

# Myotonic Dystrophy: Linkage with Apolipoprotein E and Estimation of the Gene Carrier Status with Genetic Markers

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**ABSTRACT:** The genes for myotonic dystrophy (MD) and for apolipoprotein E (ApoE) belong to a chromosome 19 syntenic group of markers. A familial linkage analysis between MD and ApoE was performed using the J Ott LIPED program (IBM PC/XT, April 1984) to estimate the genetic distance between these 2 genes. Of a total of 136 individuals in 11 MD families, 81 were confirmed to be affected by the disease and 41 were asymptomatic. ApoE phenotypes were determined in 115 of these 122 individuals. No recombinant was observed out of 74 meioses which were informative for both MD and the ApoE isoproteins. A global maximal lod score Z of 19.00 was obtained at the recombination fraction  $\Theta = 0$ . The upper  $\Theta$  value at the confidence interval corresponding to the peak lod score ( $Z_{max} - 1$ ) was 0.03. This suggests that the loci for MD and ApoE are at a distance of 0 to 0.03 Morgan. Since ApoE and apolipoprotein C2 (ApoC2) have been shown by others to be about 40 kb apart, our data are therefore consistent with the distance estimate of 0.02 Morgan reported between MD and ApoC2. The D19S19 (LDR152) polymorphic DNA sequence is also tightly linked to MD on chromosome 19. The segregation of ApoE isoproteins and of ApoC2 and D19S19 DNA polymorphism was utilized for evaluating the probability for individuals at risk of inheriting the disease gene in MD families. Data are presented on 3 families to emphasize the usefulness of genetic markers to estimate the MD gene carrier status of asymptomatic individuals and also for those presenting a partial syndrome. The limitations of such approach are also discussed.

**RÉSUMÉ: Dystrophie myotonique: linkage avec l'apolipoprotéine E et estimation du statut de porteur du gène à l'aide de marqueurs génétiques** Les gènes de la dystrophie myotonique (DM) et de l'apolipoprotéine E (ApoE) font partie d'un groupe synténique de marqueurs situés sur le chromosome 19. Une analyse de linkage familial entre DM et ApoE a été faite à l'aide du programme LIPED afin d'estimer la distance génétique entre ces deux gènes. D'un total de 136 individus parmi 11 familles, 81 ont été confirmés atteints par DM et 41 étaient asymptomatiques. Les phénotypes de l'ApoE ont été déterminés chez 115 de ces 122 individus. Aucun recombinant n'a été observé sur 74 méioses informatives pour DM et pour les isoprotéines de l'ApoE. Un "lod score" global maximal Z de 19.00 a été obtenu à la fraction de recombinaison  $\Theta = 0$ . La valeur  $\Theta$  supérieure correspondant à l'intervalle de confiance de  $Z_{max} - 1$  unité de "lod score" était de 0.03. Ceci suggère que les loci de DM et d'ApoE sont séparés par une distance de 0 à 0.03 Morgan. Comme les gènes de l'ApoE et de l'apolipoprotéine C2 (ApoC2) sont connus comme étant à environ 40 kb l'un de l'autre, nos données sont consistantes avec celles qui indiquent que la distance génétique entre DM et ApoC2 serait de 0.02 Morgan. La séquence d'ADN polymorphique au locus D19S19 (LDR152) est également étroitement liée à DM. La ségrégation des polymorphismes de la protéine ApoE et de l'ADN aux loci ApoC2 et D19S19 a été utilisée pour évaluer la probabilité que des individus à risque dans des familles DM héritent du gène de la maladie. Nous présentons des données sur 3 familles afin de démontrer l'utilité des marqueurs génétiques pour estimer le statut de porteur du gène DM chez des individus à risque asymptomatiques et aussi pour ceux présentant un syndrome partiel. Les limitations d'une telle approche sont également discutées.

