Am. J. Hum. Genet. 59:445–452, 1996

Founder Effect and the Prevalence of Myotonic Dystrophy in South Africans: Molecular Studies

Andrea Goldman, Amanda Krause, Michele Ramsay, and Trefor Jenkins

Department of Human Genetics, School of Pathology, The South African Institute for Medical Research and University of the Witwatersrand, Johannesburg

Summary

A high prevalence of myotonic dystrophy (DM) has been described in South African Caucasoid Afrikaans-speaking families in the northern Transvaal. Evidence is presented for a strong founder effect, with a single haplotype occurring on 68% of all Caucasoid DM chromosomes; among the Afrikaans speakers, the proportion was 83%. In addition to this major haplotype, five minor DM haplotypes in the Caucasoids and two minor haplotypes in DM individuals of mixed ancestry were found. All DM chromosomes, however, had a common haplotype core, namely, *Alu* (ins), *Hin*fI-2 (intron 9), and *Taq*I-2 (D19S463). We have detected significant linkage disequilibrium between the DM mutation and particular alleles of the extragenic markers D19S112 and D19S207. Significant differences were found in allele and haplotype distributions in the Caucasoid DM and non-DM chromosomes and Negroid non-DM chromosomes. These findings together with the strong association of allele 3 at the D19S63 locus on 93% (14/15) of the South African DM chromosomes suggest that the majority of present-day DM mutations in South African Caucasoids may have originated from a common initial founder who introduced one of the European ancestral mutations.

Introduction

Myotonic dystrophy (DM) is one of several disorders known to be caused by expansion of a trinucleotide repeat. An unstable CTG-repeat DNA sequence occurs in the 3′ noncoding region of the myotonin protein kinase gene, located on chromosome 19q13.3 (Aslanidis et al. 1992; Brook et al. 1992*a*; Buxton et al. 1992; Fu et al. 1992; Harley et al. 1992; Mahadevan et al. 1992). This mutation is thought to account for >99% of DM cases with no other mutation type having been described. DM occurs with an average prevalence of 2.4–5.5/100,000 in European populations (Harper 1989). The disorder has been described in many countries worldwide, including Japan, China, India (Harper 1989), South Africa (Caucasoid families only) (Lotz and van der Meyden 1985), Nigeria, and also in black American families (Harper 1989; Ashizawa and Epstein 1991).

The epidemiology of DM in southern Africa is interesting: no single proven DM case has been described in an indigenous southern African Negroid or Khoisan individual (Lotz and van der Meyden 1985; Ashizawa and Epstein 1991). Both populations were found to have significantly fewer large-repeat-length alleles than do European Caucasoid and Japanese populations, which may, in part, explain the absence of DM in them (Goldman et al. 1994, 1996). The prevalence of the disease in the Indian population or in people of mixed ancestry in South Africa is still uncertain, although a number of cases have been diagnosed. A high prevalence of DM has been described in South African Caucasoid Afrikaans-speaking families in the northern Transvaal, with a minimum prevalence rate of 14.3/100,000 (Lotz and van der Meyden 1985). Both the geographical distribution of families and a previous genealogical study in the area suggested that a founder effect is the likely explanation for this high prevalence of DM.

Haplotype and mutation studies are useful in attempting to understand the mechanisms that lead to the high frequency of genetic disorders in populations. Intragenic as well as extragenic markers were used to examine patterns of allelic association with respect to the DM mutation, and haplotypes were constructed to determine whether a founder chromosome could be identified in the Caucasoid DM families. Haplotypes were constructed for non-DM Negroid families in an attempt to explain the apparent absence of DM in this population.

Subjects and Methods

Subjects

A total of 19 South African Caucasoid DM families of European origin (12 Afrikaans-speaking and 7 English-speaking families, where the surname was used as an

Received August 23, 1995; accepted for publication May 3, 1996.
Address for correspondence and reprints: Andrea Goldman, Department of Human Genetics, SAIMR, P.O. Box 1038, Johannesburg, South Africa. E-mail: 058agm@chiron.wits.ac.za

