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Génétique/Genetics

Genetic heterogeneity of severe childhood autosomal recessive muscular dystrophy with adhalin (50 kDa dystrophin-associated glycoprotein) deficiency

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Hétérogénéité génétique de la dystrophie musculaire autosomique récessive de l'enfance avec déficit en adhaline (glycoprotéine de 50 kDa associée à la dystrophine)

ABSTRACT

Severe autosomal recessive muscular dystrophy (SCARMD), McKusick n° 253700, has been originally described in North-African populations, in which significant linkage has been established with DNA markers mapping to the proximal region of the long arm of chromosome 13, without evidence for heterogeneity of the SCARMD locus in these populations. A striking feature of this disease is the isolated deficiency of adhalin, a sarcolemmal 50 kDa dystrophin-associated glycoprotein. We report a non-inbred French family with a milder progressive form of muscular dystrophy affecting subjects of both sexes. The parents are not affected suggesting an autosomal recessive transmission. In 4 siblings displaying mild to overt clinical signs of muscular dystrophy, serum creatine kinase was high, and muscle specimens showed variable degree of necrosis-regeneration with little fibrosis. In the 4 cases adhalin was completely absent in muscle sections, whereas dystrophin and the other members of the dystrophin-associated protein complex were normal, except for the 35 kDa dystrophin-associated glycoprotein which was decreased as usually observed in SCARMD. Linkage and homogeneity analysis using 4 microsatellite markers of chromosome 13q that are linked to the North-African SCARMD locus were performed in this family.

Note présentée par Jean Rosa.
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RÉSUMÉ

Une dystrophie musculaire sévère à transmission autosomique récessive (SCARMD) (MIM 253700) a été d'abord décrite dans des populations nord-africaines. Dans ces familles, une liaison significative a été démontrée avec des marqueurs génotypiques localisés en 13q12, sans hétérogénéité génétique. Un déficit en adhaline, glycoprotéine de 50 kDa appartenant au complexe sarcolemmique associé à la dystrophine, constitue une constante caractéristique de cette affection. Nous rapportons ici une famille d'origine française, non consanguine, comportant dans une même fratrie 4 sujets atteints d'une forme modérée de dystrophie musculaire progressive touchant les deux sexes. Chez ceux-ci, la créatine-kinase sérique est élevée, et les biopsies musculaires montrent une nécrose-régénération sans fibrose marquée et un déficit total en adhaline, alors que la dystrophine et les protéines membranaires associées sont normales, à l'exception de la glycoprotéine de 35 kDa qui est diminuée comme habituellement observé dans les SCARMD. Le mode de transmission est compatible avec une hérédité autosomique récessive, car les parents sont asymptomatiques et il n'existe pas d'autres cas dans la famille. Une analyse de liaison et une recherche d'hétérogénéité ont été effectuées dans cette famille à l'aide de 4 microsatellites polymorphes de la région 13q12 ayant antérieurement été trouvés liés au locus SCARMD. Les résultats montrent que le locus impliqué dans cette famille n'est pas lié aux marqueurs de cette région. Ce second locus pourrait

Results show that the morbid locus involved in this family does not map to the same region as the SCARMD locus. This second locus may be involved in sporadic cases of muscular dystrophy with adhalin deficiency that have been reported in Europe. ▲

Key words *: severe childhood autosomal recessive muscular dystrophies, Duchenne/Becker muscular dystrophy, dystrophin, dystrophin-associated proteins, adhalin (50 kDa dystrophin-associated glycoprotein), sarcolemma, linkage, genetic heterogeneity.*
Mots clés : dystrophie musculaire autosomique récessive sévère de l'enfance, dystrophies musculaires des ceintures, myopathie de Duchenne/Becker, dystrophine, protéines associées à la dystrophine, adhaline (50 kDa glycoprotéine associée à la dystrophine), sarcolemme, liaison, hétérogénéité génétique.

