Thus, these two mutations accounted for 43.8% of investigated MLD alleles. Mutation c.459+1G>A was most frequent in late-infantile MLD patients (40%), while p.P426L was most frequent in adults (42.5%), which is consistent with earlier observations, although p.P426L was also found in a few late-infantile patients (0.9%), and c.459+1G>A was present in some adults (9%). Mutation c.459+1G>A is more frequent in countries situated at the western edges of Europe, i.e., in Great Britain and Portugal, and also in Belgium, Switzerland, and Italy, which is visible as a strand ranging from North to South, and additionally in Czech and Slovak Republics. Mutation p.P426L is most prevalent in countries assembled in a cluster containing the Netherlands, Germany, and Austria. In other Central European countries, the frequency of both c.459+1G>A and p.P426L ranges from 8 to 37.5%. Our study has confirmed that c.459+1G>A and p.P426L are the most frequently found MLD-causing mutations in Europe. The data about their prevalence reflect the population variability in Europe.

* Corresponding author. Fax. +48 22 858 91 69.
E-mail address: alugipin@yahoo.com (A. Lugowska).

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doi:10.1016/j.ymgme.2005.07.010

Keywords: Metachromatic leukodystrophy; Arylsulfatase A; Mutation frequency

Introduction

Metachromatic leukodystrophy (MLD, McKusick 250100) is a genetic metabolic disorder leading to demyelination of the central and peripheral nervous systems. According to the age of onset and clinical manifestations, four types of MLD are distinguished: late-infantile, juvenile (with the subtypes of early- and late-juvenile), and adult [1].

The biochemical basis of MLD is deficient activity of the lysosomal enzyme—arylsulfatase A (ARSA; EC 3.1.6.8), leading to the storage of its substrates, especially sulfatide (galactosylceramide I^3—sulfate), which normally constitutes ca. 5% of the lipid content of the myelin sheath. A much less common form of MLD is caused by the lack of saposin B, a non-enzymatic sphingolipid activator protein, required for degradation of ARSA substrates [1].

MLD is inherited as an autosomal recessive trait. The gene coding for human ARSA is located on chromosome 22q13, is 3.2 kb long, and consists of 8 exons [2], the GenBank Accession Nos. X52150 and X52151.

So far, nearly 100 MLD-causing mutations in the gene coding for ARSA have been identified; among them are deletions, splice-site mutations, and mostly missense mutations. Two mutations, namely c.459 + 1G > A and p.P426L, appear to be the most frequent ones among European MLD patients, when studied in small groups. The first one was observed in 15–43% and the second in 16–25% of mutated alleles, respectively [3–5].

Mutation c.459 + 1G > A is a result of G → A transition at the nt 609 of the ARSA gene and the consequent destruction of the splice donor site of exon 2. Individuals homozygous for this mutation suffer from severe late-infantile MLD. In cultured skin fibroblasts from these patients ARSA RNAs were almost undetectable [3].

Transition C → T at nt 2381 of the ARSA gene leads to a Pro426 to Leu substitution (P426L). Patients homozygous for this mutation present with adult type of MLD. Mutated p.P426L-ARSA polypeptides have enzymatic activity but are rapidly degraded due to the defective oligomerization [6].

In this multicentre study, we compare the prevalence of these two mutations in 384 patients presenting with different types of MLD and originating from 16 European countries.

Materials and methods

Patients

The 384 unrelated patients presenting with different types of MLD originated from Austria, Belgium, the Czech and Slovak Republics (including former Czechoslovakia), France, Germany (Caucasians of different origin), Great Britain, Greece, Italy, the Netherlands, Poland, Portugal, Spain, Sweden, Switzerland, and Ukraine (Table 1). Among them, 186 individuals have been classified as late-infantile MLD (48%), 118 as juvenile (31%), and 80 as adult MLD patients (21%). It

