Primary adhalinopathy (α-sarcoglycanopathy):

Clinical, pathologic, and genetic correlation in 20 patients with autosomal recessive muscular dystrophy

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Article abstract—Primary adhalin (or α-sarcoglycan) deficiency due to a defect of the adhalin gene localized on chromosome 17q21 causes an autosomal recessive myopathy. We evaluated 20 patients from 15 families (12 from Europe and three from North Africa) with a primary adhalin deficiency with two objectives: characterization of the clinical phenotype and analysis of the correlation with the level of adhalin expression and the type of gene mutation. Age at onset and severity of the myopathy were heterogeneous: six patients were wheel-chair bound before 15 years of age, whereas five other patients had mild disease with preserved ambulation in adulthood. The clinical pattern was similar in all the patients with symmetric characteristic involvement of trunk and limb muscles, calf hypertrophy, and absence of cardiac dysfunction. Immunofluorescence and immunoblot studies of muscle biopsy specimens showed a large variation in the expression of adhalin. The degree of adhalin deficiency was fairly correlated with the clinical severity. There were 15 different mutations (10 missense, five null). Double null mutations (three patients) were associated with severe myopathy, but in the other cases (null/missense and double missense) there was a large variation in the severity of the disease.

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Alterations in the components of the sarcoglycan complex cause different types of muscular dystrophies. The sarcoglycans form a subcomplex in the dystrophin-glycoprotein-complex, linking the subsarcolemmal cytoskeletal proteins to the extracellular matrix. This subcomplex is composed of four transmembrane glycoproteins, one of 50, one of 43, and two of 35 kDa, named α- (or adhalin), β-, γ-, and δ-sarcoglycans,[1-4] respectively. In 1992, Matsumura et al.[5] reported adhalin deficiency in muscle biopsy specimens from Algerian and Lebanese patients presenting with a severe childhood autosomal recessive muscular dystrophy (SCARMD). The clinical phenotype of this disease was close to Duchenne (DMD) and Becker (BMD) muscular dystrophies as reported by Ben Hamida et al.[6] in 1983. Age at onset was between 3 and 15 years, and the patients presented with proximal weakness most often associated with hypertrophy of calves. The levels of serum creatine kinase were usually very high. Presence of dystrophin in the muscle biopsy specimens[7] led to a search for a deficiency in one of the dystrophin-associated glycoproteins (DAG)/dystrophin-associated proteins (DAP) and to the discovery of adhalin deficiency.[5] Several authors have subsequently reported adhalin deficiency in European,[8-10] Brazilian,[11,12] and Asian[13-15] patients with SCARMD or milder muscular dystrophies.

Genetic studies indicated a heterogeneity of adhalin deficiencies. Ben Othmane et al.[16] mapped the defective gene, responsible for the form of SCARMD prevalent in North Africa, to chromosome 13q12 and this was confirmed by Azibi et al.[17] Passos-Bueno et al.[11] reported exclusion of this locus by linkage analysis in three Brazilian families and Romero et al.[9] reported similar results in one French family, all affected by adhalin deficiency. Roberds et al.[18] subsequently mapped the adhalin gene to chromosome 17q12-q21.33 and Roberds et al.[19] identified the first missense mutations in a previously reported French family.[9] Different groups subsequently reported other cases of "primary" adhalinopathy.[12,14,20,21] Secondary adhalin deficiencies are due to mutations affecting β-, γ-, or δ-sarcoglycan genes, which are localized to chromosomes 4q12,[22,23] 13q12,[24] or 5q33.[3,4]

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Table *Main clinical, immunochemical, and molecular data on the cohort of patients with adhalin (α-sarcoglycan) deficiency*

Family: case number	Age/sex	Age of onset (yr)	Clinical grade	Alpha-sarcoglycan IF	Alpha-sarcoglycan IB	Adhalin gene mutation: localization in each allele
Group A: Europe						
1	26/F	14	III	(+)	(+)	Arg(77)Cys/Gly(68)Gln
2	3/F	5	I	0	Trace	Arg(77)Cys/Arg(77)Cys
3	25/M	13	III	(+)	Trace	Nonsense (151)/Val(247)Met
4	13/M	6	VII	0	0	Nonsense (80)/Arg(77)Cys
5	24/M	8	VI/VII	Trace	Trace	Tyr(62)His/Val(242)Ala
6a	14/M	9	VI/VII	0	Trace	Arg(98)His/Val(175)Ala
6b	16/F	12	III	0	Trace	Arg(98)His/Val(175)Ala
6c	10/F	9	I	0	Trace	Arg(98)His/Val(175)Ala
6d	8.5/F	6	II	0	nd	Arg(98)His/Val(175)Ala
7	35/F	8	VII	(+)	(+)	Arg(77)Cys/Arg(284)Cys
8a	32/F	13	III	(+)	(++)	Val(247)Met/nd
8b	34/F	15	III	nd	nd	Val(247)Met/nd
9	36/M	6	IV	(+)	(+)	Splice intron 7/Arg(98)His
10	21/F	8	VII	0	Trace	Arg(77)Cys/Arg(34)His
11	17/F	7	V	Trace	Trace	Arg(34)His/Arg(34)His
12	29/M	13	III	(++)	(++)	Arg(77)Cys/Arg(284)Cys
Group B: North Africa						
13	11/F	4	VII	0	0	Splice intron 1/splice intron 1
14	10/F	3	VI	Trace	0	Arg(34)Cys/Arg(34)Cys
15a	20/M	6	VII	0	0	Splice intron 6/splice intron 6
15b	19/F	6	VII	0	0	Splice intron 6/splice intron 6

