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Linkage Analysis of the Apolipoprotein C2 Gene and Myotonic Dystrophy on Human Chromosome 19 Reveals Linkage Disequilibrium in a French-Canadian Population

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Summary

The gene for human apolipoprotein C2 (APOC2), situated on the proximal long arm of chromosome 19, is closely linked to the gene for the most common form of adult muscular dystrophy, myotonic dystrophy (DM). Six APOC2 RFLPs (*Taq*I, *Bgl*I, *Ban*I, *Bam*HI, *Nco*I, and *Ava*II) have been identified to date. We have conducted a comprehensive DM linkage study utilizing all six RFLPs and involving 50 families and 372 individuals. The most informative RFLPs are, in descending order, *Nco*I (lod = 6.64, θ = 0.05), *Bgl*I (lod = 6.12, θ = 0.05), *Ava*II (lod = 6.02, θ = 0.03), *Ban*I (lod = 5.76, θ = 0.04), *Taq*I (lod = 4.29, θ = 0.06), and *Bam*HI (lod = 1.75, θ = 0.01). A substantial increase in the lod scores over those seen with the individual RFLPs was obtained when the linkage of the entire APOC2 haplotype (composed of the six RFLPs) was studied (lod = 17.87, θ = 0.04).

We have observed significant inter-APOC2 RFLP linkage disequilibrium. Consequently, the three most informative RFLPs have been found to be *Ban*I, *Taq*I, and either *Bgl*I, *Ava*II, or *Nco*I polymorphisms.

We also demonstrate linkage disequilibrium between DM and APOC2 in our French-Canadian population (standardized disequilibrium constant ϕ = .22, χ^2 = 5.12, df = 1, $P < 0.04$). This represents the first evidence of linkage disequilibrium between APOC2 and the DM locus.

Introduction

The gene for human apolipoprotein C2 (APOC2) is situated on the proximal long arm of chromosome 19 (Hulsebos et al. 1985; Lusis et al. 1985). Recently, APOC2 has been identified as a closely linked probe for the most common form of adult muscular dystrophy, myotonic dystrophy (DM; Shaw et al. 1985). The linkage between APOC2 and the DM gene is of sufficient degree (lod = 23.9, θ = 0.04; Naylor et al. 1985) to allow the accurate antenatal and presymptomatic diagnosis of DM (Lunt et al. 1986, 1987). Largely as a result of this linkage, a search for RFLPs in the APOC2 region has been conducted; to date, a total of six have been identified (Humphries et al. 1983; Wallis et al. 1984; Appleby et al. 1986; Korneluk et al. 1987). Given that APOC2 is used for the molecular genetic diagnosis of myotonic dystrophy and that studies utilizing all six RFLPs would be time-consuming, the identification of the most informative polymorphisms is of considerable importance. We present here the first report to utilize all six RFLPs in a comprehensive DM linkage study; this work involved 50 families and 372 individuals. On the basis of the degree of polymorphism heterozygosity and inter-RFLP linkage disequilibrium, the three most informative RFLPs have been identified.

An earlier report of linkage disequilibrium between an apolipoprotein E biochemical polymorphism and the DM locus in a French-Canadian population (Laberge et al. 1985) prompted a search for a similar phenomenon in our study group. Evidence for linkage disequilibrium between DM and APOC2 in our French-Canadian population was observed, representing the first demonstration of linkage disequilibrium between APOC2 and the DM locus. The implications of the presence of linkage disequilibrium in the search for the DM gene are discussed.

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Material and Methods

Families

A total of 50 families with myotonic dystrophy were studied; of these, 24 were French-Canadian and most of the remaining 22 were either Scottish-Canadian or Anglo-Canadian (in four families we were unable to assign the linguistic origin). The origin of the DM for a given family (i.e., French-Canadian vs. non-French-Canadian) was determined by examination of the clinical chart and, when necessary, interviews with the appropriate family members. The presence or absence of DM was evaluated clinically in all cases. As well, electromyography was performed on the majority of individuals at risk for carrying the gene, regardless of their clinical appearance. These studies were frequently complemented by an ophthalmologic examination to rule out the presence of characteristic eye signs (Harper 1979) is ostensibly unaffected individuals.