Table 1
Participation of MLD patients from Europe in this study

Country	Total no. of MLD patients	No. of late-infantile MLD patients	No. of juvenile MLD patients	No. of adult MLD patients
Austria	13	4	5	4
Belgium	12	10	2	—
The Czech and Slovak Republics	13	5	2	6
France	21[a]	14	7	—
Germany	102	34	40	28
Great Britain	18	9	7	2
Greece	7	4	2	1
Italy	32	32	—	—
The Netherlands	49	2	26	21
Poland	38	13	17	8
Portugal	17	13	1	3
Spain	32	25	2	5
Switzerland	7	6	1	—
Sweden	13	12	—	1
Ukraine	10	3	6	1
Together	384	186	118	80
%	100	48	31	21

[a] Ref. [24].

should be noted that patients investigated in this study are the ones from whom DNA samples were available. Patients in whom MLD diagnosis was confirmed biochemically but DNA analysis was impossible to perform were not included in the presented work.

Biochemical assays

Diagnosis of MLD was confirmed biochemically by demonstration of deficient ARSA activity in isolated peripheral blood leukocytes, cultured skin fibroblasts or serum [7–9].

In the majority of patients, additionally sulfatide excretion in urine was assayed by various methods [10–14]. In Sweden, the diagnosis was in all cases confirmed by quantitation of sulfatide in urinary sediment with high performance thin-layer chromatography (HPTLC) followed by detection with orcinol or a sulfatide-specific monoclonal antibody [15–17].

DNA analysis

Screening for c.459 + 1G > A and p.P426L mutations was carried out in DNA samples by means of the PCR-RFLP, sequencing, and amplification refractory mutation system (ARMS) methods [18–21].

Results

Late-infantile MLD patients

Our results showed that in late-infantile MLD patients c.459 + 1G > A was most frequent in individuals from the Czech and Slovak Republics—70% (7/10) of investigated alleles, Great Britain—67% (12/18), and Portugal—65% (17/26). It was also frequent in late-infantile patients from Germany—46% (31/68), Poland—42% (11/26), Belgium—40% (8/20), Austria—37.5% (3/8), Italy—36% (23/64), and 33%—from Switzerland (4/12) and Ukraine (2/6). This mutation accounted for 29% (7/24) of MLD alleles in late-infantile individuals from Sweden, and 24% (12/50)—from Spain [20], but was not detected in four Greek and two Dutch patients (Table 2).

Mutation p.P426L was found in only three late-infantile patients, which means 1.6% (1/64) MLD alleles in Italian, 12.5% (1/8)—Austrian, and 25% (1/4)—Dutch late-infantile patients. This mutation was not detected in late-infantile patients from other countries (Table 3).

Juvenile MLD patients

In juvenile MLD patients, mutations c.459 + 1G > A and p.P426L occurred more variably. The frequency of c.459 + 1G > A in juveniles ranged from 12% (4/34)—in Poland, through 15% (8/52) and 16% (13/80) in the Netherlands and Germany, 20% (2/10)—in Austria, 25%—in Belgium (1/4), the Czech and Slovak Republics (1/4), and Spain (1/4), to 36% (5/14)—in Great Britain and 50% (1/2)—in Switzerland. No carriers of this mutation in juvenile patients were found in Greece, Portugal, and Ukraine (Table 2).

Mutation p.P426L was the most frequent in juvenile MLD patients from Austria—50% (5/10) of mutated alleles. In Germany, it was found in 38% (30/80) and in the Netherlands—37% (19/52) of MLD-causing alleles. In Belgian MLD juveniles and in the Czech and Slovak Republics it was observed in 25% (1/4) of MLD alleles, in Ukraine—17% (2/12), in Poland—15% (5/34), and in

