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Severe childhood autosomal recessive muscular dystrophy with the deficiency of the 50 kDa dystrophin-associated glycoprotein maps to chromosome 13q12

Kemal Azibi, Lucien Bachner[1], Jacques S.Beckmann[2,3], Kiichiro Matsumura[4], Elhadi Hamouda[5], Malika Chaouch[5], Athmane Chaouch[5], Rachida Ait-Ouarab, Alain Vignal[3], Jean Weissenbach[3], Marie-Claude Vinet[1], France Leturcq[1], Huguette Collin[6], Fernando M.S.Tomé[6], Abderrezak Reghis, Michel Fardeau[6], Kevin P.Campbell[4] and Jean-Claude Kaplan[1,*]

Hôpital Bologhine, CHU Alger-Ouest, Algeria, [1]INSERM 129, Institut Cochin de Génétique Moléculaire, 75014 Paris, [2]CEPH, 27 rue Juliette Dodu, 75010 Paris, [3]Généthon, 1 rue de l'Internationale, BP 59 9102 Evry Cedex, France, [4]Howard Hughes Medical Institute, University of Iowa College of Medicine, Iowa City, IA 52242, USA, [5]Hôpital Ben-Aknoun, CHU Alger-Ouest, Algeria and [6]INSERM 153 and CNRS URA614, 17 rue du Fer-à-Moulin, 75005 Paris, France

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We have recently demonstrated the specific deficiency for the 50 kDa dystrophin-associated glycoprotrein (50DAG) in Algerian patients afflicted with severe childhood autosomal recessive muscular dystrophy with DMD-like phenotype (SCARMD). A similar disease affecting Tunisian patients was linked to chromosome 13q but the status of the 50DAG was not investigated. Here we show by linkage analysis of Algerian families that the genetic defect which leads, either directly or indirectly, to the deficiency of the 50DAG in skeletal muscle is localized to the proximal part of chromosome 13q. We have not found any evidence of genetic heterogeneity among the thirteen families studied. It remains to be demonstrated whether the 50DAG gene maps at 13q12, and to determine if it is mutated in this disease.

INTRODUCTION

Ben Hamida and Fardeau[1,2] described in Tunisia a type of muscular dystrophy phenotypically very close to Duchenne muscular dystrophy (DMD), or in some instances to Becker muscular dystrophy (BMD), but autosomally transmitted. It was characterized by onset around 5 years, muscle wasting affecting preferentially proximal groups, pseudo-hypertrophy of the calves in most cases, high serum creatine phosphokinase at early stages of the disease, progressive evolution with confinement to wheelchair by 12–15 years, and death between 20 and 30 years by respiratory insufficiency if not controlled. Unlike for DMD, females and males were equally affected, and families were often consanguineous, consistent with an autosomal recessive mode of inheritance. Later this disease was also found in surrounding countries in the Maghreb (Western part of North-Africa): Algeria[3,4] and in Morocco (Sefiani, personal communication). The disease has been referred to by several names: severe childhood muscular dystrophy, or autosomal recessive (Duchenne-like) muscular dystrophy, or severe childhood autosomal recessive muscular dystrophy (SCARMD). Its MIM number is 253700[5]. The nature and number of gene(s) involved is unknown, but dystrophin is normal in SCARMD patients[6].

In Algeria we have identified 57 patients with SCARMD, belonging to 34 families (of which 29 have more than 1 affected individual) with some large inbred pedigrees (Chaouch et al, in preparation). Three patients of this series were previously shown to be deficient in the 50DAG[7], a member of the sarcolemmal dystrophin-associated glycoprotein complex[8–10]. In this report we demonstrate, in a subset of 13 selected families, linkage of SCARMD associated with 50DAG deficiency to markers of the proximal region of chromosome 13q. These data (i) confirm the recently published primary locus assignment obtained in three extended Tunisian pedigrees[11], (ii) indicate genetic homogeneity of the disease between these two populations, and (iii) show for the first time that the genetic defect responsible, either directly or indirectly, for the 50DAG deficiency in muscle from SCARMD patients is localized to chromosome 13q.

RESULTS

Immunohistochemical analysis of dystrophin-associated proteins in patients with SCARMD (Figure 2)

The sarcolemmal 50DAG was deficient in cryosections of skeletal muscle from all patients investigated in this study (Figure 2, Table 2). In contrast, dystrophin (Figure 2) and all the other members of the sarcolemmal dystrophin associated protein complex[8–10] (not shown) were present. These results were exactly the same as those obtained from the four SCARMD patients reported previously[7], which included two siblings of family 9 and one member of family 3 (Fig. 1, Table 2).

