Original Article

Mucolipidosis II: a single causal mutation in the *N*-acetylglucosamine-1-phosphotransferase gene (*GNPTAB*) in a French Canadian founder population

Mucolipidosis (ML) II (I-cell disease) is a lysosomal storage disorder caused by a deficiency of UDP-*N*-acetylglucosamine:lysosomal enzyme *N*-acetylglucosamine-1-phosphotransferase. MLII is an autosomal recessive disease with a carrier rate estimated at 1/39 in Saguenay–Lac-Saint-Jean (SLSJ) (Quebec, Canada), which is the highest frequency documented worldwide. To identify the causing mutation, we sequenced *GNPTAB* exons in 27 parents of 16 MLII-deceased children from the SLSJ region as obligatory and potential carriers. We also performed a genealogical reconstruction for each parent to evaluate consanguinity levels and genetic contribution of ancestors. Our goal was to identify which parameters could explain the high MLII frequency observed in the SLSJ population. A single mutation (c.3503_3504delTC) was found in all obligatory carriers. In addition, 11 apparent polymorphisms were identified. The mutation was not detected in genomic DNA of 50 unrelated controls. Genealogical data show six founders (three couples) with a higher probability of having introduced the mutation in the population. The frequency of the mutation was increased as a consequence of this founder effect and of the resulting population structure. We suggest that c.3503_3504delTC is the allele causing MLII in the SLSJ population, and its high carrier rate is most likely explained by a founder effect.

M Plante[a], S Claveau[b], P Lepage[c], È-M Lavoie[a], S Brunet[c], D Roquis[c], C Morin[d,e], H Vézina[a] and C Laprise[b,e]

[a]Département des sciences humaines, Université du Québec à Chicoutimi, Saguenay, Québec, Canada, [b]Université de Montréal Community Genomic Medicine Center, Chicoutimi Hospital, Saguenay, Québec, Canada, [c]Sequencing Platform Team, McGill University and Génome Québec Innovation Centre, Montreal, Québec, Canada, [d]Department of pediatric, Chicoutimi Hospital, Saguenay, Québec, Canada, and [e]Département des sciences fondamentales, Université du Québec à Chicoutimi, Saguenay, Québec, Canada

Mucolipidosis (ML) II (MIM 252500) or I-cell disease is a rare autosomal recessive lysosomal storage disorder caused by a deficiency in the UDP-*N*-acetylglucosamine:lysosomal enzyme

N-acetylglucosamine-1-phosphotransferase (GlcNAc-phosphotransferase, EC 2.7.8.17) activity, an enzyme required for the mannose 6-phosphate marker tagging to newly synthesized lysosomal

enzymes (1). This initial step is essential for proper intracellular targeting of lysosomal hydrolases to the lysosome (2). Undegraded materials made of lipids and mucopolysaccharides form lysosomal deposits, recognized to cause the spectrum of clinical manifestations described in the literature (3). These include severe delayed development, short stature, coarse facial features with hyperplastic gums, dislocation of the hips, generalized hypotonia and thick and tight skin (3). Patients show dysostosis multiplex on radiological evaluation. They rarely survive the first decade of life (4).

GlcNAc-phosphotransferase is a hexameric enzyme made of three distinct subunits (α_2, β_2, and γ_2) (5). It is encoded by two separate genes: *GNPTAB*, encoding the α/β-subunit precursor, and *GNPTG*, which codes for the γ subunit (6–8). A frameshift mutation in *GNPTG* was shown to segregate with MLIIIC (6). *GNPTAB* spans 85 Kb on chromosome 12q23.3 and contains 21 exons (7, 8). A spectrum of mutations in *GNPTAB* was recently reported to cause MLII and MLIIIA (7, 9–12). The first study of importance on *GNPTAB* mutations was reported by Tiede et al. (7) and identified seven MLII-causing mutations in six patients. Another study by Kudo et al. (12) on 18 pedigrees with MLII or MLIIIA identified 15 mutations of which distinct combinations allowed to define each of these two disorders.