DNA Analysis

DNA was extracted from either peripheral blood samples or lymphoblast cultures by using a modification of the method of Madisen et al. (1987). Appropriate amounts (approximately 5 μg) of DNA were digested with restriction enzymes according to manufacturers' specifications (Amersham and BRL) and run on 0.8% agarose gels (Ultrapure;® BRL); blotted on nylon membranes Hybond-N;® Amersham), and probed. DNA probes were prepared by the oligolabeling method of Feinberg and Vogelstein (1983) with α^{32}P dCTP (>3,000 Ci/mmol; Amersham). Blots were hybridized with radiolabeled probe in 6 × SSC, 5 × Denhardt's solution and 100 μg salmon sperm DNA/ml at 65 C for at least 12 h. The final wash of the hybridized blots consisted of 0.2 × SSC – 0.1% SDS at 65 C for approximately 20 min. Washed blots were exposed to X-ray film (Kodak X-OMAT AR) at – 70 C for 1–4 days. Dupont Lightning Plus® screen intensifiers were used.

A total of six APOC2 RFLPs were screened; *Taq*I, 3.8/3.5 kb (Humphries et al. 1983); *Bgl*I, 12/9 kb (Wallis et al. 1984); *Ban*I, 2.5/1.6 kb; *Bam*HI, 6/4.9 kb (Frossard et al. 1985); *Nco*I, 14.5/11.5 kb (Frossard et al. 1986); and *Ava*II, 0.6/0.4 kb (Korneluk et al. 1987). The *Bgl*I RFLP was detected with the genomic probe pSC11 (Wallis et al. 1984), a gift of S. E. Humphries. The remaining RFLPs were detected with a full-length cDNA APOC2 probe, kindly provided by F. E. Baralle (Sharpe et al. 1984).

Linkage Analysis

Lod scores were determined using LIPED with age-at-onset capability (Ott 1974) and employing a straight line approximation of Harper's DM age-at-onset data (Harper 1979). The APOC2 RFLP population allelic frequencies used in the program were calculated from the total number of haplotypes studied (189 in all), which included both the myotonic and nonmyotonic chromosomes. The 145 nonmyotonic haplotypes were obtained both from married-in members of the various families and from unaffected chromosomes from individuals with DM. The DM gene frequency used in the program was taken as 1/10,000, the lowest frequency permitted by LIPED and close to the published value of 1.3/10,000 (Todorov et al. 1970).

The presence or absence of a polymorphic restriction-enzyme site at a given locus is designated by a plus sign (+) or a minus sign (–), respectively. Similarly, for the DM locus, the (+) allele represents the presence of the DM defect while the (–) allele indicates its absence.

Statistical Analysis

Statistically significant differences in allelic frequencies were detected by calculating the normal deviate z for two given frequencies P_a and P_b as follows:

$$z = \frac{P_a - P_b}{[P_c \cdot (1-P_c) \cdot (1/n_a + 1/n_b)]^{1/2}}, \quad (1)$$

where

$$P_c = \frac{n_a P_a + n_b P_b}{n_a + n_b}, \quad (2)$$

and n_a and n_b are the number of "a" and "b" allele chromosomes, respectively (Lapin 1980).

The presence of linkage disequilibrium was ascertained by means of the χ^2 test for independence of variables. Specifically, the number of each haplotype (i.e., ++, +–, –+, or ––) observed for the two loci in question (i.e., the DM locus and the APOC2 RFLP) was compared with the number expected given the allelic frequency and assuming total linkage equilibrium. Linkage disequilibrium was also quantified by means of the standardized disequilibrium statistic ϕ, where

$$\phi = \frac{n_1 \cdot n_4 - n_2 \cdot n_3}{[(n_1 + n_2) \cdot (n_3 + n_4) \cdot (n_1 + n_3) \cdot (n_2 + n_4)]^{1/2}} \quad (3)$$