Table 2
Distribution of mutation c.459 + 1G > A in MLD patients from Europe

Country	Late-infantile MLD patients		Juvenile MLD patients		Adult MLD patients		MLD patients together	
	No. of alleles	%	No. of alleles	%	No. of alleles	%	No. of alleles	%
Austria	3/8	37.5	2/10	20	1/8	12.5	6/26	23
Belgium	8/20	40	1/4	25	n.d.	n.d.	9/24	37.5
Czech and Slovak Rep.	7/10	70	1/4	25	2/12	17	10/26	38
France	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6/42	14
Germany	31/68	46	13/80	16	5/56	9	49/204	24
Great Britain	12/18	67	5/14	36	1/4	25	18/36	50
Greece	0/8	0	0/4	0	0/2	0	0/14	0
Italy	23/64	36	n.d.	n.d.	n.d.	n.d.	23/64	36
Netherlands	0/4	0	8/52	15	0/42	0	8/98	8
Poland	11/26	42	4/34	12	1/16	6	16/76	21
Portugal	17/26	65	0/2	0	3/6	50	20/34	59
Spain	12/50	24	1/4	25	2/10	20	15/64	23
Sweden	7/24	29	n.d.	n.d.	0/2	0	7/26	27
Switzerland	4/12	33	1/2	50	n.d.	n.d.	5/14	36
Ukraine	2/6	33	0/12	0	0/2	0	2/20	10
Together	137/344	40	36/222	16	15/160	9	194/768	25

n.d., no data; for France only data for all types MLD patients together are available from [24].

Table 3
Distribution of mutation p.P426L in MLD patients from Europe

Country	Late-infantile MLD patients		Juvenile MLD patients		Adult MLD patients		MLD patients together	
	No. of alleles	%	No. of alleles	%	No. of alleles	%	No. of alleles	%
Austria	1/8	12.5	5/10	50	5/8	62.5	11/26	42
Belgium	0/20	0	1/4	25	n.d.	n.d.	1/24	4
Czech and Slovak Rep.	0/10	0	1/4	25	2/12	17	3/26	11.5
France	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6/42	14
Germany	0/68	0	30/80	38	32/56	57	62/204	30
Great Britain	0/18	0	2/14	14	1/4	25	3/36	8
Greece	0/8	0	0/4	0	0/2	0	0/14	0
Italy	1/64	1.6	n.d.	n.d.	n.d.	n.d.	1/64	1.6
Netherlands	1/4	25	19/52	37	14/42	33	34/98	35
Poland	0/26	0	5/34	15	8/16	50	13/76	17
Portugal	0/26	0	1/2	50	0/6	0	1/34	2.9
Spain	0/50	0	0/4	0	4/10	40	4/64	6
Sweden	0/24	0	n.d.	n.d.	2/2	100	2/26	8
Switzerland	0/12	0	0/2	0	n.d.	n.d.	0/14	0
Ukraine	0/6	0	2/12	17	0/2	0	2/20	10
Together	3/344	0.9	66/222	30	68/160	42.5	143/768	18.6

n.d., no data; for France only data for all types MLD patients together are available from [24].

Great Britain—14% (2/14). This mutation was found in only one allele of the single juvenile patient from Portugal and was not found in juvenile patients from Greece, Spain, and Switzerland (Table 3).

Adult MLD patients

In adult patients, mutation p.P426L was most frequently observed in Austria—62.5% (5/8) of MLD alleles, in Germany—57% (32/56), and in Poland—50% (8/16). In adult MLD patients from Spain, p.P426L accounted for 40% (4/10), from the Netherlands—for 33% (14/42), whereas in Great Britain and in the Czech and Slovak Republics this mutation accounted for 25 and 17% (1/4 and 2/12) of mutated alleles, respectively. One adult patient from Sweden was homozygous for p.P426L. This mutation was not found in adult MLD patients from Greece, Portugal or Ukraine (Table 3).

Mutation c.459 + 1G > A was detected in only 6% (1/16) of MLD alleles in adult patients from Poland, in 9% (5/56) from Germany, and in 12% (1/8) from Austria, in 17% (2/12) from the Czech and Slovak Republics, but it was more frequent in patients from Spain—20% (2/10), Great Britain—25% (1/4), and Portugal—50% (3/6), in the latter case always appearing in compound heterozygosity with p.I179S. Mutation c.459 + 1G > A was absent in material from adults from Greece, the Netherlands, and Ukraine (Table 2).