In most population, MLII is quite rare. It is estimated at 1/123,457 live birth in Portugal (13), 1/252,525 live birth in Japan (14) and 1/625,000 live birth in the Netherlands (15). In the young founder population of the northeastern region of Quebec [Saguenay–Lac-Saint-Jean (SLSJ)], a high MLII prevalence at birth of 1/6184 with an estimated carrier rate of 1/39 was found (16). The purpose of this study was to investigate the cause of this high prevalence of MLII through the identification of the causal mutation. Because MLII is fatal in childhood, this required the analysis of the parental obligate carriers. We found that all the obligate carriers had a single common mutation in exon 19 of *GNPTAB*. With genealogical reconstructions and ancestor genetic contributions calculations, we were then able to characterize these mutation origins and to explain its high frequency observed in the SLSJ population.

Materials and methods

MLII carriers

The parents of 16 children diagnosed with MLII at the paediatric clinic of SLSJ (Fig. 1) (Quebec,
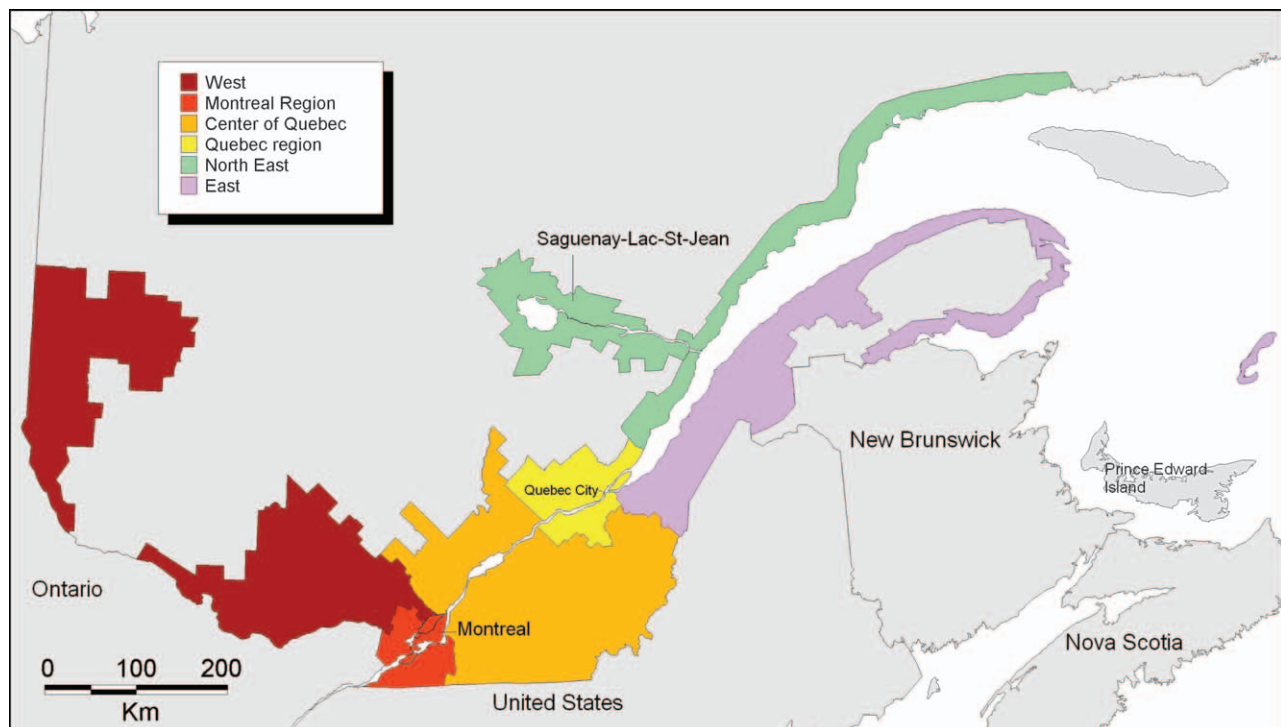

Fig. 1. Localization of Saguenay–Lac-St-Jean and of the six regional groupings used to investigate the genetic contribution of potential introducers.

Canada) were contacted by the Genetic Council Services of the Chicoutimi Hospital and were asked to participate in the present study. All those 16 affected children showed the classical MLII phenotype. A total of 27 obligatory carrier parents (27 different pedigrees) (84%) agreed to donate a blood sample for genomic DNA isolation and to provide family information for genealogical reconstruction. The Chicoutimi Hospital Local Ethics Committee approved the study and all the subjects (or their parents) provided their informed consent.

