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Characteristics of Myotonic Dystrophy in Istria: Molecular Genetic Approach. Part II: Analysis of Genetic Polymorphisms

I. Medica[1,2], N. Logar[1] and B. Peterlin[1]

[1] Division of Medical Genetics, Department of Obstetrics and Gynecology, University Medical Center Ljubljana, Slovenia
[2] Outpatient Paediatric Department Pula, Croatia

A B S T R A C T

One of the world highest prevalence estimates of myotonic dystrophy (DM) has been reported in the Croatian region Istria. To analyse the population genetic characteristics of DM locus in Istria, two intragenic and three extragenic polymorphic markers were tested. The Southern blot technique was used for D19S63 locus analysis, whereas PCR analysis was performed for CKMM, Alu polymorphism, DMPK (G/T) intron 9/HinfI polymorphism, and D19S207 genetic markers. The compound haplotypes segregating with DM were established. A complete association between the DM mutation and D19S63, D19S207, intron 9/HinfI polymorphism and Alu polymorphism markers were found. In all DM chromosomes: D19S63 and Alu markers had the allele 1 in common; D19S207 had the allele 3 in common, DMPK (G/T) intron 9/HinfI marker had the allele 2 in common. The analysis of CKMM polymorphism revealed genotype heterogeneity; in DM chromosomes either allele 2 or allele 4 were found.

The haplotype analysis in the population of Croatian Istria supports the linkage disequilibrium between the DM mutation and Alu polymorphism, intron 9/HinfI polymorphism, D19S63 and D19S207 markers as reported worldwide. The results of the haplotype analysis suggest a common origin of the mutation in Istrian population.

Introduction

Myotonic dystrophy (DM) is the most common muscular dystrophy in adults and one of the most frequent autosomal dominant diseases[1]. Great differences in prevalence estimates worldwide could be explained by ascertainment bias and by the absence of specific diagnostic tests provided by recent molecular genetic techniques.

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The approach to the localization of the DM gene was based on the principles of the reverse genetics. Over the last years, several informative DNA markers have been identified for the mapping and cloning of the DM gene [2–4]. The genetic defect underlying DM is now known to be the expansion of the CTG repeats at the 3' end of myotonin protein kinase gene[5–11]. These molecular tools have recently shed light on the origins, maintenance and population genetic characteristics of DM.

A significant linkage disequilibrium between DM mutation and some nearby DNA polymorphic loci has been detected in different investigated inbred and outbred populations: English, French, Spanish, Finnish, French-Canadian[4,12–14]. These findings suggest that most of mutated alleles are the consequence of a unique or a small number of ancestral mutations. On the other hand, the increased number of (CTG) motifs as the cause of DM was found in almost all chromosomes from all investigated populations[5–11,15]. Therefore, molecular investigations support the idea of a unique DM mutation.

In Istria, a northwest Croatian region, a high DM prevalence of 18/100 000 was estimated after the inclusion of mutation analysis as a diagnostic test[16]. Usually, a high DM prevalence in genetic isolates has been explained by the founder effect[17].

To investigate the population genetic characteristics of DM in Istria, the intragenic genetic markers Alu[18] and DMPK (G/T) intron 9/HinfI polymorphism[19] and the extragenic markers D19S63[4] and D19S207[20], all in linkage disequilibrium with the DM mutation, and the extragenic CKMM[21] marker, the most distant to DM mutation proximally, were used.

Patients and Methods

Patients

DM patients were ascertained as reported previously[22]. All living Istrian patients identified as DM were included in the study.

The study sample consisted of 40 subjects from 9 families: 27 DM patients and 13 first-degree relatives; additionally, 10 healthy spouses were analyzed.

Methods

The genetic polymorphism analysis was aimed at defining the haplotypes around the DM gene characteristic of the disease and involved the analysis of five genetic markers close-linked to DM mutation: CKMM and D19S63 loci proximal to the DM gene, the intragenic Alu polymorphic locus distal to the DM mutation, the intragenic intron 9 (G/T) HinfI polymorphism, and D19S207 telomeric to the DM gene.

The PCR analysis was performed to amplify the CKMM region. After the double digestion with NcoI and TaqI, four different haplotypes resulted: haplotype 1 = TaqI and NcoI site absent; haplotype 4 = both sites present; haplotype 3 = only TaqI site present; haplotype 2 = only the NcoI site present[21].

The marker D19S63, in linkage disequilibrium with the DM mutation[4], was analyzed using Southern blot method. The probe pD10 (D19S63) has been described in details[4]. Seven g of DNA were digested with PvuII, transferred to a nylon membrane and hybridized with DIG-labelled pD10 probe. The detection was done using non radioactive DIG luminescent detection kit (Boehringer Mannheim, Germany)[16]. The probe pD10 shows 3 allelic PvuII polymorphic fragments of sizes 1: 6.0 kb, 2: 5.6 kb and 3: 5.4 kb, respectively.