All types MLD patients together

Generally, c.459 + 1G > A accounted for 25% (194/768) of mutated alleles in European patients suffering from different types of the disease, whereas p.P426L accounted for 18.6% (143/768). Thus, these two mutations together are responsible for 43.6% of MLD-causing alleles in Europe. In northern parts of Europe (i.e., in Sweden), c.459 + 1G > A was found in 27% of MLD alleles (7/26). Distribution of c.459 + 1G > A in MLD patients originating from Central European countries is similar and ranges from 21% (16/76)—in Poland, through 23% (6/26)—in Austria, and 24% (49/204)—in Germany, to 36% (5/14)—in Switzerland, 37.5% (9/24)—in Belgium, and 38% (10/26)—in the Czech and Slovak Republics. It is higher in Great Britain and Portugal with the prevalence of 50% (18/36) and 59% (20/34), respectively, but in Italy it equals 36% (23/64), and in Spain 23% (15/64). On the contrary, in the Netherlands, Ukraine, and France mutation c.459 + 1G > A was identified in as little as 8% (8/98), 10% (2/20), and 14% (6/42) of all MLD alleles, respectively (Table 2).

Prevalence of p.P426L ranges from 1.6% (1/64) in Italy (only late-infantile patients were examined), 2.9% (1/34) in Portugal, and 4% (1/24)—in Belgium, 6% (4/64)—in Spain, through 8%—in Great Britain (3/36) and in Sweden (2/26), 10% (2/20)—in Ukraine, 11.5% (3/26)—in the Czech and Slovak Republics, 14% (6/42)—in France, 17% (13/76)—in Poland, 30% (62/204)—in Germany, to 35% (34/98)—in the Netherlands, and 42% (11/26)—in Austria. This mutation was not found in patients from Switzerland (Table 3).

In total, c.459 + 1G > A accounted for 40% of MLD-related alleles in late-infantile patients, 16% in juvenile, and 9% in adults. Mutation p.P426L was identified in 42.5% of MLD alleles in adults, 30% in juvenile, and 0.9% in late-infantile patients (Tables 2 and 3).

It is noteworthy that neither c.459 + 1G > A nor p.P426L was detected in MLD patients from Greece.

It is important to point out the influence of the number of patients in investigated groups on the outcome of

the results. Obviously, less numerous groups gave 'odd' results, e.g., 50% (2/4) of c.459 + 1G > A in adults from Portugal or 25% (1/4) of p.P426L in Dutch late-infantile patients (Table 1).

Discussion

Our study has shown that mutations c.459 + 1G > A and p.P426L are the most frequently found MLD-causing mutations in Europe. The data about their prevalence reflect the population variability in Europe. Mutation c.459 + 1G > A was most frequent in late-infantile MLD patients (40%), while p.P426L was most frequent in adults (42.5%), which is consistent with earlier observations, although p.P426L was also found in a few late-infantile patients and c.459 + 1G > A was present in some adults. It has already been reported by Regis et al. [22] that p.P426L can also occur on the background of the arylsulfatase A pseudodeficiency allele, which would make the resulting allele a late-infantile MLD-causing allele. When in heterozygosity with other severe MLD-causing mutation this would result in a late-infantile phenotype. Mutation c.459 + 1G > A accounted for 25% of all examined MLD alleles in Europe, whereas p.P426L accounted for 18.6%, giving 43.6% of mutant alleles in total. These data are in accordance with results described previously by Polten and Gieselmann, who found the frequency of c.459 + 1G > A to be 27%, p.P426L—26% (together 53%) in their previous study of European MLD patients [3,23].

Since c.459 + 1G > A is more frequent (25%) one can speculate that this mutation is older. It should be kept in mind, however, that there were 2.4 times less adult MLD patients than late-infantile patients in our study. This indicates that patients with adult type MLD are most possibly often misdiagnosed or undiagnosed.

