Homozygosity by descent for a rare mutation in the myophosphorylase gene is associated with variable phenotypes in a Druze family with McArdle disease

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Abstract

We examined a large consanguineous Druze family with McArdle disease for mutations in the glycogen myophosphorylase (PYGM) gene. All affected subjects were autozygous for a single G to A transition that abolishes the 5' consensus splice site in the first nucleotide of intron 14. The G to A transition is a rare mutation, with only one previous report in a single white subject heterozygous for this mutation and another, more common, mutation at codon 49. The kindred in our study is the first family reported in which disease is caused by homozygosity for this rare mutation. This kindred was originally reported as the first familial case of McArdle disease in the Druze.

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McArdle disease is a rare hereditary disorder resulting from the absence of functional muscle glycogen phosphorylase. Patients with the disease are characterised by a lifelong history of exercise intolerance, muscle pain, stiffness on exertion, and recurrent myoglobinuria. The disease has an autosomal recessive mode of inheritance, although a few families with an autosomal dominant mode of inheritance have also been documented.^{1,2} The gene for glycogen myophosphorylase (PYGM) has been localised to 11q13³ and has been cloned and sequenced.^{4,5} At least 10 different mutations in the PYGM gene are associated with McArdle disease.⁶⁻⁹ The most common mutation observed is a nonsense mutation resulting from a C to T transition at codon 49 (R49X), resulting in arginine (CGA) being changed to a stop codon (TGA). This mutation can occur on both chromosomes of affected subjects or in combination with other rarer mutations that are not complemented in *trans*.^{8,9} Several mutations have been well characterised in white populations and Japanese,^{6,7} but strong genotype-phenotype correlations have not emerged since the full range of clinical variability is seen in patients with the same mutation.⁸

A three generation consanguineous Druze family with McArdle disease was examined for

mutations in the PYGM gene and haplotypes were constructed with 14 markers including and encompassing the PYGM gene. This kindred was originally described in a case report as the first family with McArdle disease in the Druze population.¹⁰ Inheritance of the disease in this family is consistent with an autosomal recessive mode, and Sarova-Pinhas and Sadeh¹⁰ have described a marked variability in the clinical findings among family members.

Materials and methods

FAMILY DATA

Genomic DNA was extracted from Epstein-Barr transformed lymphoblastoid cell lines in available members of the kindred (fig 1) by standard procedures. Informed consent was obtained from all family members by established protocols at the Sackler School of Medicine, Tel Aviv University. Detailed descriptions of the clinical and biochemical features of affected members of this family are provided in an earlier paper.¹⁰ Affected subjects varied in clinical severity. Two subjects, BT999 and BT937, experienced permanent weakness, three others, BT945, BT942, and BT936, experienced moderate to severe exercise intolerance into adulthood, while one subject, BT938, was the least affected with only mild and transient exercise intolerance in childhood. BT941 was diagnosed with McArdle disease at the age of 5 years, at which time exercise intolerance and cramping pains after exertion were observed. Clinical data were not available on BT941 after that time.

LINKAGE AND HAPLOTYPE ANALYSIS

Previously published markers were used to perform linkage analysis and to construct haplotypes. In all, 13 dinucleotide short tandem repeats (STRPs) were tested on genomic DNA of all available family members using primers and PCR conditions specified previously.¹¹⁻¹⁹ The markers tested were D11S1368, D11S1392, D11S905, D11S903, UT5150, D11S956, D11S480, D11S1783, PYGM (CA), INT2, D11S916, D11S787, D11S533, and a VNTR polymorphism PYGM (AT).

Pairwise lod scores were calculated between seven markers on proximal 11q and McArdle disease using LIPED.²⁰ An autosomal recessive model for the disease was assumed, with com-

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Figure 1 Pedigree of the Druze family with McArdle disease. Solid symbols represent affected subjects and open symbols unaffected subjects. The haplotype of 14 markers on chromosome 11 is shown for subjects on whom DNA was available. The order of markers in the haplotype is D11S1368 - D11S1392 - D11S905 - D11S903 - UT5150 - D11S956 - D11S480 - D11S1783 - PYGM (AT) - PYGM (CA) - INT2 - D11S916 - D11S787 - D11S533. Presence of the 1844+1G→A mutation is indicated by a + symbol underneath each chromosome, while absence of the mutation is indicated by a - symbol. We were unable to determine phase and hence assign haplotypes to three spouses, BT954, BT946, and BT952. The genotypes for these three are shown with open (unshaded) bars. Owing to extensive haplotype sharing in this consanguineous family we were unable to determine exactly where recombination had occurred in subjects BT937, BT953, BT942, BT951, and BT947. Thus, the broadest possible region of recombination is indicated in these subjects by a narrow black line.

plete penetrance, and a disease gene frequency of 0.05. In calculating lod scores, equal allele frequencies were initially used for each repeat. For all the loci, the relevant members of the kindred were fully typed making the results nearly to completely insensitive to allele frequencies.