*Can. J. Neurol. Sci. 1989; 16: 134-140*

The pleiotropic expressivity of MD has made difficult the identification of an abnormal gene product thus preventing direct analysis of the genetic mutation. Efforts have been made

to identify a polymorphic DNA marker system linked to the MD locus on chromosome 19. The genes for ApoE and ApoC2 have been mapped on the long arm of chromosome 19 (19 cen

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— 19q13.2).<sup>1-5</sup> Linkage analyses have indicated that the DM gene is at 0.02 Morgan from the ApoC2 gene<sup>6</sup> and that it is in close proximity (0% recombination) to the D19S19 (LDR152) genomic DNA polymorphic marker.<sup>7</sup> More recently, it has been shown that the genes for ApoE and ApoC2 span a physical distance of about 48 kb, both being approximately 40 kb apart.<sup>8</sup>

The prevalence of MD is at least 30 times higher among French Canadians in a northeastern area of the province of Québec than in European and American populations.<sup>9</sup> In order to define the genetic characteristics of affected families in this area, ApoE was used as a marker belonging to the syntenic MD linkage group. ApoE exhibits polymorphism at a single gene locus; 3 alleles code for 3 major isoproteins E2, E3, E4 and determine 6 ApoE phenotypes (Zannis et al in Moorjani<sup>11</sup>). Using ApoE polymorphism, we have estimated the distance separating ApoE and MD loci. The data were calculated from recombinants searched in informative meioses within 11 families. Other analyses on ApoE in MD families are presented in this issue.<sup>10,11</sup>

Data are also presented on MD families typed for ApoC2 and D19S19 DNA polymorphisms and in one occasion for the ApoE isoproteins to estimate the risk of asymptomatic individuals and of those presenting a partial syndrome of having inherited the MD gene.

#### MATERIAL AND METHODS

After having signed an informed consent form, the probands and key members of their family were involved in the present analyses. Clinical assessment was performed on individuals for which data were not yet available. Blood was drawn from each individual to determine ApoE phenotypes and ApoC2 and D19S19 DNA restriction fragment length polymorphisms (RFLPs).

#### Phenotyping of Apolipoprotein E

Phenotyping of apolipoprotein E (ApoE) was performed as described by Moorjani et al in this issue.<sup>11</sup> The Québec north-east superdeme of MD patients provided informative kindreds in a preliminary ApoE phenotype screening of individuals belonging to 40 out of 88 known MD affected families. Eleven informative pedigrees were retained for linkage analysis between ApoE and MD. This analysis was performed using the April 1984 version of the IBM/PC LIPED program (J Ott).

#### DNA Preparation from Frozen Blood

Total blood DNA was prepared from frozen venous blood. Briefly, 15 ml of thawed whole blood was diluted with 35 ml of cold TE Buffer (10 mM Tris, 10 mM EDTA) and centrifuged (3500 × g, 15 min) at 4°C. The pellet was washed in 50 ml of TE buffer. The white pellet was resuspended in 10 ml of TE and digested overnight with 150 µg/ml proteinase K and 0.4% SDS at 37°C. Proteins were removed by phenol/chloroform and chloroform/isoamyl alcohol extractions. DNA was then precipitated by addition of 2 volumes of 95% ethanol with ammonium acetate salt (0.3 M final) and spooled onto a glass rod. Ethanol precipitate was gently resuspended in TE buffer (pH 8.0). The yield of DNA ranged between 15 and 25 µg per ml of whole blood.

#### Endonuclease Digestion of Total Genomic DNA and Transfer to Nylon Membrane

Samples of extracted genomic DNA (10 µg per restriction enzyme) were digested to completion with an excess of the endonucleases *Taq* I, *Pst* I, *Nco* I, *Bgl* I and *Bgl* II. The digested DNA fragments were separated by an overnight electrophoresis in 1% agarose gels. After depurination of the gels, DNA fragments were transferred by Southern blotting to duPont Gene Screen Plus nylon membranes (GSP) using alkaline conditions.<sup>12</sup>

#### Preparation of Probes

cDNA APOC2 and genomic pSCII (ApoC2) fragments, subcloned in PUC 8, were supplied as recombinant bacteria,<sup>13,14</sup> and pSP152, subcloned in pSP64, as a plasmid.<sup>7</sup> *Escherichia coli* HB101 were transformed with this plasmid, using the calcium chloride procedure.<sup>15</sup> Cultures of *E. coli* were grown and plasmidic DNA was prepared by the alkaline lysis method. Plasmids were digested with the appropriate endonucleases and DNA fragments were separated by electrophoresis in agarose gels. Inserts were extracted from the gels using the quick freeze phenol method,<sup>16</sup> further purified by ethanol precipitation and utilized to probe genomic DNA. The Amersham Multiprime DNA labelling kit<sup>17</sup> was used to label the excised inserts to a specific activity of  $2 \times 10^9$  cpm/µg.