The intragenic Alu marker is in complete linkage disequilibrium with the DM mutation. The PCR technique was performed using the conditions described elsewhere[18]. This polymorphism is due to insertion/deletion event of Alu repeats,

and consequently, the amplification provides the following results: allele 1, 1008 bp, and allele 2, 494 bp.

The DMPK intron 9/HinfI polymorphism is a RFLPs polymorphism due to single nucleotide substitution (G/T)[19]. The altered enzyme restriction site is detected after PCR amplification[23]. The two alleles detected are: allele 1: 434 bp, allele 2: 354 bp.

The microsatellite marker GJ-VSSM2 (D19S207) located 15 kb telomeric to the DM gene was analysed as described by Jansen et al.[20]. Significant linkage disequilibrium between the DM mutation and certain alleles of this marker was observed[23,24]. After PCR amplification five (CA)n repeat alleles were observed at the microsatellite: allele 1: 157 bp, allele 2: 153 bp, allele 3: 151 bp, allele 4: 149 bp, allele 5: 145 bp[20].

Compound haplotypes segregating with DM were also established.

Results

Twenty-seven DM patients, 13 close relatives and 10 healthy spouses were analyzed with five nearby polymorphic genetic markers: CKMM polymorphism, D19S63 polymorphism, Alu polymorphism, intron 9/HinfI polymorphism and D19S207 polymorphism.

Allele frequencies of the five DNA polymorphisms studied are shown in Table 1.

TABLE 1
FREQUENCIES OF THE ALLELES FOR 5 DNA POLYMORPHISMS ON DM AND NON-DM CHROMOSOMES

			DM patients		Healthy subjects	DM and non-DM subjects
Locus	Enzyme	Allele	DM chrom.	non-DM chrom.	both chrom.	non-DM chrom.
D19S63	PvuII	1	23 (1.00)	9 (0.39)	15 (0.35)	24 (0.36)
88chrom.		2	0 (0.00)	12 (0.52)	20 (0.47)	32 (0.49)
		3	0 (0.00)	2 (0.008)	7 (0.16)	9 (0.13)
			23	23	42	65
CKMM	TaqI	1	0 (0.00)	4 (0.15)	8 (0.18)	12 (0.17)
98 chrom.	NcoI	2	17 (0.63)	4 (0.15)	8 (0.18)	12 (0.17)
		3	0 (0.00)	11 (0.40)	18 (0.41)	29 (0.40)
		4	10 (0.37)	8 (0.30)	10 (0.22)	18 (0.25)
			27	27	44	71
Alu		1	27 (1.00)	16 (0.59)	27 (0.61)	43 (0.60)
98 chrom.		2	0 (0.00)	11 (0.40)	17 (0.38)	28 (0.33)
			27	27	44	71
Intron9	HinfI	1	0 (0.00)	15 (0.55)	20 (0.45)	35 (0.49)
98 chrom.		2	27 (1.00)	12 (0.44)	24 (0.54)	36 (0.50)
			27	27	44	71
D19S207		1	0 (0.00)	0 (0.00)	9 (0.20)	9 (0.12)
98 chrom.		2	0 (0.00)	6 (0.22)	11 (0.25)	17 (0.23)
		3	27 (1.00)	0 (0.00)	10 (0.22)	10 (0.14)
		4	0 (0.00)	9 (0.33)	8 (0.18)	17 (0.23)
		5	0 (0.00)	12 (0.44)	6 (0.13)	18 (0.25)
			27	27	44	71

For Alu polymorphism, the allele 1 was shared by 100% of DM chromosomes and 60% of non-DM chromosomes.

For intron 9/HinfI polymorphism the allele 2 was shared by 100% of DM chromosomes and 50% of non-DM chromosomes.

Likewise, all DM chromosomes shared the allele 1 for D19S63 polymorphism, whereas the same allele was shared by 36% of non-DM chromosomes.

For D19S207 polymorphism all DM chromosomes shared the allele 3, the same allele was found in 14% of non-DM chromosomes.

The analysis of CKMM polymorphism revealed a genotype heterogeneity: the allele 2 was found in 63% of DM chromosomes and the allele 4 in 37% of DM chromosomes; in non-DM chromosomes the allele 2 was found in 17%, and the allele 4 in 25%.

The polymorphisms at the five different genetic markers were used to construct compound haplotypes for DM and non-DM chromosomes.