* To whom correspondence should be addressed

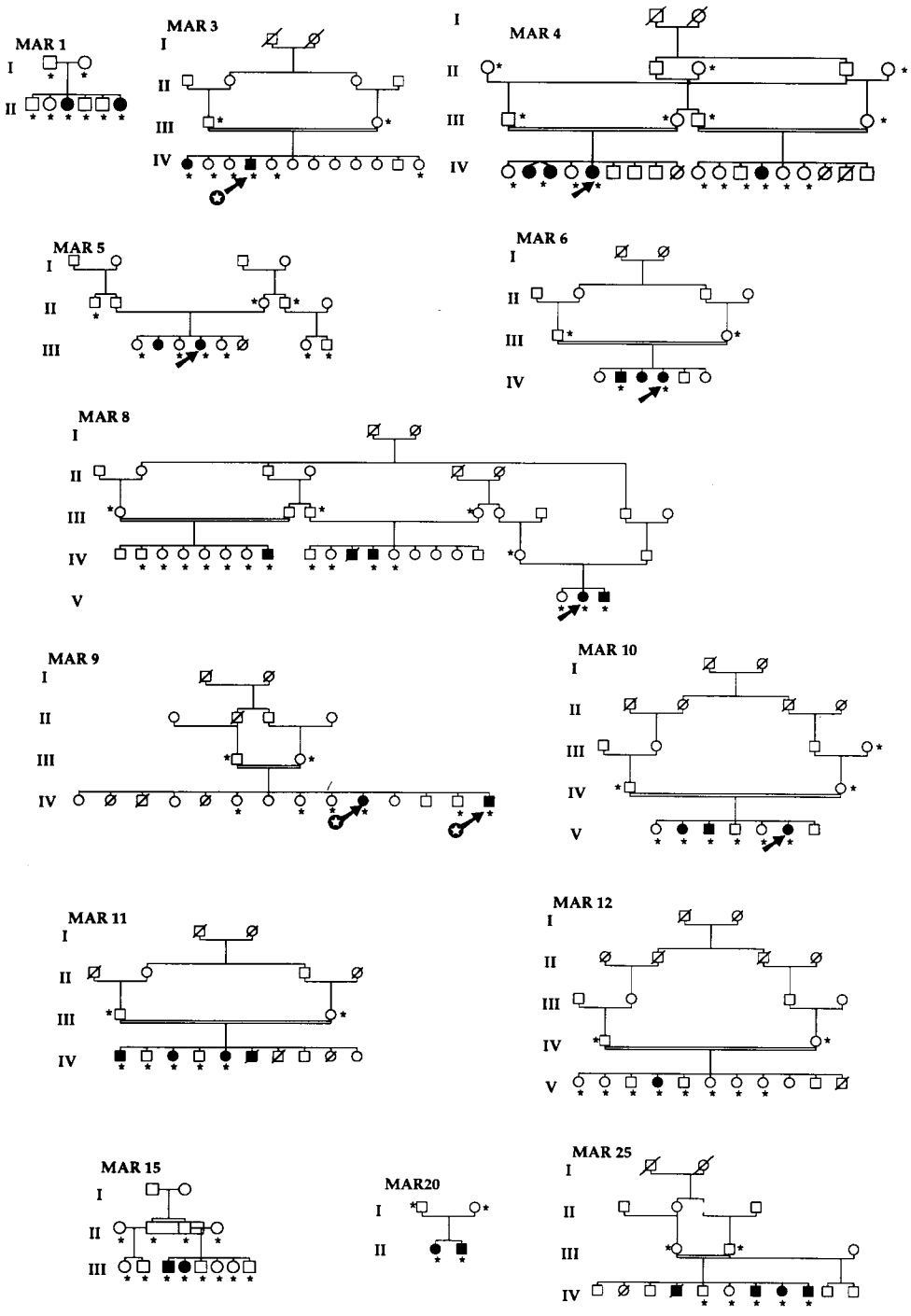

Figure 1. Pedigrees of 13 SCARMD families. Black stars indicate individuals that were genotyped for linkage analysis. Arrows show patients in which the 50DAG was investigated (white stars indicate patients already reported in ref. 7).

Linkage analysis of 13 pedigrees and evidence of mapping to chromosome 13

Negative lod scores were obtained with 85 random markers from chromosomes 1, 2, 3, 4, 5, 6, 13, 15, 22 (data available upon request), except for marker D13S175 (Table 1). Positive lod scores were subsequently obtained with other markers mapping to the same juxta-centromeric region of chromosome 13 long arm (Tables 1 and 2). Table 2 also lists the lod scores per family at 0.1% recombination and at the arbitrary value of 10%. Altogether four families (n° 4, 6, 8 and 12) yielded lod scores higher than one.