and n_1–n_4 are the numbers of ++, +–, –+ and ––

haplotypes, respectively (Hill and Robertson 1968). The parameter ϕ is a correlation coefficient which equals 0 when the two loci in question are in complete equilibrium and which equals +1 or −1 when there is total linkage disequilibrium (positive when there is an excess of ++ and −− haplotypes and negative when the +− and −+ haplotypes are overrepresented). As outlined by Rao (1965, pp. 362–363) and utilized by Chakravarti et al. (1984), if $\phi = \tanh z$, then $(n-3)^{1/2}z$ is a unit normal deviate for the null hypothesis $\phi = 0$ (where n is the sample size). The probability of linkage disequilibrium for a given ϕ can thus be estimated.

Results

Allelic Frequencies

The non-DM chromosomal allelic frequencies for the six APOC2 RFLPs shown in table 1 are in good agreement with those of past studies (Wallis et al. 1984; Williams et al. 1985; Appleby et al. 1986; Bird et al. 1987; Korneluk et al. 1987). The allelic frequencies for the DM chromosomes are only slightly different from those for the non-DM chromosomes. However, when the DM chromosomes were subdivided into French-Canadian and non-French-Canadian categories, significant differences emerged. The largest difference is seen in the *Nco*I, *Bgl*I, and *Ava*II polymorphic sites, which are present in 25% of the French-Canadian DM chromosomes ($n = 24$) compared with 70% of the non-French-Canadian DM chromosomes ($n = 20$, $P < .01$). A statistically significant difference is also seen in the frequencies of the *Taq*I polymorphism ($P < .02$; table 1). These differences in allelic frequencies between the two language groups are peculiar to the DM chromosomes and are not seen in the non-DM chromosomes.

Perhaps of greater importance is the fact that for four of the six RFLPs there are statistically significant differences in allelic frequencies between the non-DM and DM chromosomes within the French-Canadian group. As shown in table 1, the allelic frequency of the *Taq*I polymorphic site in the non-DM group is .44 ($n = 86$); for the DM chromosomes this frequency is .71 ($n = 24$, $P < .03$). The *Nco*I, *Bgl*I, and *Ava*II polymorphic frequencies are .49 and .25 for the DM and non-DM chromosomes, respectively ($P < .03$). Within the non-French-Canadian group, differences between the non-DM and DM chromosomes for the *Nco*I, *Bgl*I, and *Ava*II polymorphism allelic frequencies are also observed. However, none of these differences are statistically significant.

The *Bam*HI site is so rarely absent as to preclude

Table 1

Allelic Frequencies of the APOC2 RFLPs (+ Allele)

	Non-DM		DM	
	French-Canadian ($n = 86$)	Non-French-Canadian ($n = 59$)	French-Canadian ($n = 24$)	Non-French-Canadian ($n = 20$)
*Nco*I	.49[a]	.47	.25[a,b]	.70[b]
*Bgl*I	.49[c]	.47	.25[c,d]	.70[d]
*Ava*II	.49[e]	.47	.25[e,f]	.70[f]
*Taq*I	.44[g]	.49	.71[g,h]	.35[h]
*Ban*I	.24	.27	.21	.20
*Bam*HI	1.00	.98	1.00	.95

NOTE.—Differences between the French-Canadian and non-French-Canadian DM allelic frequencies are statistically significant for the *Nco*I, *Bgl*I, *Ava*II, and *Taq*I polymorphisms, as are the differences between the non-DM and DM French-Canadian chromosome allelic frequencies for the same polymorphisms (see significance values in footnotes below). No other statistically significant differences were observed.

[a] $P < .03$.
[b] $P < .003$.
[c] $P < .03$.
[d] $P < .003$.
[e] $P < .03$.
[f] $P < .003$.
[g] $P < .03$
[h] $P < .02$.

Table 2

Number of Observed APOC2 RFLP Haplotypes for All DM and Non-DM Chromosomes Studied

Haplotypes	Total (n = 189)	Non-DM (n = 145)	DM (n = 44)
NcoI/BglI/AvaII:			
+ + ...	89	69	20
− − −	97	73	24
Others	3	3	0
NcoI/BglI/AvaII/TaqI:			
+ + + −	87	68	19
− − − + ...	88	65	23
Others	14	12	2

meaningful statistical analysis with respect to this polymorphism.