Isolation of genomic DNA and sequencing

Genomic DNA from parents was extracted from leukocytes by the standard Qiagen DNA extraction columns (Qiagen, Valencia, CA) according to the manufacturer's instructions.

Polymerase chain reaction (PCR) primers for all the 21 coding exons were designed with the PRIMER3 software and corresponded to those reported previously for *GNPTAB* (9, 12, 17). The sequence of the PCR primers is available on request. PCR reactions were performed in a total volume of 25 μl with 100 ng of genomic DNA, 20 pmol of each PCR primers, 1 U of Taq polymerase (Qiagen), 1.5 mM of $MgCl_2$ and 200 μM of each deoxyribonucleotide triphosphate (dNTP). The PCR conditions were as follows: 15 min at 94°C; 35 cycles of 20 s at 94°C, 20 s at 53–63°C and 20 s at 72°C; and a final extension of 5 min at 72°C. Unincorporated dNTPs and primers were removed by filtration through Montage PCR96 filter plates (Millipore, Bedford, MA). Sequencing of filtered PCR products was performed with BigDye Terminator v3.1 (Applied Biosystems, Foster City, CA) and analysed on the ABI Prism 3100® Genetic Analyzer (Applied Biosystems). Sequences were processed with the SEQUENCING ANALYSIS version 5.0 software (Applied Biosystems), assembled and analysed with the PHRED/PHRAP/CONSED package (18, 19).

All coding regions were sequenced in 12 parents and 12 unaffected control individuals selected from the Centre d'étude du polymorphisme humain (CEPH) (20) Utah HapMap samples (derived from US residents with northern and western European ancestry). These samples were provided by the National Institute of General Medical Sciences Human Genetic Cells Repository at the Coriell Institute for Medical Research (Camden, NJ). Heterozygous variants present in all 12 parents were subsequently sequenced in all other parents (*n* = 27) and CEPH

controls (*n* = 50) as they might be potential causes for MLII.

Genealogical reconstruction

Genealogical reconstruction relied on the BALSAC population registry and on the BALSAC–RETRO database that contains linked and validated genealogical information on nearly 375,000 individuals married in Quebec between the beginning of the 17th century and the present day (21, 22). Genealogy of each of the 27 obligatory carriers was completed as far back as sources allowed for, which was back to the first European settlers in most instances. For comparison purposes, 27 control individuals were selected in the BALSAC–RETRO database. They were matched to cases according to place and year of marriage of their parents.

Descriptive parameters were calculated on both cohorts to ensure that they were similar and amenable to comparison of their demogenetic characteristics. These parameters included ancestors' counts, completeness index and mean genealogical depth. Genetic structure was analysed using frequency distribution and genetic contribution of ancestors to probands and controls. The goal was to see if a subset of ancestors with a higher probability of having introduced the mutation in the Quebec population could be identified. Inbreeding coefficients among children of obligate carriers were also measured in order to see if an increased frequency of consanguineous unions among carriers could explain, at least in part, the diffusion pattern of the mutation. These coefficients could be calculated only when both parents accepted to participate in the study (22 out of 27 carriers forming 11 couples). Results were compared with those obtained with a set of 11 control couples selected in the BALSAC–RETRO database and matched for date and place of marriage. All genealogical indices were computed as described previously (23, 24).

Results

Mutation identification

Direct sequencing of the 21 exons of *GNPTAB* gene was performed on PCR products amplified from genomic DNA. DNA samples were obtained from 12 parents of the probands. The parents' DNA were assumed to have one mutant allele in the *GNPTAB* gene based on the MLII transmission model (autosomal recessive disorder) (3). CEPH DNA samples (*n* = 12) were