Multipoint linkage analysis

The position of D13S115 relative to a map of four Genethon markers was determined by typing this probe in eight CEPH families. The two best supported orders are shown on figure 3A.

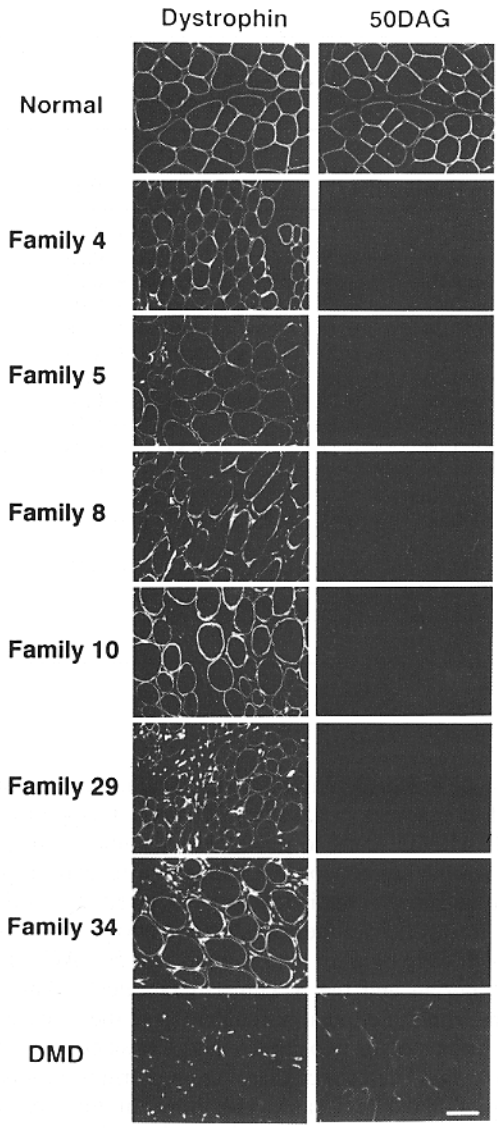

Figure 2. Immunohistochemical analysis of dystrophin and the 50DAG in biopsied skeletal muscle specimens. Transverse cryosections (7 μm) were immunostained with antibodies against dystrophin and the 50DAG. Patients from families 4, 5, 8, 10, 29 and 34 (Table 2 and Figure 1) are shown, together with a normal human and a DMD patient. Patients from families 3, 6, 9, 16, 24, 31 (not shown) exhibited the same pattern. The light labelling in the DMD muscle is probably due to cross reaction of the dystrophin antibody with DRP in blood vessels. Bar, 80μm.

Relative to the chromosome 13 map of Bowcock et al[12] it can be deduced that markers D13S175 and D13S221 map between D13S115 and the more distal D13S120 marker. Centromeric marker D13Z1 was not informative in our families.

We next mapped the disease locus against the three possible couples of markers used in the two point analyses (Figure 3B). The results are suggestive of a distal localisation of SCARMD 11 cM from D13S221, or 11 cM proximal to D13S115, but the relative odds between these two locations not yet significant. All three-point generalized lod scores (base 10) are above the critical threshold value of 3. To perform four-point analysis within reasonable computing time limits, we had to simplify the pedigrees structure by removing all consanguinity loops. This treatment did not affect significantly the two-point lodscores, proving negligible loss of information (data not shown). Four point analysis, using the map of markers on figure 3AI, yelded the results shown on figure 3C with odds against the distal location of 5/1. Analysis using the map on figure 3AII gave similar results (not shown).

Heterogeneity testing

A search for genetic heterogeneity among the 13 families was carried out with the HOMOG program (version 3.31, ref 13). Analysis was done on the three sets of lod score values. No evidence for heterogeneity was found among informative families (data not shown). Furthermore no heterogeneity was found by combining the lod scores obtained for probe D13S115 on our families to those recently published by Ben Othmane et al[11] on Tunisian families. Thus the data with this marker were cumulated, raising the lod score to 10.26 (Table 1). As only a subset of Algerian families was available for biopsy and amenable to 50DAG analysis, we also used the Morton's likelihood ratio test for heterogeneity among groups of families as implemented by the MTEST program (version 3.10, ref 13). No significant evidence in favour of heterogeneity was obtained, for each one of the three chromosome 13 microsatellites, among any of the groups tested. In particular an examination of the 50DAG-deficient families and those of unknown 50DAG status, with respect to either D13S115, D13S175, or D13S221, yielded chi square values ranging from 0.0 to 0.41, 1d.f.