0002-9297/96/5902-0022$02.00

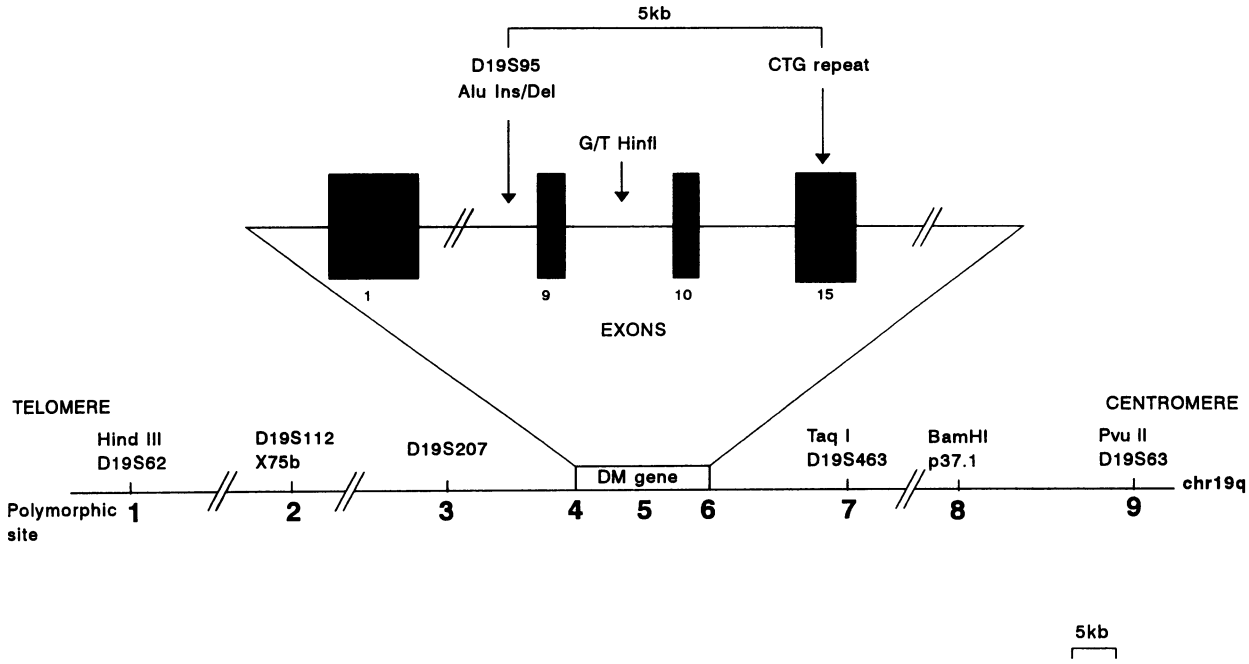

Figure 1 Schematic map of genomic DNA showing the relative positions of the intragenic and extragenic polymorphisms associated with the DM locus. The approximate distances between the DM gene and D19S112, D19S62, and D19S63 are 90 kb, 280 kb, and 140 kb, respectively (Jansen et al. 1992; Brook et al. 1992*b*; Crow et al. 1992).

indication of the linguistic origins), 1 Indian DM family (Hindu-Gujerati origin) and 3 DM families of mixed ancestry (often referred to as "Coloured") were investigated. It was difficult to determine the precise geographical origin of the families since they were referred from many different sources. If the DM mutation is passed through the maternal line, the true origin in terms of language affiliation may be obscured. The clinical diagnosis of DM was confirmed in all cases by the presence of the triplet repeat expansion (10 families previously reported by Goldman et al. [1995*a*]). Non-DM chromosomes from unrelated individuals in 23 DM and 4 non-DM families constitute the source of Caucasoid non-DM haplotypes. In addition, haplotypes were determined in seven non-DM South African Negroid families (with no known Caucasoid admixture).

DNA Extraction

Genomic DNA was extracted from peripheral blood using a salting-out procedure (Miller et al. 1988).

Haplotype Analysis

Nine polymorphisms comprising three intragenic and six extragenic markers were typed in Caucasoid (DM and non-DM), Indian and mixed-ancestry DM chromosomes and Negroid non-DM chromosomes. Their positions are shown in figure 1, and details of each marker are presented in table 1.

PCR Analysis

The conditions used for PCR are as described elsewhere (see table 1 for references). The *Taq*I polymorphism (D19S463) was detected as previously described by Neville et al. (1994), except that PCR conditions were 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min for 10 cycles; followed by 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min for 20 cycles. Two $(AC)_n$ systems were used: D19S112 (Jansen et al. 1992) and D19S207 (Jansen et al. 1993). The PCR reaction was performed in a 25-μl reaction volume containing 100 ng genomic DNA; 10 mmol Tris-HCl, pH 8.8; 50 mmol KCl; 1.5 mmol $MgCl_2$; 0.1% Triton-X; 125 μmol of dATP, dTTP, and dGTP; 2.5 μmol of dCTP; 50 ng of each primer; 20–30 nCi (α-^{32}P) dCTP; and 2 U *Taq* DNA polymerase. The PCR conditions for the D19S112 system were 94°C for 48 s, 57°C for 48 s, and 72°C for 48 s for 25 cycles, and a final extension of 10 min at 72°C, carried out using a Perkin Elmer Cetus DNA thermal cycler. The reaction conditions for D19S207 were the same as described above, with the exception of an annealing temperature of 68°C. It was difficult to size and distinguish alleles 1–5 reliably, but allele 6 could confidently be sized. For the purpose of this study, alleles 1–5 are pooled and are referred to as allele 1.