VERSION ABRÉGÉE

Une forme de myopathie sévère, ressemblant à la dystrophie musculaire de Duchenne (DMD) mais à transmission autosomique récessive (SCARMD) (MIM 253700), a été originellement décrite en Tunisie, puis retrouvée dans d'autres pays d'Afrique du Nord (Algérie, Maroc) et du Moyen-Orient. Chez ces malades, la dystrophine est normale, mais il existe un déficit isolé en une glycoprotéine de 50 kDa (50-DAG ou adhaline). Celle-ci fait partie d'un complexe de 6 protéines du sarcolemme associées à la dystrophine (DAP), constituant un lien entre le cytosquelette intracellulaire et la laminine, principal composant de la matrice extracellulaire. Le déficit en adhaline chez les sujets atteints de SCARMD est constant et caractéristique, car (1) il ne touche pas les autres membres du complexe, (2) il n'a pas été observé dans d'autres myopathies génétiquement transmises. On ignore encore si ce déficit constitue le défaut primaire ou si, comme pour le déficit global en DAP observé dans la myopathie de Duchenne, il s'agit d'un effet secondaire. Jusqu'à présent les analyses de liaison visant à localiser le ou les locus SCARMD ont été effectuées dans des familles de populations maghrébines (en Tunisie, en Algérie, au Maroc). Elles ont toutes conclu à une localisation en 13q12, sans indication d'une hétérogénéité génétique.

Récemment cinq cas sporadiques de myopathie ont été décrits chez des malades d'origine européenne, où la symptomatologie, l'histopathologie et le déficit en adhaline constituaient un ensemble très évocateur de SCARMD. Se trouvait dès lors posée la double question d'une répartition géographique non limitée aux populations endogames nord-africaines et d'une hétérogénéité génétique. Celle-ci ne pouvait être étudiée à partir de cas sporadiques. La découverte de plusieurs cas dans la famille française que nous rapportons ici nous a incités à étudier la liaison aux marqueurs de la région 13q, à la recherche d'une hétérogénéité génétique.

La famille en question est remarquable par l'importance de la fratrie : 8 sujets issus d'un couple de Français non consanguins constitué par un père originaire du nord de la France et une mère originaire d'Alsace, sans consanguinité retrouvée sur 4 générations. Le propositus (sujet II-3 de la *Fig. 1*) est un garçon vu pour la première fois à l'âge de 11 ans pour des difficultés à marcher et des crampes apparues vers 9 ans. L'examen révélait un déficit musculaire prédominant au niveau des ceintures, un signe de Gowers et une hypertrophie des mollets. Sa créatine-kinase sérique était de 50 fois la normale. Une étude systématique des autres membres de la fratrie fut alors entreprise et révéla l'existence d'une sœur de 2 ans plus âgée ayant présenté à partir de 11 ans des symptômes similaires, et montrant les mêmes signes cliniques que le propositus avec une élévation de la créatine-kinase. Deux sœurs plus jeunes (6 et 5 ans) ne présentaient qu'une discrète hypertrophie des mollets ; cependant, leur taux de créatine-kinase sérique était aussi très élevé *(Fig. 1)*. Aucun de ces 4 sujets ne présentait de retard mental, ni de signes d'atteinte cardiaque, à l'exception du propositus, où l'on notait sur l'ECG des ondes T négatives compatibles avec une hypertrophie ventriculaire gauche débutante, non manifeste à l'échocardiographie. Les 4 autres sujets de la fratrie étaient normaux, mais 2 d'entre eux présentaient un taux de créatine-kinase modérément élevé (3 à 8 fois la médiane normale, *Fig. 1*). Une biopsie musculaire a été effectuée chez les sujets à créatine-kinase très élevée (II-2, II-3, II-6, II-7) et a montré des lésions de nécrose-régénération d'intensité variable selon les individus, avec très peu de fibrose, ce qui témoigne d'une prolifération très peu active du conjonctif interstitiel, à la différence de ce qui est caractéristiquement observé dans les biopsies provenant de sujets atteints de myopathie de Duchenne ou de SCARMD typiques. L'étude immunohistologique des coupes à congélation, effectuée à l'aide d'une batterie d'anticorps dirigés contre la dystrophine et les protéines du complexe des DAP, a montré une réaction normale avec les différents antigènes, à l'exception de l'adhaline (50-DAG) qui était totalement absente. Ce déficit complet diffère de l'aspect obtenu dans les formes habituelles de SCARMD, où le marquage du sarcolemme par l'anticorps anti-adhaline n'est pas complètement aboli.