Intra-APOC2 Linkage Disequilibrium

Profound linkage disequilibrium between three of the six RFLPs is seen, with 98% of the *NcoI/BglI/AvaII* haplotypes being represented by the complementary +++ or −−− haplotypes (table 2). This polymorphism cluster is in only slightly weaker disequilibrium with the *TaqI* polymorphism: *NcoI/BglII/AvaII/TaqI* +++− and −−−+ haplotypes represented 92% of the total number of observed haplotypes (table 2). The *NcoI/BglI/AvaII/TaqI* array is in moderate linkage disequilibrium with the *BanI* site. This is reflected in the magnitude of the disequilibrium constants (ϕ) for the four pairings between *BanI* and the *NcoI*, *BglI*, *AvaII*, and *TaqI* sites. For all four combinations we calculate ϕ values of approximately $\pm.57$.

This pattern of linkage disequilibrium between the APOC2 RFLPs is seen in all categories—DM and non-DM, French-Canadian and non-French-Canadian. A more in-depth analysis of the disequilibrium pattern seen within the APOC2 gene region will be the subject of a future communication.

Linkage Analysis

Unequivocal identification of the APOC2 RFLP haplotype for the DM chromosome could be made in all but one of the 50 families studied; hence, linkage analysis was performed on a total of 49 families. In addition to lod scores for the six individual APOC2 RFLPs, a lod score for the entire APOC2 haplotype was obtained. The APOC2 haplotype composed of the six RFLPs was ascertained for each family member; this compound marker was then analyzed for linkage with DM. This approach is justifiable as the polymorphic sites are all quite close to one another and no inter-polymorphic recombinations were observed in our data.

Table 3 shows lod scores for each of the six polymorphisms studied. The most informative RFLP was *NcoI*, which showed a lod score of 6.64 at a recombination fraction of .05. The low degree of heterozygosity at the *BamHI* site is reflected in the low lod scores for this polymorphism (lod = 1.75, θ = .01). The relatively wide range of maximum recombination fractions for the six RFLPs (.01–.06) is a result of varying numbers of informative meioses and recombinant events being detected by the different polymorphisms. Four recom-

Table 3

Lod Scores Characterizing the Relationship between the DM Locus and APOC2 RFLPs

	Lod Scores for Individual APOC2 RFLPs						Lod Score for
θ	*NcoI*	*BglI*	*AvaII*	*TaqI*	*BanI*	*BamHI*	Compound Haplotype
.01	5.981	5.349	5.826	3.443	5.503	*1.754*	16.752
.02	6.400	5.800	5.986	3.908	5.694	1.722	17.535
.03	6.658	5.998	*6.020*	4.121	5.753	1.691	17.809
.04	6.631	6.089	6.001	4.229	*5.755*	1.658	*17.869*
.05	*6.635*	*6.120*	5.952	4.280	5.724	1.626	17.809
.06	6.602	6.112	5.884	*4.294*	5.671	1.594	17.670
.07	6.542	6.076	5.801	4.281	5.601	1.561	17.476
.08	6.461	6.018	5.708	4.248	5.520	1.528	17.239
.09	6.365	5.944	5.606	4.201	5.428	1.528	16.968
.10	6.255	5.856	5.497	4.141	5.328	1.462	16.670

Note.—Numbers in italics are maximum lod scores.

binant meioses were observed among the 104 informative meioses studied, yielding a $\theta \approx .04$, in agreement with earlier studies (Naylor et al. 1985). The maximum lod score for the DM-APOC2 compound haplotype relationship was 17.87 at a recombination fraction of .04.

