

MUTATION IN BRIEF

The Frequency of Mucopolidosis Type IV in the Ashkenazi Jewish Population and the Identification of 3 Novel MCOLN1 Mutations

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Mucopolidosis type IV (MLIV) is a neurodegenerative lysosomal storage disorder that occurs in an increased frequency in the Ashkenazi Jewish (AJ) population. The frequency of the disease in this population has been established by the testing of 66,749 AJ subjects in the Dor Yeshorim program, a unique premarital population-screening program designed for the Orthodox Jewish community. A carrier rate of 0.0104 (95% C.I. 0.0097-0.011) was found. The distribution of the 2 AJ founder mutations, namely, c.416-2A>G and c.1_788del, was determined to be 78.15% and 21.85%, respectively. Three novel mutations were identified in non-Jewish MLIV patients: a missense mutation c.1207C>T, p.Arg403Cys; a 2bp deletion, c.302_303delTC; and a nonsense, c.235C>T, Gln79X. © 2005 Wiley-Liss, Inc.

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INTRODUCTION

Mucopolidosis type IV (MLIV, MIM# 252650) is a neurodegenerative lysosomal storage disorder characterized clinically by severe psychomotor retardation, ophthalmological abnormalities, elevated blood gastrin and iron deficiency (Amir et al. 1987, Bach, 2001, Altarescu et al. 2002). Patients with milder psychomotor manifestations were also described (Chitayat et al. 1991, Casteels et al. 1992, Reiss et al. 1993). The progression of the disease is very slow despite the early age of onset (1-2 years) and most patients remain in an apparent steady-state for their first 2-3 decades of life if blood iron is appropriately controlled. Life expectancy is not clear as yet and patients in their thirties and forties are repeatedly described (Newell et al., 1975, Chitayat et al., 1991, Bargal et al., 2002). The disease is rare in the general population but has been observed in an increased frequency in the Ashkenazi Jewish (AJ) population (Bach 2001, Altarescu 2002).

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The disease is caused by mutations in the MCOLN1 gene (GenBank accession number for cDNA: AF287269) which was identified a few years ago (Bargal et al., 2000, Bassi et al., 2000, Sun et al., 2000). Over 15 MLIV-causing mutations have been described (Bach 2001, Altarescu et al., 2002, Slaugenhaupt 2002, Bach 2004), two of which, a splice mutation (c.416-2A>G), and a partial gene deletion (c.1_788del) were identified as founder mutations in the AJ population (Slaugenhaupt et al., 1999, Bargal et al., 2000). These mutations comprise 95% of the MLIV alleles in this ethnic group. MLIV is one of some 10 fatal and/or devastating recessive disorders that are found in high prevalence in the AJ population as expected for an isolated population for over a millennium. Population screening programs for the detection of high risk couples (two heterozygotes for one of these diseases), before the birth of the first affected offspring, have been operated in recent years in countries of high concentrations of this ethnic group, primarily in Israel and the U.S. A unique screening program for the orthodox Jewish community, the Dor Yeshorim program, is principally a premarital testing program (Broide et al., 1993, Ekstein and Katzenstein, 2001, Ekstein et al., 2004). The large number of AJ subjects tested by this program enabled an accurate assessment of the gene frequency of MLIV in this population as well as an accurate estimation of mutation distribution. Previous reports for the frequency of MLIV were based on much smaller numbers that permitted less significant statistical evaluations. We also report the detection of 3 novel mutations in the MCOLN1 gene in non-Jewish MLIV patients.

MATERIALS AND METHODS

The subjects tested in this study participated in the Dor Yeshorim screening program. In addition to MLIV this program offers the testing for 9 other recessive disorders that are prevalent in the AJ population. Genotyping for the 2 AJ MLIV mutations was performed in the following laboratories; The Department of Human Genetics, Hadassah Medical Center, Jerusalem; Genehelp laboratory, Dor Yeshorim, Jerusalem; and at Tepnel Diagnostics Ltd., Abingdon.

The analysis of the 2 MLIV founder mutations in the AJ population (c.416-2A>G and c.1_788del) at Hadassah and Genehelp laboratories was performed by a multiplex PCR reaction as previously described (Bargal et al., 2001). Tepnel uses allele specific amplification in the Amplification Refractory Mutation System (ARMS™) (Newton et al., 1989).

The data summarizes the outcome of this program during the years 2000-2004. The testing is performed confidentially, the program does not record any names and the only personal information available is the AJ origin and birth dates. We have no information of any relationship among them and there was no selection for individuals related to MLIV patients. Thus, this group represents a random, unbiased, sample for the AJ population.

To estimate carrier frequency we divided the number of detected carriers by the number of the total tested subjects. The allele frequency was calculated from the total number of carriers.

Mutations analysis: Two patients were referred with the typical clinical symptoms of MLIV (Amir et al., 1987, Altarescu et al., 2002), including severe psychomotor retardation, cornea opacity and retina degeneration. Electron microscopy examination of a conjunctiva biopsy indicated the presence of the typical storage bodies for MLIV (Berman et al., 1974). The first patient (AB) was a 24 years old female of Druze origin from consanguineous parents (first cousins). The 2nd patient (CD) is a 4 years old female from France, not Jewish. Her parents were not available for further analysis. DNA from peripheral blood was prepared by standard methods. Genomic DNA was amplified and analyzed for MCOLN1 mutations as previously described (Bargal et al., 2001). Restriction analysis of the novel mutations was performed on genomic DNA using the primers outlined in Table 1. The identified mutations are presented in the cDNA.