#### Hybridization of Restriction Fragments

The restriction fragments transferred onto GSP membranes were hybridized with the [<sup>32</sup>P]-labeled ApoC2-cDNA (*Taq* I and *Nco* I), pSC11 (*Bgl* I) and pSP152 (*Bgl* II, and *Pst* I) probes. The hybridization was performed at 42°C using the formamide procedure. Membranes were washed at high stringency (65°C and 0.1 X SSC, 0.1% SDS) and exposed to Kodak XAR-5 films for autoradiography. The size of the DNA fragments were estimated using the lambda phage DNA markers.

For the estimation of the MD gene carrier status, the phase of marker alleles was established by hand on MD and normal chromosomes following the segregation of alleles in at least two informative generations for each family.

#### RESULTS

#### Linkage between MD and ApoE

The data on ApoE protein polymorphism among MD patients and their relatives showed that 11 families segregated for both MD and ApoE (Table 1). The clinical Steinert disease status was diagnosed in 81 subjects, 41 were asymptomatic at the time of medical examination. ApoE phenotypes were determined for 115 of these 122 individuals. No recombinant was observed among 74 meioses informative for both MD and ApoE.

A global maximal lod score (Z) of 19.00 was obtained at the recombination fraction  $\Theta_m$  and  $\Theta_f$  of 0 (Table 2-a). The confidence interval at the minus 1 peak lod score gave the 0.03 upper value for  $\Theta$  (Table 2-b). The general (both sexes combined) lod score Z at recombination values  $\Theta$  of 0 to 0.4 are represented by the diagonal in Table 2 (a-b). The marginal scores are represented in italic in Table 2-a. Marginal lod scores were also calculated

ed according to the recommendations of Conneally et al<sup>18</sup> (Table 2-c).

**Estimation of the DM Gene Carrier Status with Genetic Markers**

The segregation of ApoE polymorphisms, of *Taq I*-, *Nco I*, *Bgl I* - ApoC2 or of *Pst I*, *Bgl II* - D19S19 RFLPs was analysed in 3 families of individuals at risk seeking genetic counselling. These data are presented together with the DM status in the pedigrees illustrated in Figures 1, 2 and 3.

In the 002 family (Figure 1), the mother (normal) was homozygous for each marker and transmitted to each of her children the haplotype 2-2-3 for markers D19S19, ApoC2 and ApoE respectively together with a normal allele (-) at the MD locus. The children who were affected received from their father the haplotype 2-2-4 with the MD gene (mutant allele +). The paternal 1-1-3 haplotype from the father's normal chromosome was transmitted to his normal children as well as to those at risk. This suggested that the siblings at risk did not inherit the father's MD mutant allele. Although the data suggest that neither of the uncles carried the MD gene since they did not have the 2-2-4 haplotype associated with the MD gene, comparison

**Table 1: Individuals Clinically Informative in 11 Families Segregating for MD and ApoE and Informative Meioses**

Family number*	Number of subjects	ApoE Typing	Diagnoses DM		Informative meioses	
			Normal	Normal	Male	Female
002	14	12	8	4	10	
005	21	17	11	8	12	
026	16	14	9	5		8
029	28	25	16	10	14	3
034	5	5	4	1	3	
045	9	7	6	3		3
053	12	9	6	4	6	
077	12	9	8	2	2	3
103	9	7	6	1	4	
146	5	5	4	1	3	
150	5	5	3	2		3
11	136	115	81	41	54	20