Linkage Disequilibrium between APOC2 and DM

Evidence of linkage disequilibrium between four of the APOC2 RFLPs (*Nco*I, *Bgl*I, *Ava*II, and *Taq*I) and the DM locus was observed in the French-Canadian population (table 4). In contrast, the *Ban*I polymorphism appears to be in equilibrium with DM within the French-Canadian group ($\phi = .03$, $\chi^2 = .46$, df = 1). While no evidence for statistically significant linkage disequilibrium between DM and the APOC2 within the non-French-Canadian group was observed, differences bordering on statistical significance were recorded for the same four RFLPs (table 4). As with the French-Canadian population, the *Ban*I polymorphism appeared to be in equilibrium with the disease locus for the non-French-Canadian population.

Given that the large majority (92%) of the *Nco*I/*Bgl*I/*Ava*II/*Taq*I haplotypes are represented by the complementary haplotypes +++− and −−−+, an analysis for an association between this haplotype and both the French-Canadian and the non-French-Canadian DM loci was conducted. A nonrandom association between the DM locus and the *Nco*I/*Bgl*I/*Ava*II/*Taq*I −−−+ haplotype in the French-Canadian population and between the disease locus and the *Nco*I/*Bgl*I/*Ava*II/*Taq*I +++− haplotype in the non-French-Canadian population was observed ($\chi^2 = 7.56$, $n = 42$, df = 1, $P < .01$; table 5).

Table 4

ϕ and χ^2 Values Characterizing the Five DM Locus–APOC2 RFLP Pairings for French-Canadian and Non-French-Canadian Groups

	French-Canadian ($n = 110$)		Non-French-Canadian ($n = 79$)	
	ϕ	χ^2	ϕ	χ^2
*Nco*I	−.20[a]	4.31[b]	.19	3.08
*Bgl*I	−.20[a]	4.31[b]	.19	3.08
*Ava*II	−.21[a]	4.66[b]	.19	3.08
*Taq*I	.22[a]	5.13[b]	.12	1.23
*Ban*I	.03	.46	.07	.43

NOTE.—Evidence for linkage disequilibrium between four of the five polymorphisms (*Nco*I, *Bgl*I, *Ava*II, and *Taq*I) and DM in the French-Canadian group is seen (see significance values in footnotes below). No other statistically significant values were observed.

[a] $P < .04$.

[b] $P < .04$.

Table 5

Number of APOC2 *Nco*I/*Bgl*I/*Ava*II/*Taq*I RFLP Haplotypes Observed on the DM Chromosomes for the French-Canadian and Non-French-Canadian Study Populations

Nco/*Bgl*/*Ava*/*Taq* Haplotypes	French-Canadian	Non-French-Canadian
+++−	6 (10.4)	13 (8.5)
−−−+	17 (12.7)	6 (10.4)

NOTE.—The numbers in parentheses represent the expected number of haplotypes in association with the DM chromosomes for the two linguistic groups. This was calculated by using the frequencies of the haplotypes in the 42 DM chromosomes showing this type of haplotype and by assuming their random distribution between the linguistic groups. $\chi^2 = 7.56$, $P < .01$, df = 1 for independence of these haplotypes and the French-Canadian and non-French-Canadian DM loci.

Discussion

Allelic Frequencies

The frequencies of the six APOC2 polymorphic enzyme sites in both non-French-Canadian and French-Canadian non-DM chromosomes were all comparable with previously reported values (Wallis et al. 1984; Williams et al. 1985; Appleby et al. 1986; Bird et al. 1987; Korneluk et al. 1987). The APOC2 RFLP allelic frequencies for the DM chromosomes taken as a whole show no pronounced difference from those seen for the non-DM chromosomes. However, when the DM chromosomes were categorized on the basis of language groups and compared with one another, statistically significant differences emerged for four of the six RFLPs (*Bgl*I, *Ava*II, *Nco*I, and *Taq*I). Furthermore, within the French-Canadian group there were statistically significant differences between the non-DM and DM groups for the same four polymorphisms. A probable explanation for these observations would be the presence of a founder effect, as has been suggested for the French-Canadian population of a region in eastern Quebec (Laberge at al. 1985; Veillette et al. 1986; see discussion below).