DISCUSSION

With its homogeneous phenotypic presentation strikingly similar to Duchenne muscular dystrophy, and high prevalence in North-

Table 1. Two point lod scores (sex averaged) between the SCARMD locus and three microsatellite markers of 13q12

θ Marker	0.0	0.001	0.01	0.05	0.1	0.15	0.2	0.3	0.4	Zmax	θ_{Zmax}	Confidence interval (−1 lod)
D13S175	$-\infty$	−9.87	−4.60	0.25	2.00	2.49	2.43	1.59	0.56	**2.52**	0.166	0.070−0.26
					1.10	*1.55*	*1.60*	*1.12*				
D13S221	$-\infty$	−8.85	−2.98	1.43	2.64	2.78	2.51	1.56	0.61	**2.80**	0.136	0.090−0.28
					2.45	*2.37*	*2.07*	*1.30*				
D13S115	$-\infty$	−4.85	−1.34	1.60	2.45	2.49	2.21	1.37	0.58	**2.53**	0.128	0.055−0.26
					1.99	*1.97*	*1.77*	*1.15*				
D13S115 cumulated	$-\infty$	n.a.	n.a	10.26	10.26	9.25	7.87	4.32	2.05	n.a.	n.a.	n.a.

Lod scores for families in which the 50DAG deficient status was proven (Table 2) are shown *in italics*. Cumulated lod scores for D13S115 (last row) were obtained by adding our data to those reported in Tunisian families[11]. Other microsatellites from this area (Weissenbach et al, unpublished) were genotyped in these families, but, probably due to the high degree of consanguinity, these markers were not informative.

Table 2. SCARMD families investigated for 50DAG and/or linkage analysis

Family No	50DAG in muscle biospy	Lod scores					
		D13S175		D13S221		D13S115	
		θ		θ		θ	
		0.001	0.1	0.001	0.1	0.001	0.1
1	n.d.	− 1.60	+ 0.18	n.d.		+ 0.80	+ 0.48
3	**deficient** (M*)	− 1.41	− 0.31	− 2.79	− 0.19	− 1.07	− 0.33
4	**deficient** (F)	+ 0.02	**+ 1.00**	**+ 2.21**	**+ 1.70**	+ 0.54	+ 0.82
5	**deficient** (F)	+ 0.07	+ 0.05	+ 0.37	+ 0.22	+ 0.05	+ 0.04
6	**deficient** (F)	**+ 1.08**	+ 0.78	**+ 1.24**	+ 0.93	+ 1.08	+ 0.78
8	**deficient** (F)	− 3.91	+ 0.38	− 1.62	− 0.30	**+ 1.43**	**+ 1.19**
9	**deficient** (1M*, 1F*)	+ 0.21	+ 0.47	− 1.26	+ 0.39	− 2.65	− 0.27
10	**deficient** (F)	− 2.84	− 0.53	− 0.30	0.10	− 0.54	− 0.24
11	n.d.	− 0.93	− 0.31	− 3.25	− 0.25	− 0.89	− 0.29
12	n.d.	**+ 1.08**	+ 0.77	**+ 1.08**	+ 0.77	**+ 1.33**	+ 0.88
15	n.d.	− 0.23	− 0.14	n.d.		− 2.15	− 0.27
16	**deficient** (F)	n.d.		n.d.		n.d.	
20	n.d.	+ 0.30	+ 0.21	+ 0.60	+ 0.43	+ 0.60	+ 0.43
24	**deficient** (F)	n.d.		n.d.		n.d.	
25	n.d.	− 0.16	+ 0.18	− 3.44	− 0.75	− 3.37	− 0.77
29	**deficient** (F)	n.d.		n.d.		n.d.	
31	**deficient** (M)	n.d.		n.d.		n.d.	
34	**deficient** (M)	n.d.		n.d.		n.d.	

* 50DAG deficiency previously reported[7]
n.d.: not determined

Africa[1,2,3], SCARMD is a well-defined nosological entity, most likely due to a defect in a single autosomal gene. In a previous report[4], on the basis of linkage analysis carried out in Algerian families with SCARMD, we had already excluded three candidate loci: the recessive (LGMD2) and dominant (LGMD1) forms of limb girdle muscular dystrophy, respectively at 15q11−qter[14,15] and 5q31−q35[16], and the dystrophin-related protein gene (DRP) an autosomal homologue of dystrophin[17,18] (DMDL locus at 6q22−q23). The DRP protein had already been found to be preserved, and with normal size, in SCARMD patients[19]. We also excluded the DRP gene by using a polymorphic microsatellite within the DRP gene itself[20], that gave negative lod scores in our SCARMD families (data not shown).