An extended haplotype was constructed consisting of all 14 markers encompassing the PYGM locus in this family. The order D11S1368 - D11S1392 - D11S905 - D11S903 - UT5150 - D11S956 - D11S480 - D11S1783 - PYGM (AT) - PYGM (CA) - INT2 - D11S916 - D11S787 - D11S533, based on published maps,¹⁸ was used to construct haplotypes, assuming the fewest number of recombinants. Markers PYGM (AT) and PYGM (CA) are 1.5 kb apart and lie either in 3' or 5' untranslated regions or in an intron of the PYGM gene,¹² and their order with respect to the mutation and flanking markers is unknown. Although the order PYGM (AT) - PYGM (CA) was used in

construction of haplotypes, the reverse order is just as likely. The marker D11S1783 lies in close proximity to the PYGM locus, and its order with respect to the PYGM locus has not been resolved.¹⁸

MUTATION ANALYSIS

Genomic DNA samples from the family were screened for three known mutations at PYGM. Two of the mutations screened were the common C to T substitution at codon 49 in exon 1 (R49X) and a T to C substitution at codon 291 in exon 8 (L291P).^{6,7} These mutations were screened using restriction digests of PCR products as previously described.^{6,7} The genomic DNA was also screened for a known G to A transition at the 5' splice junction of intron 14 (1844+1GA) of the PYGM gene. Previously published primers and PCR conditions⁶ were used to amplify a 135 base pair PCR product containing the 5' splice junction of intron 14. The mutation was identified by digestion of the 135 base pair PCR product with the restriction enzyme *Nco*I.

Table 1 Pairwise lod scores of markers on 11q13 with McArdle disease

Marker	Recombination fraction							Zmax	θest
	0.000	0.001	0.050	0.100	0.200	0.300	0.400		
D11S1783	0.73	0.72	0.64	0.55	0.38	0.23	0.10	0.73	0.0
PYGM AT	0.52	0.51	0.47	0.42	0.30	0.19	0.09	0.52	0.0
PYGM CA	3.77	3.76	3.40	3.02	2.23	1.42	0.63	3.77	0.0
INT2	0.22	0.22	0.18	0.14	0.07	0.03	0.01	0.22	0.0
D11S916	3.19	3.18	2.89	2.58	1.94	1.27	0.57	3.19	0.0
D11S787	2.32	2.32	2.07	1.81	1.29	0.76	0.27	2.32	0.0
D11S533	2.35	2.34	2.11	1.85	1.31	0.75	0.24	2.35	0.0

Figure 2 Digitised image of the NcoI digest of the 135 base pair PCR product encompassing the 5' splice site in intron 14 of the PYGM gene. Lanes 1 and 2 are two sibs who are carriers for the mutation. Lanes 3-6 are unaffected spouses in the kindred. Lane 7 is an affected subject who is homozygous for the G to A transition.

Presence of the mutation results in digestion of the 135 base pair product into two fragments of 115 and 20 base pairs. Restriction digests were performed in a final volume of 15 µl; 12.5 µl of PCR product were digested with 10 U of enzyme for one hour at 37°C according to the manufacturer's instructions. Digested products were diluted with formamide loading dye in a 1:5 ratio and 1.5 µl of the diluted mixture was loaded onto a 6% native acrylamide gel (19:1 acrylamide:bisacrylamide). The gel was run at 20 W for two hours at room temperature, stained with SYBR green (Molecular Probes, Eugene, Oregon) for 30 minutes, and then scanned using a Fluorimager (Molecular Dynamics model 575).

Results and discussion

Pairwise lod scores between McArdle disease and seven markers in the 11q13 region are given in table 1. Two STR loci, PYGM (CA) and D11S916, gave lod scores greater than 3 at θ=0. Two other STR loci, D11S787 and D11S533, gave lod scores of 2.32 and 2.35 at θ=0. Three markers, INT2, D11S1783, and PYGM (AT), were not informative in the pairwise analysis. Obligate recombinants were not observed between McArdle disease and any of these seven markers.

None of the affected members in this kindred had the common C to T substitution at codon 49 in exon 1 (R49X) or the T to C substitution at codon 291 in exon 8 (L291P). A G to A transition was detected in the first nucle-

otide of intron 14 (1844+1G→A) in all affected members of the Druze family (figs 1 and 2). All affected subjects were homozygotes for this mutation. This mutation results in activation of an upstream cryptic splice site in exon 14 and deletion of 67 base pairs of the 3' end of exon 14^o and thus affects the glucose binding domain of the PYGM gene.⁴ The mutation is rare in patients of European ancestry, and has previously been observed only in the heterozygous state in an affected subject with the R49X mutation on the other chromosome.