Southern Blotting

The DM expansion was detected using Southern blotting with *Bgl*I- and *Eco*RI-digested DNA and hybridized

Table 1

DM-Associated DNA Polymorphisms Used in Haplotype Analysis

Marker	Locus	Method of Detection (S/P)[a]	Alleles	Allele Size	Reference
1	D19S62	pD8/*Hin*dIII (S)	1, 2	4.8, 2.5 kb	Brook et al. (1990*a*)
2	D19S112 (X75b)	QH128 and QH129 (P)	0–13	92–118 bp	Jansen et al. (1992); Imbert et al. (1993)[b]
3	D19S207	207(a) and 207(b) (P)	1–6	157–135 bp	Jansen et al. (1993)
4	D19S95	p5B1.4/*Eco*RI (S)	I, D	10.0, 9.0 kb	Shelbourne et al. (1992)
		pBB0.7/*Pvu*II (S)	I, D	4.0, 5.0 kb	Crow et al. (1992)
5	Intron 9	Hh71 and Hh72 (P), *Hin*fI	1, 2	434, 354 bp	Mahadevan et al. (1993*a*); Goldman et al. (1995*b*)[b]
6	CTG	DM101 and DM102 (P)	5–29	92–140 bp	Brook et al. (1992*a*)
7	D19S463	564 and 565 (P), *Taq*I	1, 2	676, 574 bp	Neville et al. (1994)
8	p37.1	p37.1/*Bam*HI (S)	1, 2	5.6, 5.3 kb	Yamagata et al. (1992)
9	D19S63	pD10/*Pvu*II (S)	1, 2, 3	6.0, 5.6, 5.4 kb	Brook et al. (1990*b*)

[a] S = Southern blotting, and P = PCR.
[b] Primer sequences described in these references.

with p5B1.4 (Shelbourne et al. 1992). The *Eco*RI/p5B1.4 system, in addition to detecting the DM expansion, was also used to detect the *Alu* insertion/deletion polymorphism (D19S95) (marker 4) (Harley et al. 1992; Mahadevan et al. 1993*b*). The presence of the *Alu* insertion/deletion polymorphism (marker 4) was detected by probing *Pvu*II-digested DNA with pBB0.7 in patients with the expansion. Methods were performed as described elsewhere (see references in table 1) but with minor modifications.

Statistical Analysis

Statistical significance was determined using the χ^2 test, with Yates's correction where necessary.

Results

Allele Association on Non-DM and DM Chromosomes

The allele frequencies in non-DM Caucasoid and Negroid populations as well as those in the Caucasoid DM families are given in table 2. The allele frequencies for Indian and mixed-ancestry chromosomes are not presented because of the small sample sizes. Non-DM allele frequencies did not differ significantly between the South African Caucasoids and Negroids, possibly because of the small Negroid sample size. They were, with a few exceptions, similar to previously reported allele frequencies in other populations (Harley et al. 1991; Buxton et al. 1992; Yamagata et al. 1992; Neville et al. 1994). Both the South African Caucasoid and Negroid allele frequencies differed significantly from those in the Japanese ($P < .01$; and $P < .05$, respectively) for markers 8 and 9 (Yamagata et al. 1992). The allele frequencies of the South African Negroids also differed significantly from the Spanish population at marker 9 ($P < .01$) (Cobo et al. 1992).

A new allele was found in South African Negroids at the D19S112 locus. The newly reported allele is 4 bp larger than the reported allele 11 (142 bp) and is designated allele 13 (146 bp) (see table 3).

The DM expansion mutation in the South African Caucasoid, Indian, and mixed-ancestry patients is in complete linkage disequilibrium with the *Alu* (ins), *Hin*fI-2, and *Taq*I-2 (D19S463) alleles. We detected significant differences between the allele frequencies at extragenic markers D19S112, D19S207, and D19S63 when comparing DM and non-DM chromosomes (tables 2 and 3).

Haplotypes

Haplotypes were constructed using nine polymorphic sites for DM chromosomes (table 4) and non-DM Caucasoid and Negroid chromosomes (table 5).