In Europe, a pattern can be observed. First, mutation c.459 + 1G > A is more frequent in countries situated at the western edges, i.e., Great Britain and Portugal, and also in Belgium, Switzerland, and Italy, which is visible as a strand ranging from North to South, and additionally in Czech and Slovak Republics (Fig. 1). This pattern is very similar to the prevalence of mutation ΔF508 in

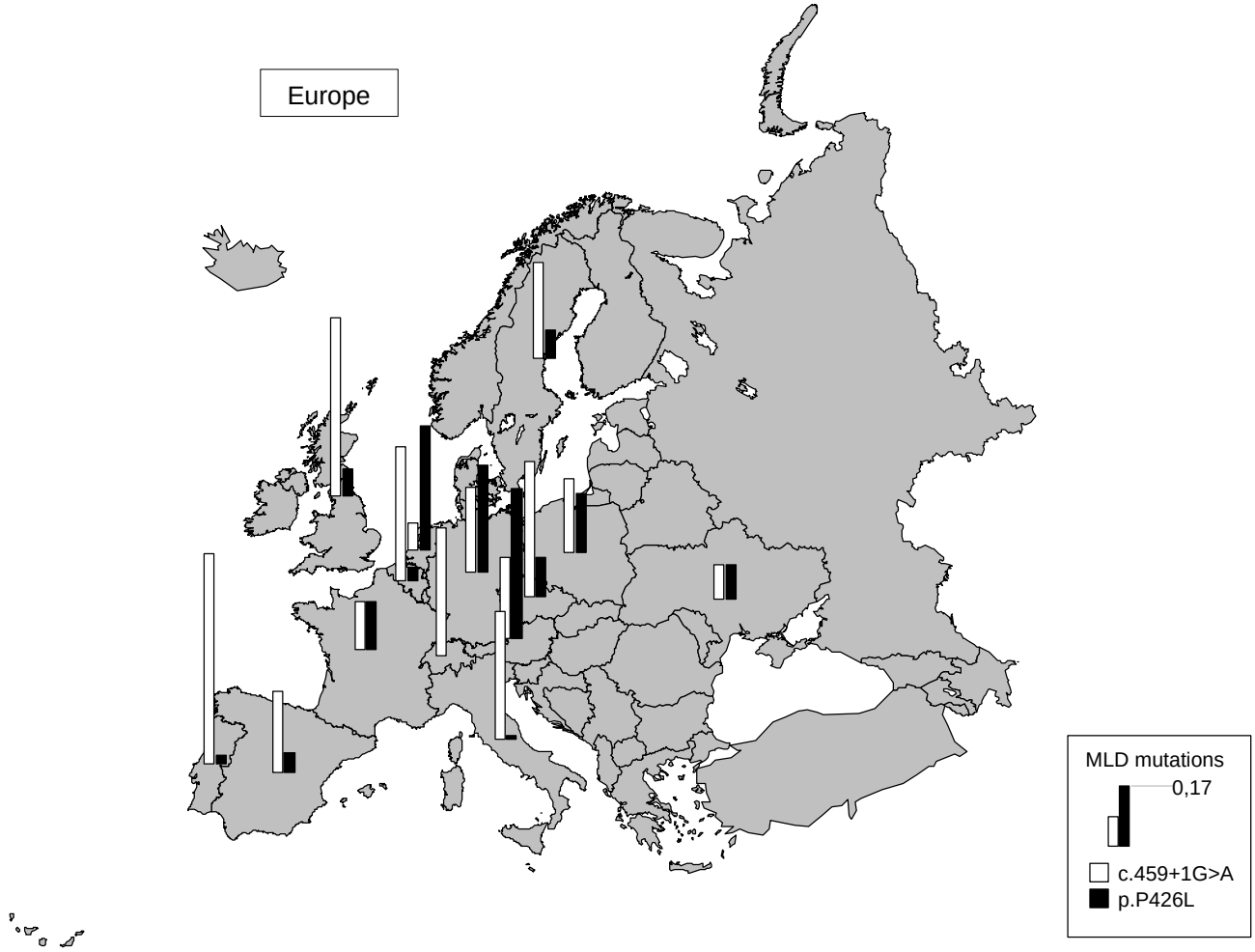

Fig. 1. Prevalence and distribution of c.459 + 1G > A and p.P426L in MLD patients from Europe. Results from former Czechoslovakia are located in the Czech Republic. The scale is expressed as decimal fraction (e.g., 0.17 means 17%).