To determine the haplotype on which the G to A substitution in the 5' splice site of intron 14 occurred, all family members were typed for 14 markers at or near the PYGM locus, including four proximal short arm makers (D11S1368, D11S1392, D11S905, D11S903) and 10 long arm markers (UT5150, D11S956, D11S480, D11S1783, PYGM (AT), PYGM (CA), INT2, D11S916, D11S787, D11S533), both proximal and distal to the PYGM locus (fig 1, table 2). This extended haplotype detected several current crossovers and at least one past recombination event in this family that led to shuffling of the mutation onto a second haplotype background. All affected subjects shared a haplotype (A: 1-3-4-7-3) at markers PYGM (CA) - INT2 - D11S916 - D11S587 - D11S533 on at least one chromosome. In one affected subject, BT942, a different haplotype at the same markers on the long arm of chromosome 11 (B: 1-3-3-3-5) segregated with the disease state. This haplotype differed from the A haplotype at the three most distal markers, D11S916, D11S787, and D11S533, and the most distal short arm marker D11S1368. This haplotype was also observed on the mutation bearing chromosome in two unaffected subjects, BT951 and BT950. The unaffected chromosome in these two subjects was inherited from the mother and bears a different haplotype (C: 7-3-1-3-5). Using the data from all the extended haplotypes in BT942's generation we were able to determine that the father of subject BT942, BT999, had two different haplotypes, A and B, with the same mutation. The two different haplotypes, A and B, were probably created owing to recombination events on either side of the mutant PYGM gene in previous generations. Since the extent of the conserved haplotype entering this kindred from D11S1392 to INT2 is at least 15 cM, it seems likely that the parents of BT999 were related within only a few generations. Furthermore, because several new recombination events in subjects BT937, BT953, BT942, BT951, and BT950 were found in the extended haplotype, it is suspected that the region of haplotype sharing will be reduced in succeeding generations.

Affected subjects in this family are the first patients with McArdle disease who are homozygous for this mutation. Variable clinical severity, comparable to that seen in patients with McArdle disease caused by other mutant genotypes, was observed in affected family members in this kindred, the exception being the fatal infantile form of the disease, which was not observed in members of this kindred.

Table 2 Haplotype information for the five most distal markers, PYGM(CA)-INT2-D11S916-D11S587-D11S533, on chromosome 11q13 for each subject in Druze kindred by affected status

ID No	Haplotypes for affected subjects			Haplotypes for unaffected subjects
	Mild disease	Moderate disease	Severe disease	
BT999			A/B	
BT949				A/other
BT937			A/A	
BT945		A/A		
BT938	A/A			Other/other
BT939				A/C
BT953				Other/other
BT954				
BT942		A/B		
BT946				Other/other
BT936		A/A		
BT951				B/C
BT950				B/C
BT952				Other/other
BT941	A/A			
BT944				A/other
BT940				A/other
BT947				A/other

In this table and in the text, A corresponds to haplotype (1-3-4-7-3), B corresponds to haplotype (1-3-3-3-5), and C corresponds to haplotype (7-3-1-3-5) at markers PYGM(CA)-INT2-D11S916-D11S587-D11S533 at 11q13. Other corresponds to any haplotype other than A, B, or C.

The phenotypic variability in these patients is illustrated by the clinical profile of two affected male sibs, BT936 and BT938, both of whom are homozygous (autozygous) for the same mutation and encompassing chromosomal segment. The former, BT936, had considerable exercise intolerance persisting into adulthood, while the latter, BT938, had a very mild form of the disease which did not significantly affect his life beyond childhood. Thus, despite all affected subjects being homozygous for the same rare mutation, and showing significant haplotype sharing from D11S1368 to D11S533, a genotype-phenotype correlation could not be established. This implies that the clinical variability in this family cannot be attributed to differences in regulatory elements or functional polymorphisms within the PYGM gene. In some diseases, like cystic fibrosis, clinical variability and lack of genotype-phenotype correlation have been attributed to differences in the chromosomal background,²¹ while in other diseases, like limb-girdle muscular dystrophy, differences in the mitochondrial background are thought to play a role.²² We propose that interactions with other genes or the mitochondrial background of the affected subject alter the severity of McArdle disease. Additionally, other factors such as the dysfunctional vitamin B6 metabolism²³ and environmental factors may play a significant role in the disease. As McAr-

dle disease is rare, this family is a valuable resource for studying the contribution of other genes or environment to the variability in phenotype of this disease.

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