DM Chromosomes

One major haplotype (haplotype I) occurred on 68% (13/19) of all Caucasoid DM chromosomes and 83% (10/12) of the Afrikaans-speaking families. In addition to this major haplotype, five (haplotypes II–VI) minor Caucasoid DM haplotypes were found, and one haplotype (haplotype VII) occurred on one Indian chromosome and two chromosomes from DM individuals of mixed ancestry. One incomplete haplotype on a mixed-ancestry DM chromosome was consistent with Caucasoid haplotype VI (table 4). The four common Caucasoid non-DM haplotypes were not found in the DM population.

Non-DM Chromosomes

Three major Caucasoid haplotypes were observed (haplotypes 12, 23, and 29) (table 5). These haplotypes account for 44% of chromosomes in the non-DM Cau-

Table 2

Allele Frequencies on DM and non-DM Chromosomes in South African Populations

		Caucasoid Chromosomes						Negroid Chromosomes (Non-DM)	
		DM		Non-DM		DM versus Non-DM			
Marker[a]	Allele	*n*	Frequency	*n*	Frequency	χ^2	*P*	*n*	Frequency
1	1	1	.06	20	.37	4.2	.040	7	.23
	2	15	.94	34	.63			23	.77
3	1–5	1	.08	57	.88	30.19	$<10^{-6}$	29	.94
	6	11	.91	8	.12			2	.06
4	I[*Alu*(ins)]	17	1.0	51	.53	11.36	.0008	22	.71
	D[*Alu*(del)]	0	.0	45	.47			9	.29
5	1	0	.0	39	.49	11.19	.0008	16	.5
	2	16	1.0	41	.51			16	.5
7	1	0	.0	44	.49	10.69	.001	10	.31
	2	15	1.0	46	.51			22	.69
8	1	3	.19	14	.21	.033	.856	6	.19
	2	13	.81	54	.79			25	.81
9	1	1	.07	35	.56	31.53	$<10^{-6}$	16	.52
	2	0	.0	16	.26			13	.42
	3	14	.93	11	.18			2	.06

[a] Numbers refer to markers referred to in table 1. Marker 2 allele frequencies are presented in table 3.

casoid population ($N = 62$). Most of the remaining 19 haplotypes occurred infrequently. Similarly, in the Negroid population ($N = 26$), three common haplotypes (haplotypes 12, 14, and 29) account for 42% of chromosomes and most of the remaining 13 haplotypes occurred infrequently. The common haplotypes have a range of alleles at the hypervariable D19S112 and CTG loci associated with them, probably because of recent slippage events. The divergent non-DM haplotypes are the result of multiple recombinations that have produced random association between the alleles over a long period of time. In a total of 26 non-DM Negroid

Table 3

Number (Frequency) of Chromosomes Observed for Each Allele for D19S112

	Caucasoid Chromosomes				Negroid Chromosomes (Non-DM)
	SA Caucasoid		European[a]		
Allele	Non-DM	DM	Non-DM	DM	
0	15 (.17)	2 (.13)	57 (.15)	5 (.05)	1 (.03)
1	...	...	3 (.01)	...	...
2	...	...	3 (.01)	...	2 (.06)
3	8 (.09)	...	29 (.07)	...	4 (.13)
4	3 (.03)	...	21 (.05)	7 (.07)	2 (.06)
5	11 (.12)	3 (.20)	66 (.17)	35 (.35)	3 (.09)
6	21 (.23)	10 (.67)	76 (.19)	44 (.44)	8 (.25)
7	13 (.12)	...	49 (.13)	6 (.06)	7 (.22)
8	14 (.16)	...	55 (.14)	3 (.03)	2 (.06)
9	4 (.05)	...	22 (.06)	1 (.01)	...
10	...	...	10 (.03)	...	2 (.06)
11	1 (.01)	...	1 (.01)	...	...
13	...	...	...	...	1 (.03)
Total	90	15	392	101	32

[a] Jansen et al. (1992).

Table 4

DM-Associated Haplotypes in South African Populations

	Polymorphic Sites[a]									No. of Chromosomes	
Haplotype	1	2	3	4	5	6	7	8	9	Caucasoid	Other
I	2	6	6	I	2	+	2	2	3	13[b]	...
II	2	5	6	I	2	+	2	1	3	2	...
III	2	0	6	I	2	+	2	1	3	1	...
IV	2	5	6	I	2	+	2	2	1	1	...
V	1	0	6	I	2	+	2	2	3	1	...
VI			1	I	2	+	2			1	1[c]
VII	1	5	6	I	2	+	2	1	3	...	3[d]
Total										19	4

[a] Numbers refer to markers referred to in table 1.