Table 1. SLSJ genetic variants identified in GNPTAB gene

Genetic variants[a]	Location	Predicted effect on mRNA and protein	SLSJ genotype distribution[b]	CEPH genotype distribution[b]
c.-41-39delGGC	5′UTR[d]	ND	Seven hetero and five homo	Six wt and six hetero
c.137G>A	Exon 2	Non-synonymous mutation p.R46Q	11 wt and one hetero	12 wt
IVS2+40A>G	Intron 2	ND	11 wt and one hetero	12 wt
IVS4ins100_102CA (rs4015837)	Intron 4	ND	Four wt and eight hetero	12 wt
IVS4-233T>C (rs6539012)	Intron 4	ND	Seven wt and five hetero	12 wt
IVS10-167C>T (rs7963747)	Intron 10	ND	Six wt and six hetero	Five wt and seven hetero
c.1932G>A (rs10778148)	Exon 13	Synonymous mutation	Four wt and eight hetero	Eight wt and four hetero
IVS15+5T>C (rs759935)	Intron 15	ND	Six wt and six hetero	Eight wt and four hetero
IVS17-25G>A (rs3736476)	Intron 17	ND	Three wt and nine hetero	Three wt and nine hetero
3503_3504delTC (rs34002892)[c]	Exon 19	Frameshift, premature stop codon p.L1168QfsX5	27 hetero	50 wt
IVS19-344G>T (rs1811338)	Intron 19	ND	Five wt and seven hetero	Seven wt and five hetero
c.4966A>C	3′UTR[d]	ND	11 wt and one hetero	Eight wt and four hetero

CEPH, Centre d'étude du polymorphisme humain; ND, not determined; SLSJ, Saguenay–Lac-St-Jean; wt, wild type.
[a]Identified relative to the first nucleotide in the ATG start codon of cDNA as nucleotide +1 (GenBank accession number NM_024312). Intron genetic variant identification is described in accordance with ENSEMBL v37 accession number ENST00000299314. Mutations are given in bold.
[b]Hetero indicates heterozygous, which correspond to one minor allele and one frequent allele. wt correspond to two frequent alleles. Homo indicates homozygous and represents two minor alleles.
[c]3503_3504delTC has been genotyped in 27 SLSJ parents and 50 CEPH controls.
[d]Untranslated region.

sequenced as *GNPTAB* wild-type control for electrophoregram comparison. Sequence analysis led to the identification of 12 genetic variants that are reported in Table 1. One genetic variant was present in 12 parents, so the 15 remaining parents ($n = 27$) and 50 CEPH control subjects were sequenced. Polymorphism and mutation identification is based on the nomenclature suggested by den Dunnen and Antonarakis (25, 26) and complemented with the dbSNP rs number when it is available.

Mutation analysis

The genetic variants and the genotype frequency observed in the sampled population are listed in Table 1. A mutation located in exon 19 was present in all parents (100%). The electrophoregram showed a two-nucleotide deletion (c.3503_3504delTC), causing a frameshift mutation that led to a premature stop codon (p.L1168QfsX5). All parents were heterozygous for this deletion. None of the 50 CEPH control subjects presented the deletion.

Among the 12 genetic variants identified in the SLSJ genotype, eight were previously reported in dbSNP (rs4015837, rs6539012, rs7963747, rs10778148, rs759935, rs3736476, rs34002892 and rs1811338) (27). Seven variants are located into introns, and five variants are located into exons. These five exon variants are c.-41-39delGGC, c.137G>A, rs10778148 (which are not expected to have a deleterious effect on the encoded protein), c.3503_3504delTC and c.4966A>C. The variants were investigated considering their location in the gene, their predicted effect on messenger RNA (mRNA) or encoded protein and their prevalence in the sample. A three-nucleotide deletion located in the 5′ untranslated region (UTR) was identified (c.-41_-39delGGC) in the sequenced samples of MLII probands' relatives, seven were heterozygous and five were homozygous for the deletion, which is documented as a polymorphism and observed in six CEPH controls (heterozygous) (12). Some variants were observed in single individuals (p.R46Q, IVS2+40A>G, and c.4966A>C) and were not considered to be the causal mutation in SLSJ population, given that MLII is an autosomal disease. Of these 12 genetic variants observed in SLSJ genotype, only seven were present heterozygously in some CEPH genotype (c.-41-39delGGC, rs7963747, rs10778148, rs759935, rs3736476, rs1811338 and c.4966A>C) (Table 1). Although the effects of these variants on mRNA or protein have not been investigated, they are not the predominant cause of MLII in this population. Thus, c.3503_3504delTC is the causal mutation of MLII in the sampled population.