For the *Bgl*I, *Ava*II, *Nco*I, and *Taq*I polymorphisms within the non-French-Canadian population, considerable allelic-frequency differences between the non-DM

and DM chromosomes were also observed. However, these differences are not statistically significant.

Implications for RFLP Diagnosis

As in the past studies (Wallis et al. 1984; MacLeod et al. 1987), significant linkage disequilibrium between RFLPs within the APOC2 gene region was observed. This has practical implications for the genetic diagnosis of DM. The disequilibrium between the *Bgl*I, *Ava*II, and *Nco*I polymorphisms is so profound (table 2 and data to be presented elsewhere) that only in exceptional cases does more than one of these polymorphisms need to be sought. *Taq*I is also in disequilibrium with the *Bgl*I, *Ava*II, and *Nco*I cluster (table 2 and unpublished data). However, the analysis of the *Taq*I RFLP reveals a slight increase in APOC2 heterozygosity, and consequently we have found it to be a useful polymorphism in our diagnostic linkage studies. Furthermore, given the low frequency of the rare *Bam*HI (−) allele (2%), performance of this RFLP analysis does not appear justified. Thus, of the six polymorphisms discussed in this work, only three appear worth analyzing for DM linkage on a routine basis: *Ban*I, *Taq*I, and either *Bgl*I, *Ava*II, or *Nco*I. Analysis of these combinations of RFLPs (i.e., *Nco*I/*Taq*I/*Ban*I, *Bgl*I/*Taq*I/*Ban*I, or *Ava*II/*Taq*I/*Ban*I) as compound haplotypes yields PIC values of .64 (Botstein et al. 1980).

Two RFLPs can be analyzed with only a modest decrease in informativeness: *Ban*I and either the *Bgl*I, *Ava*II, or *Nco*I RFLPs have a PIC value of .59, and the *Ban*I-*Taq*I RFLP pairing has a PIC value of .58.

Linkage between APOC2 and DM

The lod scores obtained for the six APOC2 RFLPs were all less than 7—numbers that appear to be low given the 50 families studied. This is largely due to the small size of the families, a reflection of the fact that many come to our attention through individuals requesting genetic counseling. While the lod scores are disproportionately low with small family sizes, a relatively large sampling of myotonic haplotypes is obtained, something that is of value when assaying the presence of linkage disequilibrium.

The compound haplotype was consistently more informative than the individual markers. This is reflected in a lod score for the compound haplotype (17.87, $\theta = .04$) that showed a marked increase over that for the individual RFLPs (maximum lod score of 6.64 for the *Nco*I RFLP, $\theta = 0.05$). The 90% confidence interval for compound haplotype lod score ($.01 < \theta < .10$) contains the maximum θ's for all the individual RFLPs with the exception of *Bam*HI ($\theta_{max} = .01$). The *Bam*I lod score of 1.75, however, is reflective of only a relatively small number of informative meioses and is not truly representative of the linkage of APOC2 to the DM locus.

Linkage Disequilibrium between APOC2 and DM

Evidence for linkage disequilibrium between the *Bgl*I, *Ava*II, *Nco*I and *Taq*I polymorphisms and the DM locus was observed within the French-Canadian group. Such a disequilibrium might be predicted if there were a founder effect, as has been suggested for the French-Canadian population of the Saguenay/Lac Saint-Jean region of eastern Quebec (Laberge et al. 1985; Veillette et al. 1986). Certainly the low mutation rate of DM is compatible with the existence of such a phenomenon (Harper 1979). Evidence for a French-Canadian founder effect is seen both in the very high prevalence of DM in this region (162/100,000 vs. 5/100,000 seen elsewhere; Veillette et al. 1986) and in the presence of unequivocal linkage disequilibrium between biochemical polymorphisms of apolipoprotein E (APOE) and DM in this population (Laberge et al. 1985).