cystic fibrosis, which shows a northwest to southeast gradient in Europe [25]. From the population genetics it is known that fossils and archeological records, backed up by the paleoclimatic data, clearly indicate that prehistoric populations in Europe experienced recurrent demographic changes and movements. The three potentially most important large-scale phenomena are: the colonization of Europe by first modern humans in the early Upper Paleolithic ~40–30 thousand years ago (Kya), the Mesolithic dispersal from the glacial refugia ~16–10 Kya, and the Neolithic expansions of farmers from Levant starting ~10 Kya [26], whose major route was through Anatolia (Turkey and Asia) [27]. If mutation c.459 + 1G > A was present in the Neolithic population that spread into Europe from the Near East, traces of this mutation should be observable also in Balkan countries. However, we did not detect c.459 + 1G > A nor p.P426L in Greek MLD patients, what could suggest that c.459 + 1G > A originated in north-western Europe.

The second pattern observed in Europe for the mutation p.P426L is most prevalent in countries assembled in a cluster containing the Netherlands, Germany, and Austria (Fig. 1). In other Central European countries, the frequency of both c.459 + 1G > A and p.P426L ranges from 8 to 37.5%. These observations could be explained by the fact that Central European populations are more mixed as the result of more numerous wars and migrations, and their historical consequences in the past ages. The pattern for p.P426L prevalence presenting as a cluster with the Netherlands, Germany, and Austria resembles that one found for mutation R3500Q in familial defective apoB-100 (FDB), which is most frequently found in Belgium, the Rhein-Main area in Germany, and in northwest Switzerland. Myant et al. [27] suggest that mutation FDB_{3500Q} most probably arose 6750 years ago somewhere in continental Western Europe, followed by spread northward into Britain and Scandinavia, westward at least as far as Pyrenees, and eastward toward Russia and the Balkans.

All the above explanations of the patterns which create the data of c.459 + 1G > A and p.P426L frequencies in Europe are only our speculations. For a comprehensive attempt of explanations, a more profound study would be needed, comprising—among others—the haplotype studies and more numerous groups of investigated subjects in some countries.

Interestingly, neither c.459 + 1G > A nor p.P426L was detected in Greek patients, which indicates the existence of other MLD mutations in this population or this can be simply due to the limited number of analysed subjects (seven in both countries). With these numbers concluding about genetic isolation would be only a matter of speculation.

Results obtained and presented in this study represent a large cohort of MLD patients giving representative figures of the occurrence of c.459 + 1G > A and p.P426L mutations in the European population of Caucasian origin. It seems valuable to collect and compare data about disease-causing mutations and the haplotypic background in Europe and all over the world. This action could enable the estimation of the age of a studied mutation and knowledge of the haplotypic background could even replace sometimes the search for a mutation itself [28]. We hope that the presented study will stimulate other scientists to a profound collaboration in the field of population genetics.

Our study lacks, however, data from European countries comprising the former Soviet Union (i.e., Lithuania, Latvia, Estonia, Byelorussia, Moldova, and the European part of Russia), Balkan countries, Scandinavian countries (except for Sweden), and some other countries, e.g., Hungary, and it would be very valuable to compare data from there in the future.

Acknowledgments

Authors thank patients and their families, as well as, all clinicians for their cooperation. This work was supported by the Institute of Psychiatry and Neurology (Warsaw, Poland; Project No. 51/2002). L.B. was supported by Grant Project VZ 206100-1/58 of the Ministry of Education and Youth of the Czech Republic.

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