Table 2. Descriptive parameters of the genealogies

	Cases	Controls
Number of genealogies	27	27
Total ancestral links	172,195	155,205
Distinct ancestors	12,153	13,587
Mean number of occurrences	14.2	11.4
Mean genealogical depth	11.1	10.8
Maximum genealogical depth	17	17

Genealogical results

Descriptive parameters are summarized in Table 2. For the MLII and control groups, respectively, 172,195 and 155,205 genealogical links were identified. However, because many ancestors appear several times in the genealogies, this corresponds to 12,153 and 13,587 distinct ancestors. Genealogical depth is slightly lower for controls with a mean of 10.8 generations instead of 11.1 for carriers. Completeness is also very similar among both groups, remaining higher than 80% until the 11th generation and decreasing rapidly afterwards. Table 2 also indicates that the mean number of appearances of ancestors in the genealogies is slightly higher among carriers (14.2) than among controls (11.4), pointing to a higher level of genetic homogeneity among carriers.

Figure 2 shows inbreeding curves for children with MLII and for controls. Consanguinity was found among probands at the fourth generation of ancestry. At this generation, the parents of one child with MLII share common ancestors, meaning that they are second-degree cousins. No consanguinity was found in the controls' genealogies before the seventh generation. Mean inbreeding coefficients are higher in the MLII group at all generations starting from the fourth and, at the maximum depth, consanguinity is
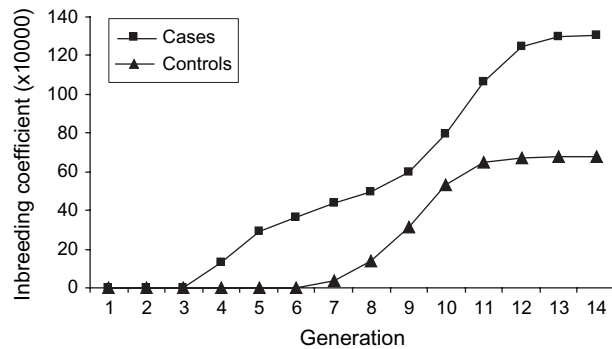
almost double that of controls (0.0130 *vs* 0.0067). The difference between both groups reaches no statistical difference, with the exception of generations 13 ($t = 2.096$, p $= 0.048$) and 14 ($t = 2.104$, p $= 0.047$), but this could be explained by the small size of the sample.

For each of the 18,612 ancestors found in the genealogies, the number of carriers and controls to which they were related as well as their genetic contribution to each group were calculated. The goal was to identify ancestors with the highest probability of having introduced the mutation on the Quebec territory based on the hypothesis that the MLII mutation was introduced by a single founder (or founder couple). Potential introducers should be related to all carriers and have a high genetic contribution to this group. Ancestors with a higher contribution when compared with controls were treated as more likely candidates.

One hundred and sixty-six ancestors appearing in all carriers' genealogies were identified, and the genetic contribution of each of these ancestors to both groups was compared. In Table 3, results are presented for the six ancestors (three couples) who came out as being the most plausible candidates. These three couples were married in the Quebec region in the second half of the 17th century. They were either children or grandchildren of immigrants and all came from France (mostly from Perche, Normandie and Aunis regions) except for one immigrant who came from Scotland. Each couple had between seven and 14 married children, most of whom settled in the Charlevoix region.

The last set of genealogical analyses aimed at estimating the genetic contribution of these three couples to the regional populations of Quebec to see the extent of variability in these contributions. Genealogical data used for these calculations came from an ongoing project on the demogenetic characteristics of the regional

Fig. 2. Mean inbreeding coefficients for subjects with MLII and controls.

Table 3. Genealogical characteristics of the three couples of ancestors with the highest probability of having introduced the mucolipidosis mutation in the French Canadian population

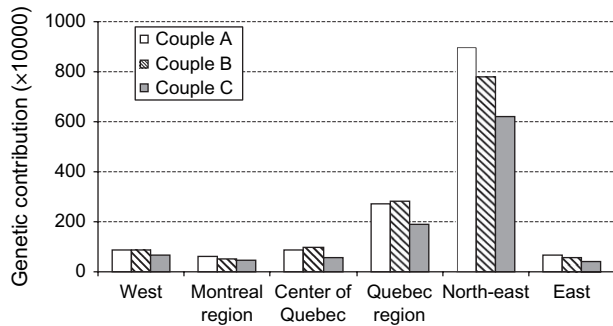| | Number of genealogies where couples appear | | Genetic contribution ($\times 10^4$) | | |
Couples	Among carriers	Among controls	To carriers a	To controls b	Difference (%) $(a - b)/a$
A	27	27	9169.92	7333.98	20.02
B	27	27	8132.32	6872.56	15.49
C	27	27	6369.63	5357.67	15.89

Fig. 3. Genetic contribution of potential introducers of the MLII mutation to Quebec regional populations.

populations of Quebec (23). Figure 3 shows the genetic contribution of the three couples to six regional groupings (mapped on Fig. 1). In each case, the highest genetic contribution is in the north-east grouping that includes the regions of Charlevoix, SLSJ and Côte-Nord. The neighbouring region of Quebec displays the second highest genetic contribution, but it is three times lower than that in the north-east region. In the four other groupings, the genetic contributions are much lower.