\*Including 1st generation parents (alive or deceased)

of the uncles haplotypes with that of their affected brother (2nd generation), as well as efforts to reconstitute the genotype of their parents (1st generation), indicate that male and female recombinations must have occurred at some point within the

**Table 2-a: Lod Scores Z at Male (m) and Female (f) Recombination Fraction  $\Theta = 0$  to 0.5**

	0.50	5.69	5.08	4.46	3.15	1.81	0.62	0
	0.40	8.10					2.71	1.83
$\Theta_m$	0.30	11.06			11.32	7.02		4.64
	0.20	13.91						7.43
	0.10	16.56		15.33				10.05
	0.05							11.28
	0.00	19.00		17.81	16.55	15.22	13.83	12.47
		0.00	0.05	0.10	0.20	0.30	0.40	0.50
		$\Theta_f$						

**Table 2-b: Lod Scores Z at Male (m) and Female (f) Recombination Fraction  $\Theta = 0$  to 0.05**

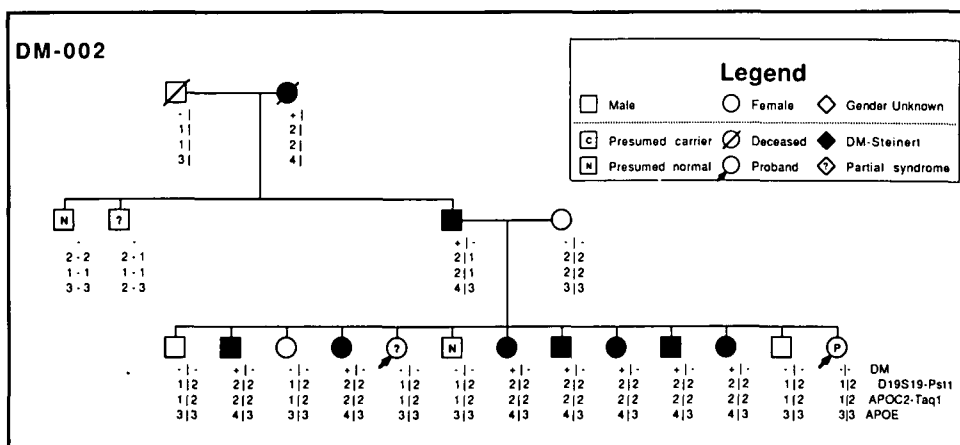
	0.05	17.80	17.68	17.57	17.45	17.33	17.21	
	0.04	18.05				17.57	17.45	
$\Theta_m$	0.03	18.29			17.93		17.70	
	0.02	18.53		18.29			17.94	
	0.01	18.77	18.65				18.18	
	0.00	19.00	18.89	18.77	18.65	18.53	18.41	
		0.00	0.01	0.02	0.03	0.04	0.05	
		$\Theta_f$						

**Table 2-c: Marginal Lod Scores  $Z(\Theta_m; \Theta_f \max)$  and  $Z(\Theta_f; \Theta_m \max)$ <sup>18</sup>**

	$\Theta$					
	0.00	0.05	0.10	0.20	0.30	0.40
$Z(\Theta_m; \Theta_f \max)^*$	13.31	12.11	10.87	8.22	5.37	2.41
$Z(\Theta_f; \Theta_m \max)^\dagger$	6.53	5.94	5.34	4.08	2.75	1.36

\* $Z(\Theta_m; \Theta_f \max = 0) = Z(\Theta_m, \Theta_f) - Z(\Theta_m = 0.5, \Theta_f \max = 0)$

† $Z(\Theta_f; \Theta_m \max = 0) = Z(\Theta_m, \Theta_f) - Z(\Theta_f = 0.5, \Theta_m \max = 0)$



**Figure 1 — Pedigree of family 002 typed for MD gene carrier estimation of at risk individuals with genetic polymorphic markers: ApoE isoproteins and ApoC2 *Taq I* and D19S19 *Pst I* DNA RFLPs using the cDNA ApoC2 and the genomic pSP152 probes. The 3 generations represented are referred to in the text: grandparents (1st generation), parents and uncles (2nd generation), children (3rd generation).**

D19S19, ApoC2 and ApoE cluster. Since the father's parents were not alive, these meioses could not be counted as recombinants in our linkage analyses between MD and ApoE. It does however indicate that with a larger sampling, recombinations between MD and ApoE (and other markers) in informative meioses should be observed.