[b] Three haplotypes are incomplete because of inability to determine phase, but are consistent with haplotype I. This figure comprises 10 Afrikaans-speaking families and 3 English-speaking families.

[c] A family of mixed ancestry.

[d] Comprises two families of mixed ancestry and one family of Asiatic Indian origin.

chromosomes, none of the complete (seven site) DM-associated Caucasoid haplotypes were found. Analysis of partial haplotypes, however, show a number of Negroid non-DM haplotypes (19; 6, 10, 12, 14, and 18) which may be consistent with two DM-associated haplotypes (IV and VI, respectively). No conclusion can, however, be drawn from these incomplete findings.

Discussion

This is the first molecular evidence for a DM founder effect in South African families. The South African finding of one predominant DM haplotype (haplotype I) suggests that, with the colonization from Europe, one of the European DM chromosomes was introduced into South Africa and increased in frequency as a result of a local founder effect. The DM haplotype I was rarely found in the non-DM Caucasoid population, probably because the chromosome was introduced into this population associated with the DM expansion. Non-DM Negroid families were also investigated to assess whether a "predisposing" DM haplotype exists in a population where DM has never been described. None of the DM-associated Caucasoid haplotypes was found. If DM mutations arise on a predisposing haplotype, that may explain the paucity of DM in the Negroid population. In Africa, DM has been confirmed in only one Nigerian family, and the DM mutation is not associated with the common European haplotype (Krahe et al. 1995). Although the Nigerian DM haplotype (*Alu*(del)–*Hin*fI-1–*Taq*I-1) is also found on South African Negroid non-DM chromosomes, it does not necessarily suggest that this is a predisposing haplotype. Krahe et al. (1995) suggest rather that it is an independent mutation event in this Nigerian family.

It is difficult to compare the South African haplotypes with haplotypes in other populations studied, because different researchers have used different markers. It is apparent, however, that all South African DM chromosomes have the same common European core haplotype (*Alu*(ins)–*Hin*fI-2–*Taq*I-2) (Mahadevan et al. 1993*b*; Neville et al. 1994; Lavedan et al. 1994). This core haplotype is also frequently found on non-DM chromosomes, suggesting that one or more ancestral DM mutations may have occurred on this haplotype. Only three intragenic markers were studied in the South African population, since strong linkage disequilibrium has been previously observed between the intragenic DM markers (Neville et al. 1994). Extragenic markers were, therefore, used to provide more information regarding the number of mutations and the origin of the South African founder haplotype.

An unexpected finding showed that a common European DM haplotype (haplotype IV), associated with allele 1 of the D19S63/*Pvu*II locus, is rare, occurring only once (7%). The European haplotype associated with allele 3 accounts for 93% of South African DM haplotypes, compared with 18% of non-DM chromosomes. This is different from the findings in the other populations, namely, British and a French Canadian subpopulation (Harley et al. 1991), Spanish (Cobo et al. 1992), Japanese (Yamagata et al. 1992), and French (Lavedan et al. 1994). In all of the above-mentioned populations, a large proportion of DM chromosomes are associated with allele 1—32%, 45%, 43%, and 45%, respectively—and allele 3—59%, 48%, 43%, and 37% of

Table 5

Haplotype Distribution of non-DM Caucasoid and Negroid Chromosomes

Haplotype Number	Polymorphic Sites[a]							No. of Chromosomes	
	1	3	4	5	7	8	9	Caucasoid	Negroid
1	1	1	I	1	2	2	2		1
2	1	1	I	1	2	2	3		1
3	1	1	I	2	2	1	1	1	
4	1	6	I	2	2	1	1	1	
5	1	6	I	2	2	1	3	1	
6	1	1	I	2	2	2	1	1	1
7	1	1	I	2	2	2	2	4	
8	2	1	I	1	1	2	1		1
9	2	1	I	1	2	2	2		2
10	2	1	I	2	2	1	1		1
11	2	1	I	2	2	1	3	2	
12	2	1	I	2	2	2	1	8[b]	4
13	2	6	I	2	2	2	1	1	
14	2	1	I	2	2	2	2	4[b]	4
15	2	1	I	2	2	2	3	2	
16	2	1	I	2	2		3	2	
17	2	6	I	2	2		3	2	
18		1	I	2	2	2	1	2	2
19		6	I	2	2	2	1		1
20		1	I	2	2	2	2	1	
21	1	1	D	1	2	1	2		1
22	1	1	D	1	1	1	3	1	
23	1	1	D	1	1	2	1	8	
24	1	1	D	1	1	2	2	3[b]	
25	1	1	D	1	1	2	3	1	
26	2	1	D	1	1	1	1	2	1
27	2	1	D	1	1	1	2		1
28	2	1	D	1	1	1	3	1	
29	2	1	D	1	1	2	1	11[b]	3[b]
30	2	1	D	1	1	2	2	3	
31	2	1	D	1	1	2	3		1
32	2	1	D	1	1	2			1
Total								62	26

[a] Marker 2 (D19S112) and marker 6 (CTG) have been excluded from haplotype construction, and details for marker 2 are shown in table 3.