Pour l'analyse de liaison, 4 microsatellites de la région 13q ayant déjà été trouvés liés au locus SCARMD ont été utilisés. Ils étaient tous informatifs dans la famille. De multiples recombinaisons ont été observées fournissant des *lod scores* négatifs en analyse à 2 points. Ces résultats sont très évocateurs d'une absence de liaison, et cette hypothèse est renforcée par l'analyse multipoints et les tests statistiques d'homogénéité (HOMOG).

L'étude de cette grande famille a fourni des indications particulièrement intéressantes par : (1) la symptomatologie moins sévère (âge de début plus tardif, évolution apparemment plus lente) que dans les formes typiques de SCARMD ; (2) la discrétion de la fibrose ; (3) le déficit complet en adhaline ; (4) l'absence de consanguinité ; (5) l'absence de liaison avec les marqueurs de 13q dans une configuration familiale où le nombre de méioses informatives aurait suffit à donner pour n'importe lequel des marqueurs, s'il avait été lié, un *lod score* de + 2,3.

Ces résultats indiquent donc que dans cette famille le locus génétique responsable de la maladie est distinct du locus SCARMD antérieurement repéré en 13q12. Ce nouveau locus pourrait correspondre aux cas sporadiques de dystrophie musculaire avec dystrophine normale et déficit en adhaline qui sont observés dans des populations d'origine européenne. Il convient à présent de déterminer si l'un de ces locus correspond au gène de l'adhaline. Récemment, une étude de 3 familles brésiliennes avec déficit en adhaline a également montré l'absence de liaison aux marqueurs de la région 13q12. ▲

A severe childhood autosomal recessive muscular dystrophy (SCARMD), MIM number 253700 [1], resembling Duchenne muscular dystrophy (DMD), has been first described in Tunisia [2]. This disease was also found in Algeria [3], in Morocco [4] and in Middle-Eastern countries [5, 6]. In SCARMD patients dystrophin is normal [7], but the 50 kDa dystrophin-associated glycoprotein (50-DAG) is deficient [8]. After its initial discovery [8], this defect has been constantly found in consecutive studies of SCARMD patients from Algeria [3] and Morocco [4]. This protein, recently named adhalin [9, 10], is one member of the large oligomeric sarcolemmal dystrophin-protein complex comprising 4 glycoproteins (35, 43, 50 and 156 kDa) and 2 non-glycosylated proteins (25 and 59 kDa) [11-13]. This complex provides a linkage between the subsarcolemmal cytoskeleton and laminin, a major component of the extra cellular matrix [12-14]. This defect seems to be constant and characteristic since : (1) it does not affect the other proteins belonging to the complex, with the exception of the 35-DAG which is decreased to a lesser extent [8], whereas in DMD the entire complex is greatly reduced as a consequence of the lack of dystrophin [15, 16] ; (2) it was not observed in other genetically determined muscular dystrophies investigated so far [8, 10, 17]. The nature and number of gene(s) involved is unknown, and it is not known yet whether or not the primary defect lies in the adhalin gene. The SCARMD locus maps to 13q12, as demonstrated first in Tunisian families [18], and confirmed in Algeria [3] and in Morocco [4]. Adhalin deficiency was also found in muscle specimens from sporadic European patients (three French, one Italian, one Greek) with SCARMD-like symptomatology [10]. This finding indicates that the geographic distribution of SCARMD, first described in endogamic North-African populations [2-6], should be reevaluated. It also raises the question of possible genetic heterogeneity, as recently observed in Brazil [19]. We report here a family of French origin with adhalin deficiency and muscle dystrophy less severe than in SCARMD, in which the disease locus is not linked to 13q12.

Materials and methods

Case reports

The pedigree of the family, including the year of birth and the serum creatine kinase level is shown in *Figure 1*. One parent originated from Northwestern France, and the other from Alsace, and no known relationship between ancestors could be traced. No Mediterranean ancestor was known on four generations.

Case 1

This proband (subject II-3) *(Fig. 1)* was normal until the age of 9, when he presented with walking difficulties and cramps. Clinical examination at 11 years of age showed proximal weakness predominating in pelvic girdle, with Gowers sign, mild scapular winging, hypertrophy of the calves and Achilleus contractures. Serum creatine kinase level was 5550 IU (normal < 100 IU).