Concurrently a candidate protein approach was followed with emphasis on the sarcolemmal proteins that have been shown to form a large oligomeric complex with dystrophin[8−10]. We found[7] that one of them, the dystrophin-associated glycoprotein of 50 kDa (50DAG), is specifically deficient in three patients, one in family 5 and two siblings from family 9.

We have now further implemented this study by investigating 10 additional unrelated patients exhibiting a typical pattern of SCARMD (Table 2). In every case, without exception, the 50DAG deficiency was confirmed, while the other tested members of the dystrophin-associated proteins were present.

Linkage analysis was performed on a panel of 13 pedigrees, including seven families in which 50DAG deficiency could be assessed (Table 1 and Figure 1). We obtained positive linkage data indicating that the disease locus maps to the proximal region of 13q. However, as a result of extensive inbreeding, no single marker was fully informative, preventing one to reach significant lod scores. Hence, the values obtained are slightly smaller than the critical threshold of 3 (Table 1). Yet, it is of interest to note, considering that these markers all map to the same chromosomal region, that four families—three of which are known to be 50DAG-deficient—yield each individual lod scores superior to 1 (fam. 4, 6, 8 and 12; Table 2). In addition, the maximum four-point lod scores were >4.0 (Fig. 3C) further supporting linkage of the SCARMD locus to these chromosome 13 markers.

These results are concordant with those recently reported on Tunisian families[11]. Statistical tests showed no evidence for genetic heterogeneity either between the two groups of families, those which were or were not examined for 50DAG, or between the 13 Algerian and the 3 Tunisian families[11]. This result is consistent with the clinical homogeneity of the disease, and the common origins of the two populations. Considering the restricted geographic area from which these families originate, it is possible that they all bear the same mutation. In this instance, the identification of markers in linkage disequilibrium with this SCARMD mutation would be instrumental in narrowing the genetic interval. It would be interesting to evaluate the involvement of this locus in SCARMD-like families originating from other parts of the world, both in Arab[21,22] and non-Arab countries[23−27]. It would also be important to verify the status of the 50DAG protein in the Tunisian SCARMD patients.

The deficiency of the 50DAG is presumed to cause a disruption/dysfunction of the dystrophin−glycoprotein complex, which is a transsarcolemmal linker between the subsarcolemmal cytoskeleton and extracellular matrix [8−10]. It is not yet proven that the 50DAG deficiency is the primary defect in SCARMD. Indeed there are several instances where a dramatic decrease of a protein is secondary to a primary defect affecting another gene, especially in the case of cyto-skeletal components. For example, in one type of spherocytic hemolytic anemia a primary defect of red-cell membrane band 3 protein leads to a secondary loss of the associated protein 4.2[35]; in hereditary elliptocytosis a sharp reduction of glycophorin C is actually due to a primary defect in the gene coding for the 4.1 protein associated to glycophorin C in the red-cell membrane[36]. In Duchenne muscular dystrophy the primary defect of dystrophin results in a secondary loss of dystrophin-associated proteins[29]. Therefore,