[b] An incomplete but compatible haplotype has been included.

DM chromosomes, respectively. The finding of almost equal proportions of DM chromosomes associated with alleles 1 and 3 in other populations suggests that a relatively old recombination/mutation event may have been responsible. In the present study, only 1 of 19 South African Caucasoid DM chromosomes was associated with allele 1. These differences are all significant at the 5% level, except the comparison with the French, where $P < .001$.

Further evidence for a founder effect can be drawn from the analysis of D19S112, where the allele association found in South African DM families was different from that previously described. Eighty-seven percent of South African DM chromosomes were associated with alleles 5 and 6, compared with 35% of non-DM chromosomes associated with these two alleles (table 3). It should be noted that 67% of South African DM chromosomes were associated with allele 6 alone, versus only 44% of European DM chromosomes, although the South African sample was small (Jansen et al. 1992).

Founder effect appears to have played a role in increasing the frequencies of deleterious mutant alleles in the Afrikaans-speaking Caucasoid population. Familial hypercholesterolemia merits singling out because molecular studies have revealed three founder mutations associated with different haplotypes, as opposed to the single DM-associated haplotype (haplotype I) (Jenkins et al. 1980; Kotze et al. 1989; Leitersdorf et al. 1989). Of the 19 Caucasoid DM families reported in this study, 12 (63%) were Afrikaans speaking. The genetic pool of the

Afrikaners is small, as there were a small number of "founders." The Dutch East India Company founded a settlement at the southern point of Africa (the Cape of Good Hope) in 1652. The total "white" population had reached 359 by 1678—composed mainly of Dutch and German immigrants. In 1688, 200 French Huguenots arrived, mainly as stable family groups. The settlers established unusually large families, and this trend persisted for several generations (Ross 1975). In this way, an extraordinary increase in the number of genetically related descendants in the total population took place. If one or two of the original "founders" had possessed the DM allele, it is not inconceivable that the gene could be present at the high frequency reported by Lotz and van der Meyden (1985).

Genealogical evidence supports the hypothesized founder effect: Lotz and van der Meyden (1985) reported that the family of Petrus Jacobus du Toit dominates the DM genealogical scene in the northern Transvaal. They were able to follow his descendants through five to seven generations to the present. Four of du Toit's affected children supply many of the surnames found to occur commonly in DM families. These data, together with the finding of one common DM haplotype (haplotype I), provide support for a single major European founder DM chromosome that was possibly introduced by this family. This would also explain the high prevalence of DM in South African Caucasoids, particularly those whose mother tongue is Afrikaans.

The rarer DM haplotypes, II–VII, share a common core of alleles for markers 3–7, which span a total of 44 kb. Two exceptions exist for marker 3, where one Caucasoid and one mixed-ancestry chromosome are not associated with allele 6 (haplotype VI). The deviation from the common haplotype, haplotype I, occurs with the markers furthermost from the DM mutation, markers 1 and 2 (~280 kb and ~90 kb telomeric from the gene, respectively) and markers 8 and 9 (~120 kb and ~140 kb centromeric from the DM gene, respectively) (see fig. 1). Subsequent mutation or recombinational events may have lead to the association of this core DM haplotype with a few extragenic alleles. Alternatively, the finding of six minor DM haplotypes suggests that new mutations may have occurred on either a predisposing haplotype or a haplotype with large predisposing CTG alleles. It is interesting to note that two non-DM chromosomes (haplotypes 5 and 13) have been identified with haplotypes similar to DM haplotypes VII and IV, respectively. Large normal sized $(CTG)_{>19}$ alleles (29 and 20 repeats, respectively) occur on both of these non-DM haplotypes, consistent with the proposal of either predisposing alleles and/or a predisposing haplotype.