Family 40 (Figure 2) was informative with ApoC2-Taq I for prediction of the MD gene carrier status of the proband. D19S19-Bgl II alleles are also represented to determine haplotypes. Comparison of the affected child genotype with those of his parents allowed the phasing of the alleles. From this information, it may be predicted that the proband was probably not a carrier of the MD gene. On the other hand, his affected mother, one aunt and three uncles all had the same 2-2 haplotype on the

MD chromosome (+) and the 2-1 haplotype on the normal chromosome (-) making possible the estimation of one chromosome haplotype from each of the grandparents. Although the clinically normal aunt and uncle of the proband had a different genotype from that of their affected siblings, they also shared with their siblings the allele 2 at each marker locus. Since the phase could not be determined in the case of these individuals because typing was not available for the grandparents, the question is now posed whether these persons are effectively noncarriers of the MD gene or asymptomatic carriers not expressing MD because of the variable penetrance of the disease.

In family 103 (Figure 3), the phase of marker alleles at the restriction sites ApoC2-Taq I, Nco I and Bgl I was established by comparing the genotype of the third generation affected child

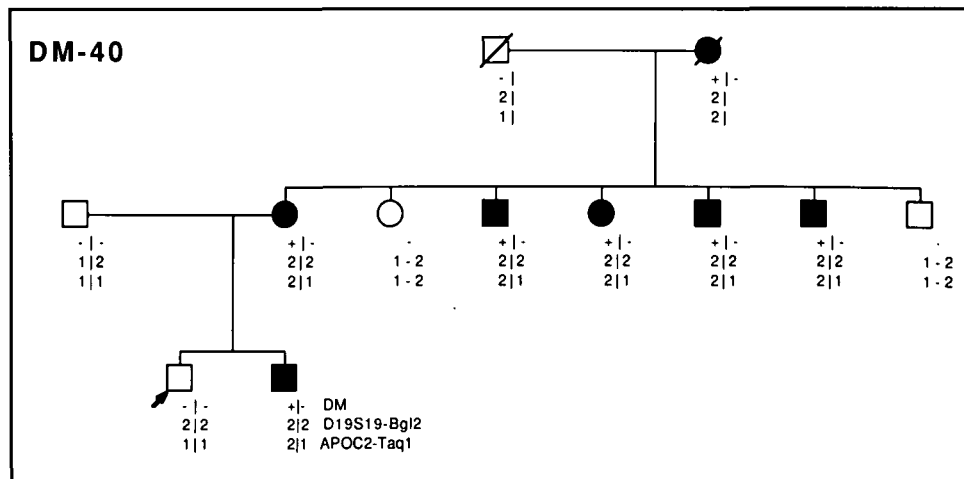


Figure 2 — Pedigree of family 40 typed for MD gene carrier estimation of at risk individuals with genetic polymorphic markers: ApoC2 Taq I and D19S19 Bgl II DNA RFLPs using the cDNA APOC2 and the genomic pSP152 probes. The 3 generations represented are referred to in the text. Refer to figure 1 for legend.