Case 2

A 13-year-old girl (subject II-2) *(Fig. 1)*, elder sister of the proband, had a normal development until 11 years of age when she manifested tiredness and gait disturbances. At 13 years, clinical examination revealed proximal weakness, mild scapular winging and hypertrophy of the calves. Serum creatine kinase level was 2385 IU.

Cases 3 and 4

These 6-year-old (subject II-6) and 5-year-old (subject II-7) younger sisters of the proband were almost clinically asymptomatic, with the exception of a moderate calf hypertrophy. However serum creatine kinase was high (6555 IU and 8370 IU in patients II-6 and II-7, respectively).

The four cases had normal intellectual development. There was no cardiac abnormality in cases 2, 3 and 4. In the proband (case 1), although the echocardiogram was normal, the ECG showed negative T waves, which might correspond to incipient left ventricular hypertrophy.

Other family members

The four other siblings of the kindred were clinically normal, but serum creatine kinase was above the upper normal limit in subject II-1, a 16-year-old boy (169 IU) and

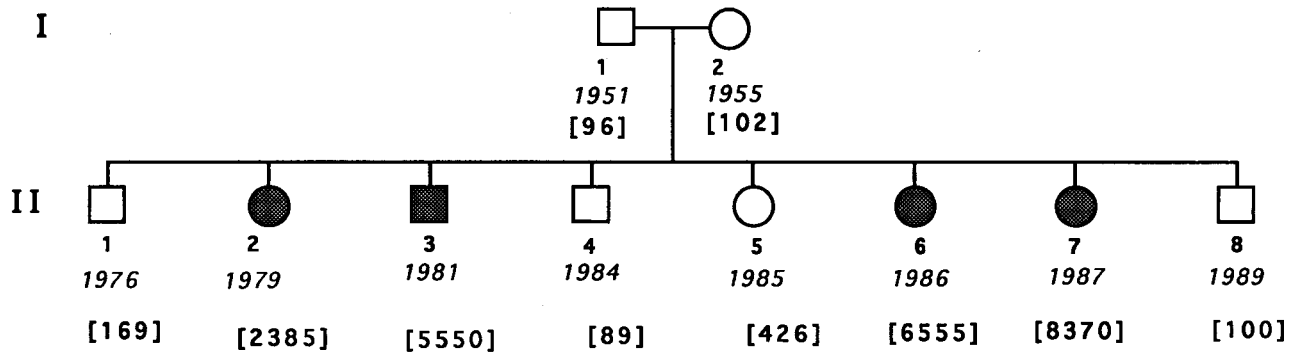

Figure 1. ***Pedigree of family H.*** *Dark symbols indicate subjects with complete absence of sarcolemmal adhalin. In italics : year of birth ; within brackets : level of serum creatine kinase [N = 25-95 units].*

subject II-5, a 7-year-old girl (426 IU), both completely asymptomatic. The parents were clinically normal, with a serum creatine kinase level at the upper normal limit (96 and 102 IU). There was no other case in the kinship.

Muscle biopsies

A biopsy of deltoid muscle was obtained for morphological and immunocytochemical studies from the four subjects with very high creatine kinase (II-2, II-3, II-6 and II-7, *Fig. 1*). A portion of the biopsies was immediately frozen in isopentane cooled in liquid nitrogen, and stored at −80 °C until processing for histoenzymological, immunocytochemical and Western-blot analysis. Conventional histological and histochemical techniques were performed in 10 μm transverse frozen cryostat sections as in previous studies [10, 20].

Immunocytochemistry

Indirect immunofluorescence analysis, exploring dystrophin (monoclonal antibodies DYS2 and DYS3 from Novocastra, and XIXC2 [15]) and five members of the dystrophin-glycoprotein complex : 50-DAG, 156-DAG, 59-DAP, 43-DAG, 35-DAG (affinity-purified polyclonal or monoclonal antibodies), was performed as in already described protocols [10, 20], and as specified in the legend of *Figure 3*.

Linkage analysis

We used three microsatellite markers (D13S115, D13S175, D13S221) that we had previously found linked to the disease locus in SCARMD families from Algeria and Morocco [3, 4], and an additional one, D13S143 [21], also linked (unpublished results). For two- and multipoint linkage analyses we used the Linkage package of programs [22]. Genetic homogeneity was investigated by the HOMOG and MTEST programs [23].