One minor DM haplotype (haplotype VII) was found in two families of mixed ancestry as well as one family of Indian origin. Apart from a different allele at marker 1, this haplotype is the same as haplotype II, found in two of the Caucasoid families. This chromosome could have been introduced into these families by gene flow from the Caucasoid population if a recombination event occurred at marker 1 (280 kb from the DM locus). The event would have occurred in the maximum time span of 342 years, the time since peoples of European origin established the first settlement in the Cape. Alternatively, if haplotype VII has an Indian origin, the chromosome would have been introduced to South Africa only subsequent to the "Indian Diaspora," which included the immigration of Asiatic Indians to South Africa as recently as 1860 (Bhana and Brain 1990). Another minor DM haplotype (haplotype VI) occurs in one Caucasoid family and one family of mixed ancestry. This chromosome differs from the other DM chromosomes at marker 3, which is situated 15 kb from the DM gene. A recombination event therefore appears to be unlikely. The one Caucasoid family with haplotype VI is an English-speaking family with a Scottish name. This haplotype could thus have a recent European origin. It will be interesting to see whether this haplotype will be observed in the United Kingdom.

Acknowledgments

We are grateful to Dr. Helen Harley for the probes D19S63, D19S62, p37.1, and pBB0.7 and for the PCR primers used for detection of the Intron 9/*Hin*fI polymorphism. We thank Kenneth Kidd for the gift of the *Taq*I PCR primers 564 and 565 and Keith Johnson for the probe p5B1.4. We thank the patients and their families for their cooperation. These families had been referred to us via the Genetic Counselling Clinic run by our department at the Transvaal Memorial Institute (Johannesburg) as well as by the Departments of Human Genetics at the Universities of Pretoria and Stellenbosch and by the Genetic Sisters of the Department of National Health and Population Development. We are most grateful to the South African Muscular Dystrophy Association for assistance in contacting families. The South African Medical Research Council, The South African Institute for Medical Research, and the Iris Ellen Hodges Cardio-Vascular Research Trust, University of the Witwatersrand, provided financial support.

References

Ashizawa T, Epstein HF (1991) Ethnic distribution of myotonic dystrophy gene. Lancet 338:642–643

Aslanidis C, Jansen G, Amemiya C, Shutler G, Mahadevan M, Tsilfidis C, Chen C, et al (1992) Cloning of the essential myotonic dystrophy region and mapping of the putative defect. Nature 355:548–551

Bhana S, Brain JB (1990) Setting down roots: Indian migrants in South Africa, 1860–1911. Wits University Press, Johannesburg

Brook JD, Harley HG, Rundle SA, Walsh KV, Shaw DJ

(1990*a*) RFLP for a DNA clone which maps to 19q13.2-19qter (D19S63). Nucleic Acids Res 18:1085

Brook JD, McCurrach ME, Harley HG, Buckler AJ, Church D, Aburtani H, Hunter K, et al (1992*a*) Molecular basis of myotonic dystrophy: expansion of a trinucleotide (CTG) repeat at the 3′ end of a transcript encoding a protein family member. Cell 68:799–808

Brook JD, Walsh KV, Harley HG, Rundle SA, Shaw DJ (1990*b*) RFLP for a DNA clone which maps to 19q13.2-19qter (D19S62). Nucleic Acids Res 18:1086

Brook JD, Zemelman BV, Hadingham K, Siciliano MJ, Crow S, Harley HG, Rundle SA, et al (1992*b*) Radiation-reduced hybrids for the myotonic dystrophy locus. Genomics 13: 243–250

Buxton J, Shelbourne P, Davies J, Jones C, Van Tongeren T, Aslanidis C, de Jong P, et al (1992) Detection of an unstable fragment of DNA specific to individuals with myotonic dystrophy. Nature 355:547–548

Cobo A, Grinberg D, Balcells S, Vilageliu L, Gonzalez-Duarte R, Baiget M (1992) Linkage disequilibrium detected between myotonic dystrophy and the anonymous marker D19S63 in the Spanish population. Hum Genet 89:287–291

Crow SR, Harley HG, Brook JD, Rundle SA, Shaw DJ (1992) Insertion/deletion polymorphism at D19S95 associated with the myotonic dystrophy CTG repeat. Hum Mol Genet 1:451

Fu YH, Pizzuti A, Fenwick RJ Jr, King J, Rajnarayan S, Dunne PW, Dubel J, et al (1992) An unstable triplet repeat in a gene related to myotonic muscular dystrophy. Science 255: 1256–1258

Goldman A, Ramsay M, Jenkins T (1994) Absence of myotonic dystrophy in southern African Negroids is associated with a significantly lower number of CTG trinucleotide repeats. J Med Genet 31:37–40

——— (1995*a*) Molecular analysis of the CTG trinucleotide repeat in South African myotonic dystrophy families—implications for diagnosis and counselling. S Afr Med J 85: 1161–1164