Table 1. Primers used for restriction analysis of the novel mutations

Primer set	Forward	Reverse
1	5-GCAGCTCCCACCCGCAGA-3	5-GGCTGCGTGTGTAGGGATT-3
2	5-GCTGGCTGTGACATTCCG-3	5-ATGGAAGATGGCCTGGTACA-3
3	5-AAGCCCTGCAAGCTGATGC-3	5-TCTCCTCAAGGAGCACAGCA-3

RESULTS AND DISCUSSION

The testing of 66,749 AJ subjects in the Dor Yeshorim program for the 2 Ashkenazi MLIV mutations detected 691 heterozygotes; a carrier rate of 0.0104 (95% C.I 0.0097-0.011) (1:96 average). The carrier frequency predicts that the disease prevalence in the AJ population is approximately 1:40,000 births. Indeed, we have diagnosed 31 MLIV patients (new families) during the years 1975-2005. There are 80,000-100,000 births in the Jewish population in Israel/year (Statistical abstracts), of which approximately 30%, or ca30,000-40,000, births/year to couples in which both spouses are Ashkenazi Jews (Cohen et al., 2004). Thus, 31 MLIV patients were detected in 30 years and during this period there were about 1,200,000 births in Ashkenazi families; this clearly reinforces the present finding for the MLIV gene frequency.

The 2 AJ mutations comprise 95% of the mutant alleles in this population, while 5% are unique mutations for each patient (Bach, 2004), thus, the overall frequency of MLIV among AJ is 0.0108 (1:93).

The distribution of the 2 Ashkenazi mutations among the 691 carriers is presented in Table 2.

Table 2: The distribution of MCOLN1 mutations among 691 AJ carriers

Mutation	Number (%)
c.416-2A>G (major)	540 (78.15)
c.1_788del (minor)	151 (21.85)

*MCOLN1 cDNA GenBank accession number: AF287269.1. Nucleotide numbering based on 1 as the A of the translation initiation codon.

The distribution of the 2 mutations is very similar to the haplotypes distribution in the MCOLN1 gene region for AJ MLIV patients when the gene was first mapped (Slaugenhaupt et al., 1999). The lower carrier frequency reported previously (Edelmann et al., 2002, Strom et al., 2004) might stem from the low sample numbers, ca 2000 individuals tested for MLIV carrier status in each report, and furthermore, the present study reflects the frequency of MLIV in a sample that is clearly Jewish while the previous reports might be more diffuse as to the origin of the tested individuals in the U.S., as indeed stated by Strom et al., 2004.

The data presented here is the outcome of the Dor Yeshorim program which is targeted primarily for the Orthodox community. The nature of this program ensures unambiguously that the individuals tested in this program are of Jewish origin and in this case the Ashkenazi origin is distinctly verified. Although the program includes only a part of the Ashkenazi population the ethnic origin is identical to the secular part of this population. It should be emphasized that a significant proportion of the secular Ashkenazim came from orthodox or ultra-orthodox families 2-3 generations ago. The age of the MLIV mutations was estimated to be of ca 50 generations old (Risch et al., 2003).

Thus, the data presented here and elsewhere (Broide et al., 1993, Abeliovich et al., 1996) regarding mutation frequencies reflect the true gene frequencies in the Ashkenazi population with marginal differences in various concentrations of this population due to some genetic drifts in recent generations.

It should be mentioned that both mutations result in a severe phenotype either homozygotes or compound heterozygotes. The carrier rate found for MLIV is comparable to various other recessive diseases currently undergoing population screening in the AJ population, including, Niemann-Pick type A+B, Fanconi anemia, Bloom and glycogen storage type 1a syndromes (Risch et al, 2003). These diseases are offered as part of a panel of 10 different prevalent diseases in the AJ, including also Tay-Sachs, Cystic Fibrosis, Familial Dysautonomy, Canavan and Gaucher.

Mutations analysis: A missense mutation c.1207C>T, p.Arg403Cys was identified in patient AB. The patient was homozygous for the mutation and her parents were heterozygotes. It should be mentioned that the patient has 2 younger brothers with a similar clinical picture but they were not available for molecular diagnosis. A control panel of 100 individuals from this ethnic group did not detect this mutation. The mutation abolishes an AciI restriction site. Thus, the PCR fragment of 164bp, prepared by primers set 1 (Table 1), is cut to 2 fragments of 77 and 87 bp in the controls and remains uncut in homozygote for the mutation (data not shown). This confirmed the identification of the mutation. The patient is the 2nd Druze MLIV patient diagnosed by us. There is no family relationship with the other Druze patient who had a different MCOLN1 mutation (Bargal et al., 2001). This is a remarkable occurrence for a population of Ca 100,000 individuals in Israel.

Patient CD was identified as a compound heterozygote for the mutation c.302_303del TC resulting in a frame shift and premature termination. The mutation creates a Taq α 1 restriction site, thus a fragment of 130bp, prepared by primers set 2, containing the mutation is cut to 38 + 90bp fragments in the presence of the mutation and remain uncut in normals. The 2nd mutation was a nonsense c.235C>T, Gln79X. This mutation abolishes one of 2 HpyCH4V restriction sites in a fragment of 119bp, prepared by primers set 3. The subfragment of 111bp remains uncut in the presence of the mutation and is fragmented to 43 + 68bp in controls (data not shown). The parents were not available for further analysis.

The mutations identified here are summarized in Table 3.

Table 3: A summary of MCOLN1* 3 novel mutations

Patient	Mutation (cDNA)	Mutation (protein)	Restriction analysis
AB**	c.1207C>T	p.Arg403Cys	Abolishment of AciI site
CD	c.302_303delTC		Creation of a Taq α 1 site
CD	c.235C>T	Gln79X	Abolishment of HpyCH4V site

*MCOLN1 cDNA GenBank accession number: AF287269.1. Nucleotide numbering based on 1 as the A of the translation initiation codon.

**The patient is homozygous for the mutation.

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