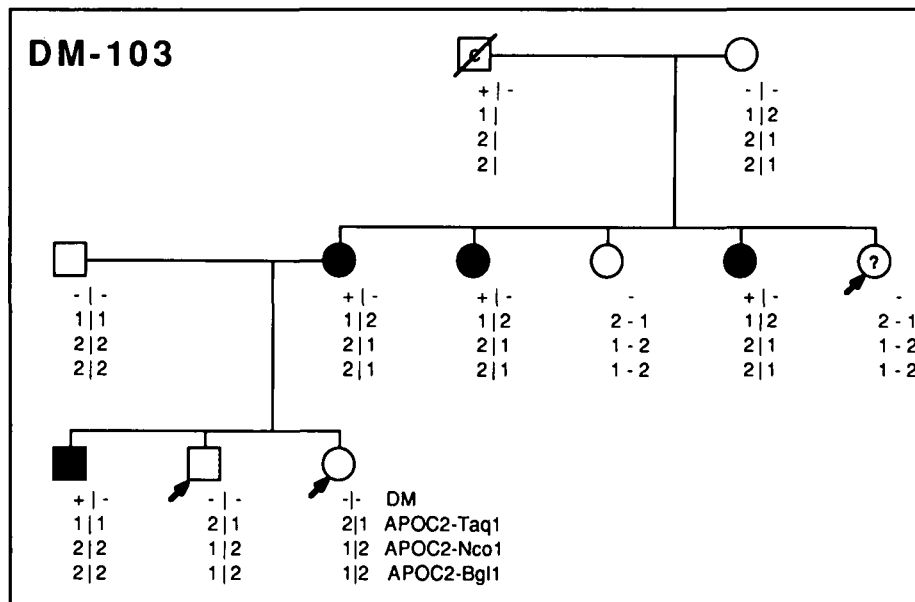


Figure 3 — Pedigree of family 103 typed for MD gene carrier estimation of at risk individuals with genetic polymorphic markers: ApoC2 Taq I, Nco I and Bgl I DNA RFLPs using the cDNA ApoC2 and the genomic pSC2 probes. The 3 generations represented are referred to in the text. Refer to figure 1 for legend.



with those of his parents. This was easily made since we had the ideal situation to phase the alleles with the MD and normal chromosomes. Indeed, the unaffected father was homozygous for the markers and the affected mother was heterozygous. Following the segregation of the alleles, it was then possible to estimate that the 2 other children consulting for genetic counselling were most probably noncarriers for the MD gene. Such prediction analysis was also requested by one aunt (2nd generation) who had a partial syndrome.<sup>20</sup> Earlier phasing of marker alleles in the affected mother allowed the haplotypes to be assigned to both chromosomes for her 2 affected sisters. Typing being available for the grandmother, she could be phased also and the haplotype associated with the MD chromosome (+) of the grandfather was predicted. Unfortunately, prediction of MD genotype with marker alleles could not be performed for this woman and also because of variable penetrance, there is a doubt as to whether the unaffected normal aunt was really a noncarrier of the gene. Indeed, all second generation sisters had exactly the same marker genotypes. Available information indicates that either the grandfather had exactly the same genotype as his wife with similar haplotypes on their 2 chromosomes and that then the women have a 50:50 chance of being noncarriers, or these 2 asymptomatic second generation women do carry the MD gene. ApoE markers will be performed in this family to supplement the available information.

### DISCUSSION

MD is a degenerative muscular disease inherited as an autosomal dominant trait with variable penetrance. As documented in this issue,<sup>19,20</sup> its phenotypic expression and age of onset are extremely variable. The first symptoms may become noticed at any age but they appear most commonly during adult life, after reproduction age has been reached. On the other hand, MD often occurs in the more severe congenital form amongst offspring inheriting the MD gene from their mother. If infants so affected survive, they manifest severe motor and mental handicaps.

Incomplete penetrance, and variations in the degree of severity and age of onset often make it difficult to determine clinically whether an individual at risk carries the MD mutant gene, particularly during the child-bearing years. Consequently, asymptomatic pregnant women carrying the MD gene can transmit the gene, encounter complications of pregnancy, and give birth to children having the congenital form of the disease.<sup>21</sup> For these reasons, individuals at risk, particularly those presenting early inconclusive signs,<sup>20</sup> desire to know their risk of carrying and transmitting the MD gene. In autosomal dominant mendelian traits such as MD, this risk is 50% of inheriting the mutant gene from the MD gene carrier parent. More precise risk estimates using linked genetic markers should be available for genetic counselling.

#### Genetic Markers Associated with the MD Gene

The primary defect in MD is unknown and there is no curative treatment for this disease. To date, there is no biochemical test than can either accurately identify gene carriers or be utilized for prenatal diagnosis. Thus, in order to better define the genetic characteristics of affected individuals in northeastern Québec, known markers or those suspected to be linked to MD

were evaluated in the informative phenotypes. ApoE appeared highly polymorphic in the population investigated in contrast to other synthetic polymorphic markers that were used. A linkage analysis between ApoE and MD was thus undertaken in informative families.