Only two haplotypes were present in DM chromosomes: haplotype 1/2/1/2/3 (for D19S63, CKMM, Alu, intron 9/HinfI and D19S207 polymorphisms, respectively) was found in 56% of DM chromosomes, and haplotype 1/4/1/2/3 in 43% of DM chromosomes.

Discussion

In this study the analysis of polymorphisms was performed to evaluate population genetic characteristics of DM in Croatian Istria. The haplotypes, linked to DM phenotype defined by DM markers with their polymorphisms, were established. In this study of Istrian population all DM patients shared the same allele distribution for D19S63 polymorphism, for Alu polymorphism, for intron 9/HinfI

TABLE 2
FREQUENCIES OF HAPLOTYPES DEFINED BY MARKERS ON DM AND NON DM CHROMOSOMES

	Alleles					No of haplotypes	
Haplotype	D19S63	CKMM	Alu	Intron 9(G/T)	D19S207	non-DM chr (65)	DM chr (23)
1	1	1	1	1	1	4	
2	1	2	1	2	3	1	13
3	1	2	2	1	1	1	
4	1	3	1	1	1	4	
5	1	3	2	1	2	5	
6	1	4	1	2	3	4	10
7	1	4	2	1	2	5	
8	2	1	1	1	2	4	
9	2	1	2	1	1	3	
10	2	2	2	2	3	4	
11	2	3	1	2	4	11	
12	2	3	2	2	2	7	
13	2	4	1	2	3	2	
14	2	4	2	2	3	1	
15	3	2	1	1	5	6	
16	3	4	1	2	3	1	
17	3	4	2	2	5	2	

No of investigated subjects: 44 No of investigated chromosomes: 88

and for D19S207 polymorphism. For the CKMM locus, two haplotypes segregating with DM were found.

The determination of the molecular basis of DM showed that the expansion of an unstable CTG repeat sequence is responsible for most cases of DM[5–11,25,26]. The finding of the same mutation in many populations suggested a common ancestry for the majority of cases[4,5]. Previously, the linkage disequilibrium was detected between polymorphic DNA markers within the gene for apolipoprotein C2 (APOC2) and the DM locus in isolated populations, such as French-Canadian and Finnish[23,24]. In 1991, Harley et al. reported the first linkage disequilibrium between DM and a tightly linked anonymous marker (D19S63) in a heterogeneous population[4]; a similar result was later reported in the Spanish and Japanese respective population[12,15]. The gene for muscle type creatine kinase (CKMM) and the DM locus are closely linked[29] but no linkage disequilibrium was proved in the Spanish and Finnish population[12,13]. Strikingly, in all DM patients studied to date, the expanded (CTG)n repeat is always asso- ciated with the 1 kb insertion allele of a two-allele Alu repeat insertion-deletion polymorphism located in intron 8 of the DM gene 5.3 kb upstream from the expanded (CTG)n repeat[5,10,14,15]. Numerous sequence polymorphisms due to a single-base change have been identified in the DM gene, recognized by altered restriction enzyme site. In intron 9 two alleles are detected by HinfI polymorphism, the larger having the frequency 0.47[19]. The analysis of the microsatellite GJVSSM2 revealed linkage disequilibrium between this extragenic marker and DM mutation[24].

All these findings prove a single ancestral mutation in DM, or a limited number of mutations on the background of a predisposing haplotype. The analysis of markers closely linked to the DM mutation locus revealed a consistent haplotype on DM chromosomes[30].

In the Croatian region Istria high DM prevalence was reported. To check the status of DM and non-DM chromosomes in Istrian patients, we analyzed different polymorphisms linked to the DM mutation and compared the results with those in some other populations (Table 3).

D19S63 is the first locus to reveal a linkage disequilibrium with DM mutation in a mixed population, and it does so with the allele 3 in British, French-Canadian, Finnish and Spanish respective populations[4,12,13], whereas in the French[14] and in our population with the allele 1. This disequilibrium might be a good indication of the founder effect or it might be the consequence of a short distance between the DM locus and the D19S63 marker on its proximal side.

Contrary to these findings in the Istrian population, 63% of DM chromosomes at the CKMM locus carry the allele 2, which is also present in 17% of unaffected chromosomes; 37% of the DM chromosomes carry the allele 4, present in 25% of non-DM chromosomes. These results make the CKMM polymorphism a poor genetic marker if applied on individual patient, but it can be useful in constructing compound haplotypes for population analyses.

The complete allelic association was found between the DM and intragenic Alu insertion polymorphism in all investigated populations and in our study as well. Our results are consistent with the hypothesis that the CTG repeat expansion occurred on one or a few ancestral chromosomes carrying a large 1 kb insertion allele.