Results

Histological and histochemical reactions on muscle biopsies from the proband (subject II-3) showed a necrotic-regenerating pattern of the muscle fibers, frequently focal. Similarly, intense necrosis was observed in muscle sections from subjects II-6 and II-7. Subject II-2 presented very few necrotic lesions and occasional hypercontracted fibers. In all cases the proliferation of interstitial connective tissue was minimal *(Fig. 2)*.

Immunocytochemical analysis of the muscle biopsy specimens of the four children using monoclonal antibodies against dystrophin revealed normal staining of the sarcolemma *(Fig. 3)*. Immunoblot analysis of dystrophin performed in skeletal muscle from patient 1 confirmed that dystrophin was normal (not shown).

The most striking change was a complete absence of immunoreactivity with the antibody against 50 kDa dystrophin-associated glycoprotein (adhalin) in the muscle fibers in all patients *(Fig. 3)*. The labeling of the other members of the sarcolemmal dystrophin-glycoprotein complex was normal, with the exception of the 35-kDa dystrophin-associated glycoprotein (35-DAG) which was

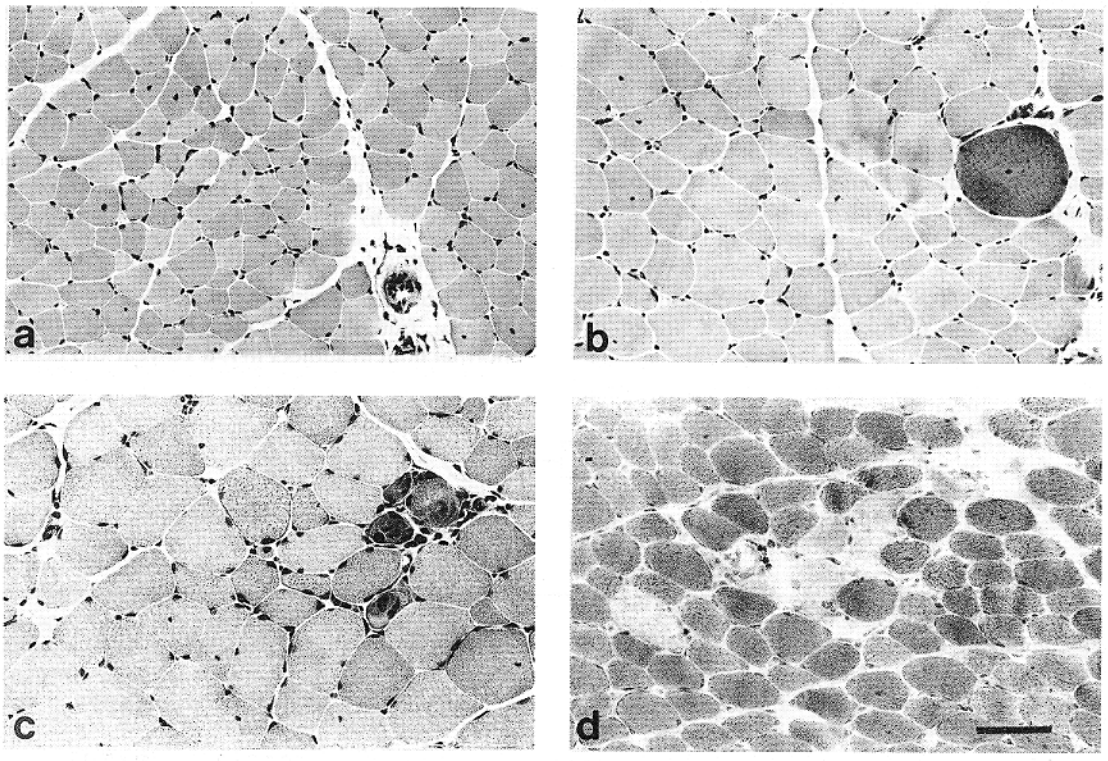

Figure 2. ***Histological changes in the 4 patient muscle biopsies.*** *Transverse 10-μm skeletal muscle cryosections stained by hematoxylin-eosin show a necrotic-regenerating pattern of muscle fibers, frequently focal, with minimal connective tissue proliferation. Occasional hypercontracted fibers are seen in one case.* ***a*** *: patient II-6 ;* ***b*** *: patient II-2 ;* ***c*** *: patient II-3 ;* ***d*** *: patient II-7. (Bar = 100 μm.)*