#### Linkage between MD and ApoE

The gene for MD has been assigned to a synthetic linkage group on chromosome 19. The genes for ApoE and ApoC2, which belong to this cluster, have been mapped on the long arm of the chromosome (19cen — 19q13.2),<sup>1-5</sup> and more recently, it has been shown that the genes for ApoE and ApoC2 span a physical distance of about 48 kb, both being approximately 40 kb apart.<sup>8</sup> Linkage analyses have indicated that the MD gene is at 0.02 Morgan from the ApoC2 gene<sup>6</sup> and very close (0% recombination) to the D19S19 (LDR152) genomic DNA polymorphic marker.<sup>7</sup>

In our linkage analysis between MD and ApoE, a global maximal lod score  $Z$  of 19.00 was obtained at the recombination fraction  $\Theta = 0$ . The upper  $\Theta$  value at the confidence interval corresponding to the peak lod score ( $Z$  max) — 1 was 0.03. This suggests that the loci for MD and ApoE are at a distance of 0 to 0.03 Morgan, which is in agreement with what could be predicted from other linkage data. Indeed, since ApoE and apolipoprotein C2 (ApoC2) have been shown to be about 40 kb apart, it indicates that our data are consistent with the distance estimate of 0.02 Morgan reported between MD and ApoC2.

As presented elsewhere in this symposium,<sup>10,11</sup> there is a significant linkage disequilibrium between the  $\epsilon 4$  allele of ApoE and MD among French Canadian families from the northeastern part of Québec. Furthermore, the ApoE locus is about as close to MD as the ApoC2 locus. Since ApoE gene is a 3 allele system with 6 haplotypes instead of the 2 allele system characterizing DNA markers, this makes it most suitable for the prediction of carriers in MD families. This is particularly so in MD families from northeastern Québec which present a disequilibrium between MD and  $\epsilon 4$ , an otherwise uncommon allele in the normal population and among normal relatives in MD families.

The prevalence of MD is much higher among French Canadians in northeastern Québec. Since demographic, historic and genealogical reconstructions suggest a large deme distribution of a MD 'founder' gene in this population, our linkage data and particularly the linkage disequilibrium, further support the notion of genetic homogeneity in patients diagnosed to date from 602 investigated individuals in 88 families.<sup>10</sup> The purpose is to find means of identifying MD carriers with appropriate informative probes. In this report, it is important to characterize this isolate since the approach of using MD-linked markers to predict the MD genotypes will be different in an isolate which evolved from founder(s) and in which genetic drifts have occurred. Thus far, the data support the theory of a large deme distribution of a founder gene, implying gene homogeneity for more than 600 patients in a population where the prevalence of MD is 40 to 80 times higher than elsewhere in the world.

#### Genetic Markers and the Prediction of MD Gene Carrier Status

Probes detecting DNA sequences closely linked to the MD locus on chromosome 19 can be used to predict the inheritance of MD. However, the degree of confidence will depend on the

distance between these loci on the chromosome. When 2 genes are close together on the same chromosome, they tend to be transmitted as a unit to the same gamete during meiosis, i.e. they do not segregate independently. Based on this characteristic, one can, from the typing of a polymorphic DNA sequence known to be localized near the MD gene locus, predict the inheritance of MD for an individual at risk by following the segregation of both the MD disease and polymorphism of the marker within the family.