APOC2, which is quite close to APOE (approximately 48 kb; Smit et al. 1988), appears to be the closer of the two apolipoproteins to DM on the basis of recombinant and linkage patterns (Brunner et al. 1987; Sarfarazi et al. 1987). Consequently, if the French-Canadian population of the present study is similar to that of the Saguenay region, even stronger linkage disequilibrium than that seen for APOE would be expected. This pattern was not seen, the disequilibrium for APOE-DM appearing more pronounced than that seen for APOC2-DM. One explanation for this apparent discrepancy would be that the population of the present study and that of the Saguenay region are not equivalent. The majority of the French-Canadian families studied in the present report reside in the Outaouais region of western Quebec, almost 700 km distance from the Saguenay region. This distance would allow some dilution of the "Saguenay DM" by DM of non-Saguenay origin to take place in the French-Canadian population of the Outaouais, leaving the disequilibrium between APOC2 and DM attenuated but intact. However, it should be noted that the APOE study is based solely on data obtained from nine families. Until a larger sample of the Saguenay French-Canadian population is studied, comparisons of the degree of linkage disequilibrium seen between APOE and the DM locus with that seen between APOC2 and DM must be made with caution.

While no statistically significant linkage disequilibrium was seen between the APOC2 RFLPs and DM for the non-French-Canadian study group, there existed a degree of disequilibrium which was close to statistical significance. This is most readily appreciated when one notes the considerable differences between the APOC2 allelic frequencies of the DM haplotypes for the two linguistic groups (table 1). This somewhat surprising finding would seem to point to the presence of a founder effect in our non-French-Canadian population, who are primarily of Scottish- and Anglo-Canadian origin.

Thus, there exists a DM-*Nco*I/*Bgl*I/*Ava*II/*Taq*I $---+$ haplotype association in our French-Canadian population. In contrast, there is a tendency toward a DM-*Nco*I/*Bgl*I/*Ava*II/*Taq*I $+++-$ haplotype association in the non-French-Canadian population. Consequently, it would appear that the original DM mutations for the two linguistic groups were distinct events. The French-Canadian mutation occurred on a chromosome with a *Nco*I/*Bgl*I/*Ava*II/*Taq*I $---+$ haplotype, and the Scottish-/Anglo-Canadian mutation occurred on a *Nco*I/*Bgl*I/*Ava*II/*Taq*I $+++-$ chromosome.

The elucidation of founder effects, both in the French-Canadian and, perhaps, in the Scottish-/Anglo-Canadian population with the associated linkage disequilibrium for closely linked markers, is of more than just academic interest. There exists potential for clinical benefit from such linkage disequilibrium. With moderate disequilibrium, such as that reported here, alterations in genetic risk for members of pedigrees in which the APOC2 RFLPs are noninformative would not be feasible. However, as probes that are closer to and show greater degrees of linkage disequilibrium with the DM locus are isolated, it will theoretically be possible to estimate altered genetic risks for such individuals on the basis of the haplotype of the DM chromosome alone. A recent example of such an approach is the haplotype analysis with DNA markers closely linked to cystic fibrosis (Farall et al. 1987).

As well, the closer to the DM gene that a given probe is situated, the fewer will be the number of recombinant events between it and the DM locus that are seen. Consequently, the rate of recombination will be of limited value in the precise localization of the disease gene. Even if a marker is so close to the DM locus that the chance of observing a recombinant event is remote, there is still a fair likelihood that one has taken place at some point in the past. If some of these past recombinant events occurred after the time of the original DM mutation, it would result in a decrease in the degree of linkage disequilibrium between the marker and the DM gene. It is clear that only when a marker is very close to the disease locus will the degree of linkage disequilibrium approach 100%. Consequently, by measuring the degree of linkage disequilibrium in these populations for new and purportedly closer probes, the search for the DM gene can be advanced.

As a case in point, we have recently identified a chromosome 19 DNA probe derived from a chromosome translocation, t(14;19), which is tightly linked to DM (lod = 10.95, θ = .0; Korneluk et al., in press). Consistent with this tight linkage, in our French-Canadian study group (ϕ = .23) this probe shows a disequilibrium with the DM locus which is of similar absolute magnitude to that seen with APOC2 (ϕ = .22). We would expect to see even greater linkage disequilibrium in our French-Canadian study population as new and closer DM markers are identified.

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