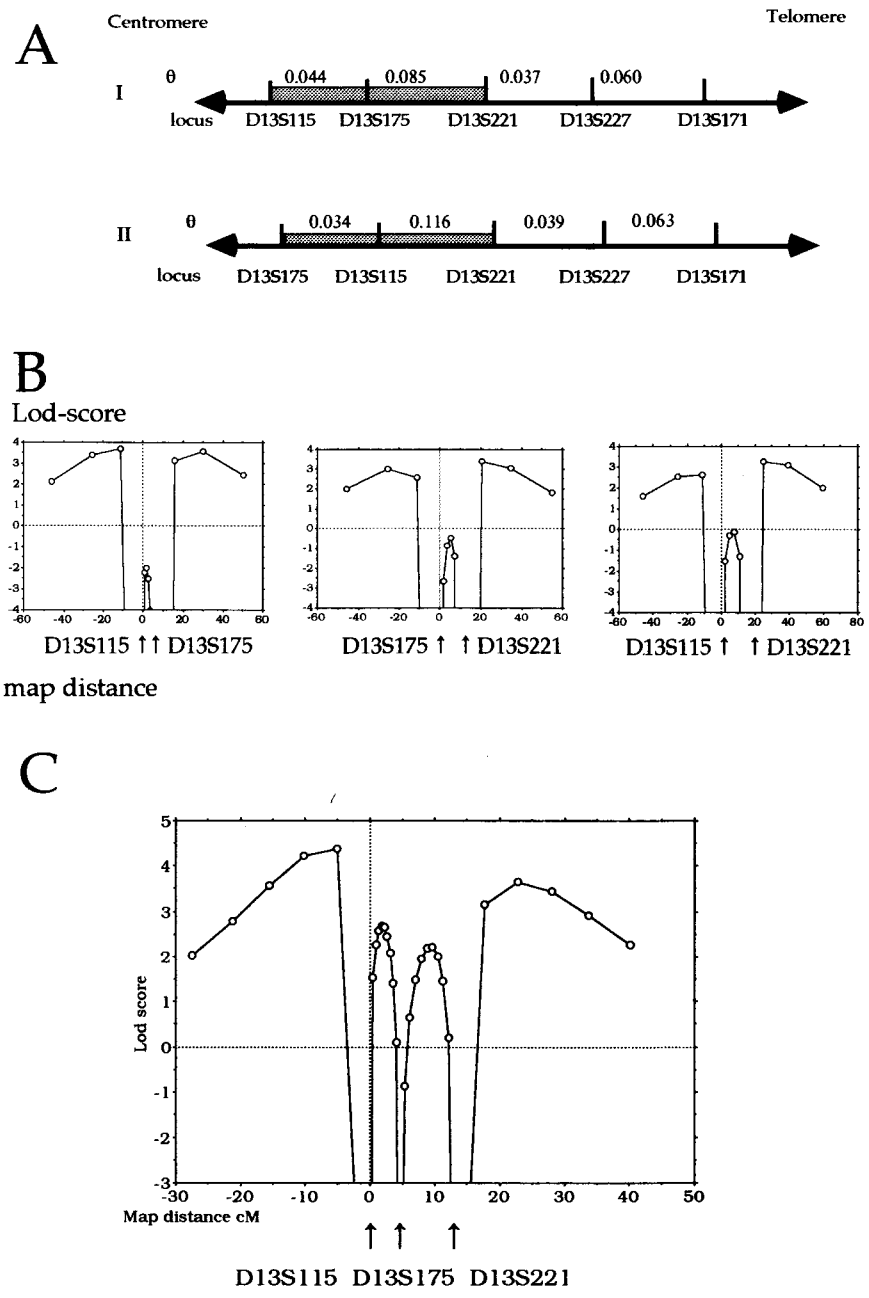

Figure 3. **A**: Genetic map of the chromosome 13 markers linked to the SCARMD locus. The best supported order is shown in I and the second best supported order in II (odds ratio = 51/1). Odds against all other orders were above 10^5. The genetic distances were slightly different from those published[32] because our figures have been computed with fewer loci. Shaded area indicates excluded region for the SCARMD locus. **B**: Three-point linkage analysis of the SCARMD locus. The graphs show the three point lod-scores (base 10) for linkage of the SCARMD locus to three fixed maps of two markers (Program LINKMAP, ref 33). Recombination fractions beetween markers have been determined on a panel of 8 CEPH families (Program CILINK, ref 33) and converted to map distances (fig. 3A) (Program MAPFUN, ref 13). The most centromeric probe is taken as the origin. Vertical arrows show marker positions. **C**: Four-point linkage analysis of the SCARMD locus. The graph show the four point lod-scores (base 10) for linkage of the SCARMD locus to the fixed map of three markers on figure 3A, I(Program LINKMAP, ref 32). This analysis has been performed on simplified pedigrees in wich all consanguinity loops have been removed Recombination fractions beetween markers have been determined on a panel of 8 CEPH families (fig. 3A) (Program CILINK, ref 33) and converted to map distances using Kosambi mapping function (Program MAPFUN, ref 13). The most centromeric probe is taken as the origin. Vertical arrows show marker positions.

in SCARMD the ultimate steps will be to check whether the 50DAG gene maps to the same region on chromosome 13 as the SCARMD locus, and to determine if it is mutated in this disease.

METHODOLOGY

Family studies

The diagnostic of SCARMD was ascertained in every patient by the same clinician (M.C.). The inclusion criteria were: (i) inheritance consistent with an autosomal

recessive pattern, with at least one affected female; (ii) muscular weakness and atrophy predominantly affecting the limb girdle; (iii) hypertrophy of the calves; (iv) clinical onset before age 10 years, and severe evolution (in our series 82% of the patients above 12 years were already wheel-chair bound); (v) increase in serum creatine kinase level; (vi) EMG and morphological examination of muscle biopsy in at least 1 affected sib showing a dystrophic process. In 13 patients (12 families) dystrophin and the dystrophin-associated proteins were investigated (Table 1). A panel of 13 families comprising 31 affected children was established for linkage analysis (pedigrees shown in Fig 1). Blood specimens were collected in Algeria from 123 subjects and lymphoblastoid cell lines established (Banque de cellules, Hôpital Cochin), followed by phenol/chloroform DNA extraction. Simulation analysis by Slink[28] showed that the number of meioses in these families was sufficient, under the assumption of homogeneity, to allow the demonstration of linkage.