Discussion

The present study reports on a *GNPTAB* mutation in 27 MLII obligatory carriers (parents of 16 affected children) in a founder population in SLSJ. To date, this is the first time that a single mutation is pointed out to cause MLII in a founder population. *GNPTAB* mutations associated with MLII and MLIIIA were recently reported in several studies including populations of various origins (7, 9, 11, 12, 28). Overall, 23 different mutations are known to cause MLII, of which only four were found in more than one pedigree (Table 4). In the SLSJ sample, where no MLIIIA cases were reported, the only mutation that can explain MLII phenotype is 3503_3504delTC because it is the only one that was observed in all the obligate carriers investigated. Although 3503_3504delTC has been observed elsewhere, it reaches the highest frequency in SLSJ [(11, 12), Table 4]. Specifically, in a group of 21 MLII patients of Israeli Arab-Muslim, Palestinian Arab-Muslim, Turkish and Irish origin, only 12 patients had the 3503_3504delTC mutation (11). In another study, eight pedigree/cell line had the 3503_3504delTC mutation, but only two of them were homozygous for that mutation (12). Hence, the single mutation responsible for the MLII phenotype in

the region of SLSJ indicates a higher genetic homogeneity compared with other populations where MLII was investigated. Similar observations were found in literature regarding SLSJ and cystic fibrosis (OMIM 219700), autosomal recessive spastic ataxia of Charlevoix–Saguenay (OMIM 270550) and tyrosinaemia type 1 (OMIM 276700) as examples (29–31).

The 3503_3504delTC mutation was the only one found in all tested carriers from SLSJ population. One MLII patient homozygous for this mutation was previously reported by Kudo et al. (12). According to the Kudo et al.'s study (12) and the carrier status of all parents, this mutation can cause a deleterious effect of the *GNPTAB* gene, resulting in MLII phenotype in the probands. It was described as a null allele, which means that protein coded (p.L1168QfsX5) do not produce any GlcNAc-phosphotransferase activity (12). The mutation yields intact α-subunits, but truncated β-subunits and a functional study through transient transfection of L1168QfsX5 plasmid in 293T cells did not show any increase in GlcNAc-phoshotransferase activity (12). According to that functional study, the presence of a homozygous 3503_3504delTC mutation is sufficient to produce MLII disease.

The SLSJ region (population 274,095 in 2006) is located 200 km north of Quebec City (Fig. 1). Settlement in this region started around 1840 with the arrival of inhabitants coming first mostly from the nearby Charlevoix region (east of Quebec City) and afterwards from other regions of the Laurentian valley (32). It is estimated that between 1840 and 1910, 75% of the 30,000 immigrants to SLSJ came from Charlevoix. Moreover, the family type of their migration contributed to give them an advantage by facilitating their settlement (33). For these reasons and because they both share an increased frequency of some otherwise rare inherited disorders, Charlevoix and SLSJ's inhabitants are often considered as belonging to the same genetic pool (for review, see 34, 35).

High inbreeding levels and founder effect are two possible explanations to elevated prevalence of genetic diseases in populations. Both hypotheses have been investigated in the SLSJ population using genealogical data. Consanguinity was somewhat higher among cases than among controls when we considered many generations of ancestry (distant consanguinity), but close inbreeding (three to four generations of ancestry) was low and very similar in both groups. A low level of close inbreeding in the SLSJ population was observed in a comparative study of the Quebec regional populations (23). Moreover, close