For the intron 9/HinfI polymorphism significant linkage disequilibrium was detected between the DM mutation and allele 2 in European, Canadian and South African Caucasoid populations[19,23,30,31].

TABLE 3
DM AND NON DM CHROMOSOME ALLELE FREQUENCIES IN DIFFERENT POPULATIONS

		Istrian		Frenh		Spanish		Finnish		British, French, Canadians		South, Africans, Caucasoid	
		DM	nDM	DM	nDM	DM	nDM	DM	nDM	DM	nDM	DM	nDM
D19S63	1	1.00	0.36	0.45	0.59	0.45	0.62	0.25	0.55	0.32	0.57	0.07	0.56
	2	0.00	0.49	0.18	0.28	0.06	0.19	0.00	0.16	0.10	0.27	0.00	0.26
	3	0.00	0.13	0.37	0.13	0.48	0.19	0.75	0.28	0.59	0.16	0.93	0.18
CKMM	1	0.00	0.17			0.00	0.00						
	2	0.63	0.17			0.25	0.25						
	3	0.00	0.40			0.27	0.25						
	4	0.37	0.25			0.48	0.49						
Alu	1	1.00	0.60	1.00	0.50					1.00	0.60		
	2	0.00	0.33	0.00	0.50					0.00	0.40		
Intron 9(G/T)	1	0.00	0.49									0.00	0.49
	2	1.00	0.50									1.00	0.51
D19S207	1	0.00	0.12										
	2	0.00	0.23										
	3	1.00	0.14										
	4	0.00	0.23										
	5	0.00	0.25							0.88	0.06		
	6	0.00										0.91	0.12

Our study confirmed a strong association between the DM mutation and allele 2.

Canadian and South African Caucasoid populations were investigated using a polymorphic microsatellite GJ-VSSM2 (D19S207). The analysis revealed a significant allelic association between the DM mutation and certain alleles. In South African Caucasoid population the smallest allele, allele 6 was found in 91% of DM chromosomes[31]. In Canadians the allele 5, was found on 88% of DM chromosomes[24]. We have found a strong association between the DM mutation and allele 3, revealed as Istrian particularity.

In conclusion, the analysis of the polymorphisms was performed with the aim of determining the characteristics of the DM mutation in Croatian Istria and to determine the ancestry of the DM mutation in Istrian population[32]. However for linkage disequilibrium calculations the number of investigated chromosomes was insufficient.

All living Istrian patients identified as DM were included in the study. After extensive genealogical reconstruction we could assign them to 9 different families. Two haplotypes segregating with DM were found in 9 Istrian DM families, differing in the result for CKMM polymorphism. As reported in other populations (Table 3), our results show a strong association between the DM locus and D19S63, Alu, intron 9/HinfI and D19S207 markers, suggesting a common origin of the mutation in the Istrian population.

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I. Medica

Outpatient Paediatric Department, Istarska 13, 52100 Pula, Croatia

ZNAČAJKE MIOTONIČNE DISTROFIJE U ISTRI: MOLEKULARNO GENETIČKI PRISTUP. DRUGI DIO: ANALIZA GENETIČKOG POLIMORFIZMA

S A Ž E T A K

Prevalencija miotonične distrofije (DM) u hrvatskoj Istri je jedna od najviših zabilježenih. Sa svrhom ustanovljenja populacijsko genetičkih osobitosti DM lokusa u Istri, analizirana su dva intragenska i tri ekstragenska polimorfna markera susjedna DM lokusu. *Southern blot* tehnika korištena je pri analizi D19S63 lokusa, a *PCR* analiza upotrebljena je za analizu CKMM, Alu polimorfizma, DMPK (G/T) intron 9/HinfI polimorfizma i za analizu D19S207 lokusa. Određeni su složeni haplotipovi koji segregiraju s DM. Potpuna udruženost s DM ustanovljena je za D19S63, D19S207 lokus, te za intron9/HinfI i Alu polimorfizam. Za lokus D19S63 svi DM kromosomi posjeduju alel 1, za Alu polimorfizam svi DM kromosomi posjeduju alel 1, za D19S207 lokus svi DM kromosomi posjeduju alel 3, za intron 9/HinfI polimorfizam svi DM kromosomi imaju alel 2. Analiza CKMM polimorfizma pokazala je genetsku heterogeničnost: u DM kromosoma nađen je alel 2 ili alel 4.

Analiza haplotipa u populaciji hrvatske Istre potvrdila je podatke o neravnoteži povezanosti Alu, intron9/HinfI, D19S63 i D19S207 polimorfnih markera s DM, kao i u studijama do sada istraživanih populacija. Rezultati naše studije analize haplotipa sugeriraju zajedničko porijeklo DM mutacije u istarskoj populaciji.