Various probes detecting polymorphic DNA sequences have been produced and are still being validated. Among these, ApoC2 and D19S19 (LDR152) present the necessary characteristics which made them suitable for preclinical testing of MD. Enough data are available to faithfully provide a probability estimate of the predictive value for both probes. Frequent RFLPs can now be detected from fragmented DNA with probes hybridizing with the ApoC2 gene.<sup>22</sup> The locus of ApoC2 is only at 2cM of the MD gene.<sup>6</sup> This marker, which can be used in carrier detection and prenatal diagnosis with an accuracy of 96 to 98%, is most useful, since its frequent RFLPs make it informative in many cases. Its use in preclinical testing and prenatal diagnosis of MD has been reported.<sup>23-26</sup> A more recent probe, D19S19, detects DNA sequences very close to the MD gene.<sup>7</sup> It gives a prediction value approaching 100% and detects also frequent RFLPs with the *Bgl* II endonuclease.<sup>27</sup>

Theoretically, as mentioned above, an offspring's risk of inheriting the gene of an autosomal genetic disease is 50%. When no further information is available, this figure is used for genetic counselling, taking into account the fact that the penetrance is not complete, that the expression and age of onset is variable, and also that children having the severe congenital form are frequently born from MD gene carrier mothers. With the typing from the ApoC2 and D19S19 probes, a risk estimate of 96 to almost 100% of inheriting or not inheriting the MD gene can be obtained. Before using linked RFLPs in counselling and prenatal testing in MD families, the MD clinical status of the family members must be carefully determined. As shown in our pedigrees, typing with linked RFLPs strongly depends on family structure. It also relies on the occurrence of heterozygosity for these DNA sequences in each given family.

Although markers are useful, they are not always informative. This means that one will often encounter difficulties in gathering sufficient information in the family of a proband to estimate its carrier status. Three families have deliberately been presented to illustrate the limitations of such analyses. Markers are known to be quite polymorphic but sometimes (e.g., Figure 3) the same haplotypes may be observed in both parents. On the other hand, since linkage disequilibrium between restriction sites at one locus is strong, the markers are often either not informative at all or all-informative. Ideally, phasing of the alleles in the case of dominant diseases such as MD requires that the affected parent be heterozygous for the markers and the normal parent be preferentially homozygous.

The family structure should also be optimal. At least 2 clinically informative generations should be available. The first living generation should consist of the parents of one affected individual. The siblings can be useful in the second generation, but not in the first. Analyses are more complicated when only one parent of the proband is alive but at times might be done in

cases where a third clinically informative generation is available to establish phases. When the requirements are not met, not only is information difficult to obtain, but analysis even creates doubt regarding the MD gene carrier status of normal (asymptomatic) individuals. Efforts must therefore be made to obtain informative data, extending the pedigrees to key members of the family and using other markers known to recognize heterozygosity. Also, respecting ethical considerations and with the individual's approval, it is usually important to store DNA samples of family members for future counselling requests.

The probands requesting for ApoC2, D19S19 and ApoE typing in our laboratory are mostly children born to affected parents who wish to know their likelihood of carrying the MD gene. Typing could eventually be offered to mildly affected or MD carrier women for prenatal diagnosis. When indicated, fetal DNA can be examined directly for DNA polymorphism when the parents are already known as ideally informative for the markers (i.e. an homozygous normal father and an heterozygous MD gene carrier mother).

#### ADDENDUM

Recent results from Ropers' group (University of Nijmegen, Netherlands) and Korneluk's group (Ottawa) indicated that presumably all currently existing markers are proximal of MD. Bases on both physical and genetic evidence the gene order should now read pJSB6,pJSB11-pLDR152-APOE-APOC2-CKMM-DM-pEFD4.2, CKMM being at 3-4 cM from MD and 0 cM from APOC2 (personal communications).

#### ACKNOWLEDGEMENTS

We would like to thank Drs. Ola Myklebost and Steve Humphries for providing us with the ApoC2 cDNA and the pSCII (ApoC2) subclones as well as Drs. Allen R. Roses and Richard J. Bartlett for the pSP152 (D19S19) subclone. Pedigrees were drawn using the version 4.0 of the Macintosh Pedigree/Draw program kindly provided by Dr. Jean W. MacCluer. We are grateful to the families who participated in the project and to the personnel of the Chicoutimi Muscular Dystrophy Clinic, of the Lipid Disease Research Center from our institution and of the Quebec Genetic Medicine Network who were involved. This work was supported by the Muscular Dystrophy Association of Canada. M.C. Thibault is a senior scholar of the «Fonds de la Recherche en Santé du Québec».

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