Immunohistochemical analysis

Immunohistochemical analysis of dystrophin and the dystrophin-associated proteins in the biopsied skeletal muscle was performed as described previously [7, 29, 30].

Genotyping

Eighty-five highly polymorphic microsatellite markers randomly distributed on chromosomes 1, 2, 3, 4, 5, 6, 13, 15, 22 were used. The chromosome 13 markers used for linkage were the following: D13S221 (AFM 248wc1)[32], D13S175 (AFM249xb1)[32] and D13S115[34]. D13S115 was typed in 8 CEPH reference families and positioned on a map of four microsatellite markers developed at Genethon: the two used for linkage analysis of the SCARMD families (D13S221 and D13S175), and two additional ones (D13S171 and D13S227)[32] .

The detailed list of the other microsatellites tested is available upon request. The primer sequences for these markers can be obtained from Genbank or GDB. Polymorphic microsatellites were visualized as described by Vignal et al[31].

Linkage analysis

For two- and multipoint linkage analyses we used the LINKAGE package of programs[33] All genotypes were downcoded to four or five equally frequent alleles, without loss of informativeness. Changing marker allele frequencies did not significantly influence the results. Assuming that SCARMD is roughly as frequent as DMD in Algeria we set the frequency of the gene to 0.01. The robustness of the assessment was also tested against changes in disease frequency. Children under 3 years have been excluded from this study, and the disease has been assumed to be fully penetrant. Genetic homogeneity was tested by the HOMOG and MTEST programs[13].