——— (1995*b*) New founder haplotypes at the myotonic dystrophy locus in southern Africa. Am J Hum Genet 56:1373–1378

——— (1996) Ethnicity and myotonic dystrophy: a possible explanation for its absence in sub-Saharan Africa. Ann Hum Genet 60:57–65

Harley HG, Brook JD, Floyd J, Rundle SA, Crow S, Walsh KV, Thibault M-C, et al (1991) Detection of linkage disequilibrium between the myotonic dystrophy locus and a new polymorphic DNA marker. Am J Hum Genet 49:68–75

Harley HG, Brook JD, Rundle SA, Crow S, Reardon W, Buckler AJ, Harper PS, et al (1992) Expansion of an unstable DNA region and phenotypic variation in myotonic dystrophy. Nature 355:545–546

Harper PS (1989) Myotonic dystrophy, 2d ed. WB Saunders, London and Philadelphia

Imbert G, Kretz C, Johnson K, Mandel J-L (1993) Origin of the expansion mutation in myotonic dystrophy. Nat Genet 4:72–76

Jansen G, Coerwinkel-Driessen M, Nillesen W, Brunner H, Wieringa B (1993) Dinucleotide repeat polymorphism at locus D19S207, close to the myotonic dystrophy (DM) gene. Hum Mol Genet 2:333

Jansen G, de Jong P, Amemiya C, Aslanidis C, Shaw DJ, Harley HG, Brook JD (1992) Physical and genetic characterisation of the distal segment of the myotonic dystrophy area on 19q. Genomics 13:509–517

Jenkins T, Nicholls E, Gordon E, Mendelsohn D, Seftel HC, Andrew MJA (1980) Familial Hypercholesterolaemia—a common genetic disorder in the Afrikaans population. S Afr Med J 57:943–947

Krahe R, Eckart M, Ogunniyi AO, Osuntokun BO, Siciliano MJ, Ashizawa T (1995) A de novo myotonic dystrophy mutation in a Nigerian kindred. Am J Hum Genet 56:1067–1074

Kotze MJ, Langenhoven E, Retief AE, Seftel HC, Henderson HE, Weichs HFH (1989) Haplotypes identified by 10 DNA restriction fragment length polymorphisms at the human low density lipoprotein receptor gene locus. J Med Genet 26:255–259

Lavedan C, Hofman-Radvanyi H, Boileau C, Bonaiti-Pellie C, Savoy D, Shelbourne P, Duros C, et al (1994) French myotonic dystrophy families show expansion of a CTG repeat in complete linkage disequilibrium with an intragenic 1kb insertion. J Med Genet 31:33–36

Leitersdorf E, Van Der Westhuysen DR, Coetzee GA, Hobbs HH (1989) Two common low density lipoprotein receptor gene mutations cause familial hypercholesterolaemia in Afrikaners. J Clin Invest 84:954–961

Lotz BP, van der Meyden CH (1985) Myotonic dystrophy. Part I. A genealogical study in the northern Transvaal. S Afr Med J 67:812–814

Mahadevan MS, Amemiya C, Jansen G, Sabourin L, Baird S, Neville CE, Wormskamp N, et al (1993*a*) Structure and genomic sequence of the myotonic dystrophy (DM kinase) gene. Hum Mol Genet 2:299–304

Mahadevan MS, Foitzik MA, Surh LC, Korneluk RG (1993*b*) Characterisation and polymerase chain reaction (PCR) detection of an Alu deletion polymorphism in total linkage disequilibrium with myotonic dystrophy. Genomics 15: 446–448

Mahadevan MS, Tsilfidis C, Sabourin L, Shutler G, Amemiya C, Jansen G, Neville C, et al (1992) Myotonic dystrophy mutation: an unstable CTG repeat in the 3′ untranslated region of the gene. Science 255:1253–1255

Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215

Neville CE, Mahadevan MS, Barcelo JM, Korneluk RG (1994) High resolution genetic analysis suggests one ancestral predisposing haplotype for the origin of the myotonic dystrophy mutation. Hum Mol Genet 3:45–51

Ross R (1975) The "white" population of South Africa in the eighteenth century. Popul Stud 29:217–230

Shelbourne P, Winqvist R, Kunert E, Davies J, Leisti J, Thiele H, Bachmann H, et al (1992) Unstable DNA may be responsible for the incomplete penetrance of the myotonic dystrophy phenotype. Hum Mol Genet 1:467–473

Yamagata H, Miki T, Ogihara T, Nakagawa M, Higuchi I, Osame M, Shelbourne P, et al (1992) Expansion of unstable DNA region in Japanese myotonic dystrophy patients. Lancet 339:692