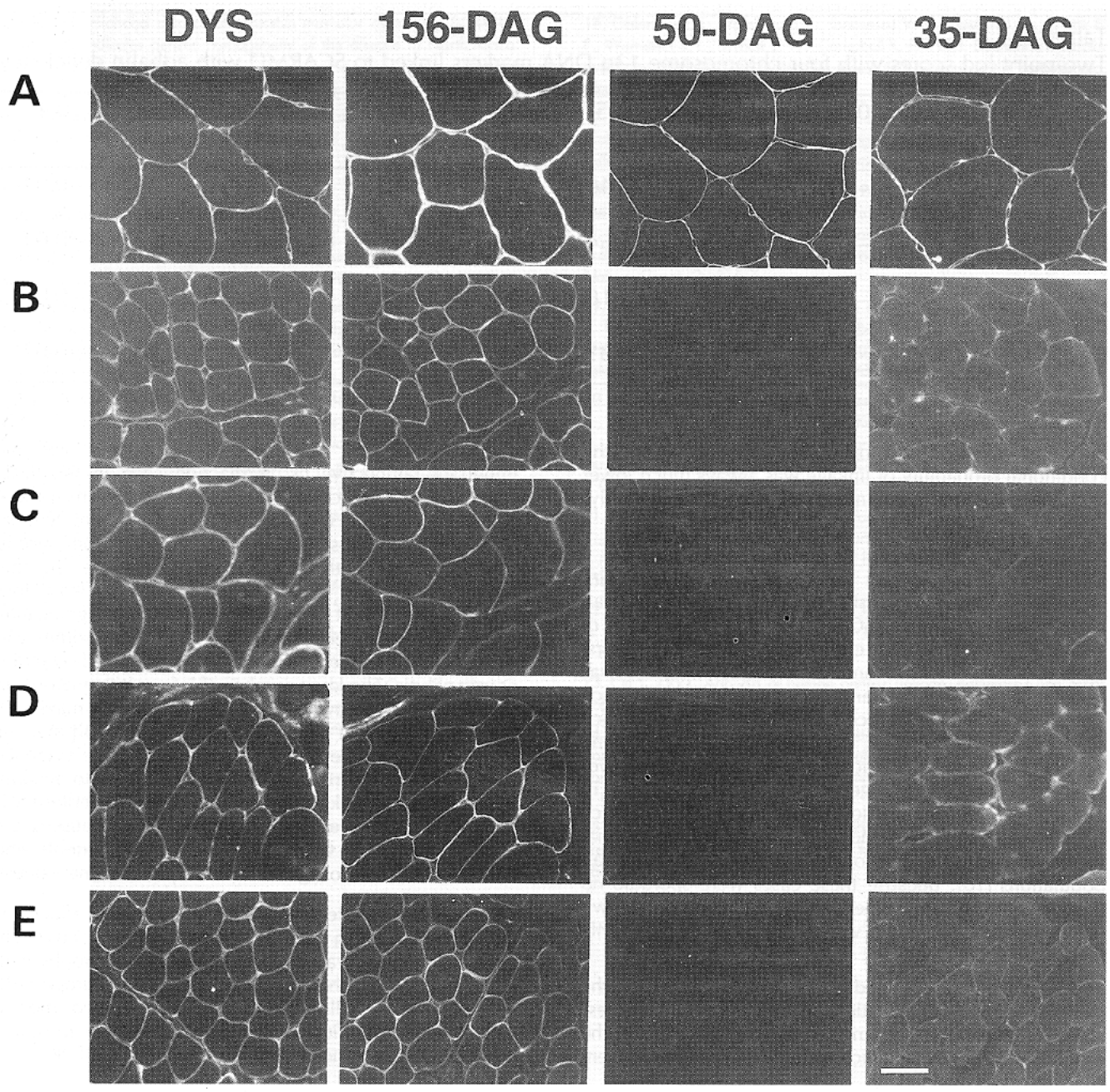

Figure 3. ***Immunofluorescence analysis of dystrophin and dystrophin-associated glycoprotein expression in the four affected members of this family.*** *Transverse 7-μm skeletal muscle cryosections from a normal individual (lane A) and from patients II-6 (lane B), II-2 (lane C), II-3 (lane D), and II-7 (lane E) were immunostained as described [8]. Antibodies used were monoclonal antibody XIXC2 against dystrophin [15], monoclonal antibody IVD3$_1$ against the 50 kDa dystrophin-associated glycoprotein [15, 24], or affinity-purified polyclonal antibodies against the 156 kDa [13] and 35 kDa [25] dystrophin-associated glycoproteins. (Bar = 50 μm.)*

decreased in all four patients relative to controls *(Fig. 3)*, as usually seen in other SCARMD patients [8, 10].