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REFERENCES

1. Ben Hamida, M. and Fardeau, M.(1980).In: Muscular Dystrophy Research: Advances and New Trends, Angelini C, Danieli GA, Fontanari D (eds), ICS n°527, Excerpta medica, Amsterdam, pp. 143–146..
2. Ben Hamida, M., Fardeau, M. and Attia, N. (1983) *Muscle and Nerve* **6**, 469–480.
3. Masmoudi, A. (1986) MD dissertation, Faculté de Médecine, Alger.
4. Azibi, K., Chaouch, M., Reghis, A., Vinet, M C., Vignal, A., Becuwe, N., Beckmann, JS., Seboun, E., Nguyen, S., Cometto, M., Fardeau, M., Tomé, F., Leturcq, F., Chafey, P., Bachner, L., and Kaplan, J-C (1991) *Cytogenetics Cell Genetics* **58**, 1907.
5. McKusick, V.(1992) Mendelian Inheritance in Man. Catalogs of autosomal dominant, autosomal recessive, and X-linked phenotypes. The Johns Hopkins University Press, Baltimore and London 10th ed.
6. Ben Jelloun- Dellagi, S., Chafey, P., Hentati, F., Ben Hamida, C., Tomé, F M S., Collin H., Dellagi, K., Kaplan, J.C., Fardeau, M., and Ben Hamida, M. (1990) *Neurology* **40**, 1903.
7. Matsumura, K., Tomé, F M S., Collin, H., Azibi, K., Chaouch, M., Kaplan, J.C., Fardeau, M., and Campbell, K P (1992) *Nature* **359**, 320–322.
8. Campbell, K.P. and Kahl, S.D. (1989). *Nature* **338**, 259–262.
9. Ervasti, J. and Campbell, K. (1991). *Cell* **66**, 1121–1131.
10. Ibraghimov-Beskrovnaya, O., Ervasti, J M., Leveille, C J., Slaughter, C A., Sernett, S W., and Campbell, K P (1992) *Nature* **355**, 696–702.
11. Ben Othmane, K., Ben Hamida, M., Pericak-Vance, M., Ben Hamida, C., Blel, S., Carter, S., Bowcock, A., Petruhkin, K., Gilliam, T., Roses, A., Hentati, F.andVance, J. (1992) *Nature Genetics*, **2**, 315–317.
12. Bowcock, A., Osborne-Lawrence, S., Barnes, R., Chakravarti, A., Washington, S. and Dunn, C. (1993) *Genomics*, **15**, 376–386.
13. Ott, J. (1991). Analysis of human genetic linkage. *The Johns Hopkins University Press, Baltimore*.
14. Beckmann, J.S., Richard, I., Hillaire, D., Broux, O., Antignac, C., Bois, E., Cann, H., Cottingham, R.W.J., Feingold, N., Feingold, J., Kalil, J., Lathrop, G.M., Marcadet, A., Masset, M., Mignard, C., Passos-Bueno, M.-R., Pellerain, N., Zatz, M., Dausset, J., Fardeau, M.and Cohen, D. (1991) *C R Acad Sci Paris*, **312**, serie III, 141–148.
15. Young, K., Foroud, T., Williams, P., Jackson, C.E., Beckmann, J.S., Cohen, D., Conneally, P.M., Tischfield, J. and Hodes, M.E. (1992) *Genomics*, **13**, 1370.
16. Speer, M.C., Yamaoka, L.H., Gilchrist, J.H., Gaskell, C.P., Stajich, J.M., Vance, J.M., Kazantsev, A., Lastra, A.A., Haynes, C.S., Beckmann, J.S., Cohen, D., Weber, J.L., Roses, A.D.and Pericak-Vance, M.A. (1992) *Amer J Hum Genet* , **50**, 1211–1217.
17. Love, D.R., Hill, D.F., Dickson, G., Spurr, N.K., Blyth, B.C., Marssden, R.F., Walsh, F.S., Edwards, Y.H.and Davies, K.E. (1989) *Nature*, **339**, 55–58.
18. Khurana, T.S., Hoffman, E.P. and Kunkel, L.M. (1990)*J Biol Chem* **265**, 16717–16720.
19. Khurana, T.S., Watkins, S.C., Chafey, P., Chelly, J., Tomé, F.M.S., Fardeau, M., Kaplan, J.-C.andKunkel, L. (1991) *Neuromuscular Disorders*, **1**, 185–194.
20. Byth, B., Love, D., Murray, J. and Davies, K. (1991). *Cytogenet. Cell Genet.* 58, 60.
21. Farag, T. and Teebi,.A.(1990). *Am. J. Med. Genet.* **37**, 290.
22. Salih, M.A.M., Omer, M.I.A., Bayoumi, R.A., Karrar, O. and Johnson, M. (1983) *Develop. Med. Child. Neurol.*. **25**, 43–52.
23. McGuire, S.A. and Fischbeck, K.H. (1991) *Muscle & Nerve* **14**, 1209–1212.
24. Gardner-Medwin, D. (1970) *J. Med. Genet.* **7**, 334.
25. Goonewardena, P., Gustavson, K.-H., Gamstorp, I., Lundstrom, N.-R. and Petterson, U. (1988) *Clin. Genet.* **34**, 299–305.
26. Zatz, M., Passos-Bueno, M. and Rapaport, D.(1989) *Am. J. Med. Genet.* **32**, 407–410.
27. Passos-Bueno, M.R., Bakker, E., Marie, S.K.N., Pavanello, R.C., Vainzof, M., Carvalho, A.A., Cohen, D., Beckmann, J. and Zatz, M. (1993) *Hum Mol Genet*, **2**, 201–202.
28. Weeks, D., Ott, J. and Lathrop, G. (1990). *American Journal of Human Genetics* **47** [Suppl], A204
29. Ohlendieck, K., Matsumura, K., Ionasescu, V., Towbin, J., Bosch, E., Weinstein, S., Sernett, S. and Campbell, K. (1993) *Neurology* (in press).
30. Ohlendieck, K. and Campbell, K.P. (1991). *J. Cell. Biol.* **115**, 1685–1694.
31. Vignal, A., Gyapay, G., Hazan, J., Nguyen, S., Dupraz, C., Cheron, N., Becuwe, N., Tranchant, M. and Weissenbach, J. (1992) *Methods in Molecular Genetics*, vol 1 (ed. Adolph, K.W.), Academic, Orlando.
32. Weissenbach, J., Gyapay, G., Dib, C., Vignal, A., Morrissette, J., Millasseau, P., Vaysseix, G.and Lathrop, M. (1992) *Nature*, **359**, 794–801.
33. Lathrop, G., Lalouel, J., Julier, C. and Ott, J. (1985) *Am J Hum Genet* **37**, 482–498.
34. Hudson, T.J., Engelstein, M., Lee, M.K. (1992) *Genomics*, **13**, 622–629.
35. Jarolim, P., Palek, J., Rubin, H.L., Prchal, J.T., Korsgren, C. and Cohen, C.M. (1992) *Blood*, **80**, 523–529.
36. Dalla Venezia, N., Gilsanz, F., Alloiso, N., Ducluzeau, M.-T., Benz Jr, E.J. and Delaunay, J. (1992) *J Clin Invest*, **90**, 1713–1717.