Table 4. Location of documented GNPTAB mutations causing MLII

Intron/exon	cDNA locus	Protein consequence	Number of pedigree affected	References
I1	118-2A>G	IVS1-2A>G (R68fsX8)	1	(11)
E3	310C>T	Q104X	1	(9)
E6	609_612delCAGA	T206YfsX6	2	(12)
E8	848delA	T284LfsX5	1	(12)
E12	1580delC	C528VfsX19	1	(12)
E13	1625insC	E542AfsX4	1	(7)
E13	1738_1741tripTATA	S581IfsX8	1	(12)
E13	2188T>AAA	L730KfsX8	1	(12)
E13	2533C>T	Q845X	1	(7)
			1	(11)
E13	2681G>A	W894X	1	(9)
E15	2916insT	Skipping exon 15 + (M9721fsX3)	1	(11)
E16	3145insC	G1049RfsX16	1	(7)
E16	3173C>G	S1058X	1	(9)
E16	3231_3234dupCTAC	Y1079LfsX3	1	(12)
I17	3249+1G>A	Skipping exon 17 + (R1112fsX2)	1	(11)
			1	(7)
			1	(12)
E17	3252delA	P1085RfsX6	1	(7)
E18	3434+1G>A	Skipping exon 18	5	(11)
E19	3474_3475delTA	H1158QfsX15	1	(9)
E19	**3503_3504delTC**	**L1168QfsX5**	**8**	**(12)**
			11	**(11)**
E19	3565C>T	R1189X	2	(9)
E19	3566insA	R1189QfsX9	1	(7)
E20	3613C>T	R1205X	1	(7)
			1	(11)
E21	3707A>T	K1236M	1	(28)

inbreeding coefficients in the MLII group were similar to levels previously reported for different genetic disorders found in Quebec (36). Hence, the high MLII frequency found in SLSJ region is not explained by a close inbreeding phenomenon but most likely by a founder effect and the resulting population structure that includes elevated levels of distant consanguinity.

The founder effect increases incidence of recessive disorders and decreases non-allelic heterogeneity in monogenic disorders (37). This is the underlying basis to the high prevalence of SLSJ genetic diseases. It is noteworthy that more than one mutation was found for diseases in SLSJ (34, 38, 39). Hence, if a disease is the result of multiple mutations, more than one ancestor is involved. However, all the SLSJ MLII parents were heterozygous for the same mutation. We cannot totally rule out the possibility of a *de novo* mutation. However, we suppose that the one founder/one gene hypothesis is the most likely scenario for the introduction of the mutation in the population despite the lack of haplotype information that could have reinforced that hypothesis. This assertion rests on the genealogical analyses of the present study as well as on the various studies conducted on the SLSJ population history and genetic structure (40, 41).

Founder effect followed by a strong population growth has been consistent to explain the high frequency of other SLSJ autosomal recessive disorders (42).

We identified three couples of founders with a higher likelihood of having introduced the MLII mutation in the contemporary SLSJ population. Two of them had ancestors who came from Perche and the other had ancestors from Normandie. This is in accordance with previous studies, where a strong genetic contribution of Perche immigrants was observed and explained by their earliest settlement in Nouvelle–France (43, 44). Moreover, the contribution of these ancestors to SLSJ gene pool is rather homogeneous, as shown by its lack of specificity: both disease and control groups are linked to these common founders. Nevertheless, genetic contribution of each of these three couples is greater in the north-east grouping, and this could explain why there is a higher prevalence of MLII in SLSJ compared with other Quebec regions. However, because these couples also contributed to the gene pool of other regions of Quebec, it does not rule out the presence of the MLII mutation outside the north-east regions. Indeed, some cases have been identified in other regions of Quebec.

Conclusions

In conclusion, the MLII mutation in SLSJ region is now identified. Indeed, our results show that only one mutation (3503_3504delTC) is present in 100% (27/27) of MLII obligatory carriers of SLSJ origin and is responsible for MLII in that population. As already reported in the literature, the SLSJ region counts some hereditary disorders with high frequencies of carriers attributed to founder effect and characteristics of the settlement history (44). These factors also explain the high prevalence of MLII and the identification of only one mutation among all obligate carriers in the present study. Attempts to identify the possible introducers of the mutation in the population point to a small group of ancestors that appears to be more specific to the northeastern regions of Quebec (SLSJ, Charlevoix and Côte-Nord). Using this information, we can confirm prenatal diagnosis earlier in the pregnancy and determine carrier status of people of SLSJ ancestry, which is of great importance for genetic counselling because there is currently no mean to cure MLII. Further analysis would be necessary to determine whether this mutation is also present in other ethnic populations and see if there could be any link between the SLSJ population structure and those populations.

Acknowledgements

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