The four microsatellite markers linked to the SCARMD locus at 13q were informative. Multiple crossovers involving these markers were detected, giving negative lod scores in 2-point linkage analysis *(Table I)*. These results exclude linkage for genetic distances that were found by previous genetic analysis on SCARMD families from North-Africa with significantly positive lod scores [3, 4, 18]. Multi-point linkage analysis reinforced the exclusion: three- and four-point analysis (LINKMAP), performed by moving the disease locus along several alternative maps of three of the four markers used, showed exclusion of linkage to the 13q12 region. Five-point analysis could not be done due to computational limits. Homogeneity testing (HOMOG) was performed by comparing two-point lod scores obtained with this family to those obtained with D13S115, D13S175, D13S221 in 22 linked informative Algerian and Moroccan families. Although

Table I
Two-point lod scores with four chromosome 13q DNA markers linked to SCARMD with adhalin deficiency

θ marker	0	0.001	0.01	0.05	0.1	0.15	0.2	0.3	0.4
D13S115	$-\infty$	−9.10	−5.12	−2.46	−1.42	−0.89	−0.56	−0.20	−0.04
D13S175	$-\infty$	−6.69	−3.70	−1.64	−0.83	−0.42	−0.18	−0.02	−0.03
D13S221	$-\infty$	−6.10	−3.13	−1.18	−0.47	−0.15	−0.02	−0.11	−0.05
D13S143	$-\infty$	−2.50	−1.50	−0.81	−0.52	−0.35	−0.24	−0.10	−0.02

no complete exclusion of linkage was obtained, the conditional probability of linkage was always much lower in this family than in any individual North-African family.

Discussion

Deficiency of the 50 kDa dystrophin-associated protein, or adhalin, has been first described in North-African patients suffering from SCARMD [8]. The linkage of the SCARMD locus to chromosome 13q12 markers, originally described in families from Tunisia [18], was also found in SCARMD families with adhalin deficiency from Algeria [3] and Morocco [4]. There was no evidence of genetic heterogeneity, suggesting a common gene defect in these North-African populations [4]. However in Brazil, genetic heterogeneity has been observed in 3 families with adhalin deficiency [19]. Isolated deficiency of adhalin was also found in patients with autosomal recessive muscular dystrophy occurring in European populations [10]. Because these cases were sporadic no linkage analysis could be performed, and it was not known whether the disease locus was the same as the SCARMD locus on chromosome 13.

The large kindred presented here is remarkable by: (1) the peculiar clinical presentation with later onset and less rapid progression than in most cases of SCARMD; (2) the distinctive histo-pathological features with prominent necrosis and very little fibrosis, contrasting with the important connective tissue reaction seen in SCARMD and DMD; (3) the complete absence of adhalin in the 4 patients, contrasting with the faint labelling of sarcolemmal adhalin usually observed on cryosections from North-African patients with SCARMD [3, 8]; (4) the fact that a French ancestry could be proven in at least four generations, without likely inbreeding; (5) the significantly negative lod scores obtained with chromosome 13q markers linked to the SCARMD locus, reinforced by multi-point analysis. The large number of informative meioses in this family would have been sufficient to yield a maximum lod score of +2.3 if any of the chromosome 13q markers used had been linked to the disease locus. The evidence of non-linkage is further increased by taking into account the serum creatine kinase level. However this criterion is difficult to use for heterozygote assessment because of the broad dispersion of values (note that the two parents who are obligatory heterozygotes have only borderline values).

These results indicate that in this family the gene affected is distinct from the 13q SCARMD locus. This new locus might account for the sporadic cases of muscular dystrophy with adhalin deficiency that have been reported in Europe [10]. Further studies will show if one of the two morbid loci already identified in autosomal recessive muscular dystrophy corresponds to the adhalin gene itself. ▼

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