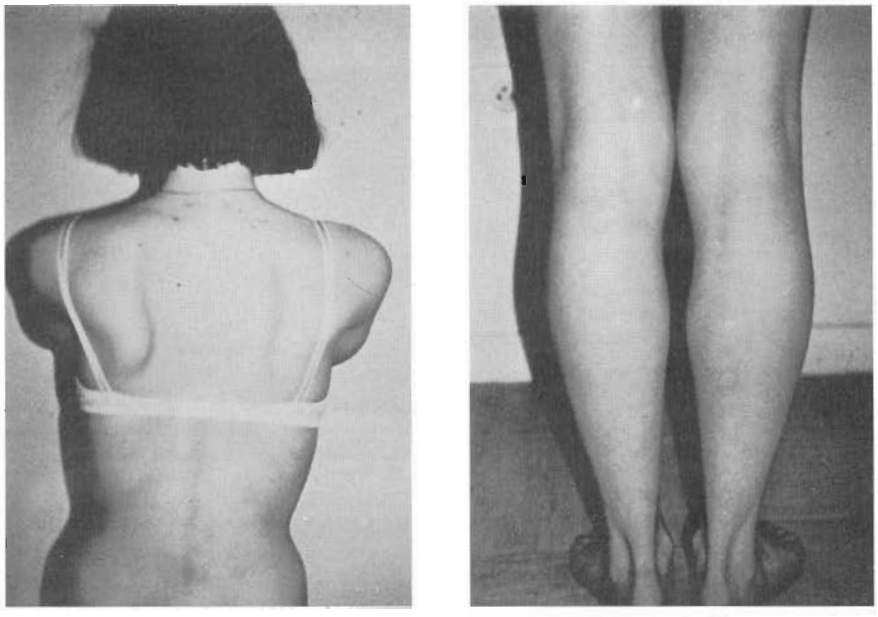
Note: families 1, 3, 4, 5, 6, 8, 9, 11, 15 were mentioned in a previous publication.[20]

IF = immunofluorescence staining; IB = immunoblotting; nd = not done.

We have identified in our group more than 80 patients with adhalin deficiency (primary or secondary). Here we report 20 cases of primary adhalinopathy, with two main objectives: characterization of the clinical phenotype and analysis of the correlations with the level of adhalin expression and the type of gene mutation. In a previous report[20] we have briefly mentioned several patients of this series.

Material and Methods. *Patients.* The 20 patients included in this study fulfilled the following selection criteria: (1) had clinical examination by one of us (12 in Salpêtrière Neuromuscular Center, two in Bologna, one in Angers, one in Saint Vincent de Paul Hospital, one in Clermont, one in Strasbourg, two in Alger), (2) had undergone a muscle biopsy of limb muscles demonstrating normal dystrophin and adhalin deficiency by immunofluorescence and by immunoblot techniques, (3) had a mutated adhalin gene. For each subject, the following parameters were obtained: age, sex, geographic origin, consanguinity, family history, distribution and severity of the weakness, presence of calf hypertrophy. The functional stage of the patients was graded from I to VII according to the scale proposed by Gardner-Medwin and Walton[25] in 1974, in which stage 7 corresponds to loss of ambulation. Manual testing of muscle strength (according to international procedures; Medical Research Council, 1943) was performed on 12 patients followed in the Salpêtrière Neuromuscular Center. Serum creatine-kinase (CK) levels were measured in all patients. Electromyographic examinations were obtained in six patients. CT of lower limb muscles was performed on nine patients. Echocarcardiogram and vital capacity were obtained in 17 and 15 patients, respectively.

Muscle biopsy. A biopsy of deltoid muscle was performed, after appropriate informed consent, from 19 patients for morphologic and immunocytochemical studies. In one family with two affected patients the muscle biopsy was performed only on one patient. The biopsy samples were frozen in isopentane cooled in liquid nitrogen and stored at −80°C. Conventional histologic and histochemical techniques were performed in 10-μm transverse frozen sections. Immunocytochemical studies were carried out on these biopsy specimens with anti-dystrophin antibodies (monoclonal NCL DYS2 and DYS 3 antibodies from Novocastra), and anti-sarcoglycan antibodies, 50 DAG [adhalin or α-sarcoglycan] (monoclonal IVD3-1[5] and NCL 50DAG



A B

Figure 1. Muscular involvement pattern (case 6b). (A) Scapular winging. (B) calf hypertrophy.

from Novocastra), 43 DAG [β-sarcoglycan] (rabbit polyclonal[22]) and 35 DAG [γ-sarcoglycan] (sheep polyclonal[26] and monoclonal 35 DAG/21B5 from L. Anderson), as previously described.[8,26] The δ-sarcoglycan was reported very recently,[3,4] and thus not studied in the present series of patients. Immunoblotting for dystrophin and for the sarcoglycan proteins were also performed as reported.[20,26] The immunostaining status was arbitrarily graded from 0 (absent) to +++ (normal expression, as observed in biopsy specimens of deltoid muscle from patients without muscle disorders).

Mutation analysis of α-sarcoglycan gene. Genomic DNA extracted from blood or lymphoblastoid cells was analyzed by DGGE after polymerase chain reaction (PCR) using oligonucleotide primers flanking the intron-exon junction of the α-sarcoglycan gene as previously described.[19,20] All mutations were checked by restriction enzyme analysis when possible or allele specific oligonucleotide (ASO) typing.

Results. *Clinical data.* Sex and age. The 20 patients (13 females and seven males, aged 3–36 years) were examined at different stages of the disease. They are listed individually in the table.

Origin. Sixteen patients were originated from Europe (14 from France, two from Italy) and four from North Africa (one from Morocco, three from Algeria; the latter belonged to two different families).

Family history. Two or more affected patients were present in each of three sibships (two from Europe, nos. 6 and 8, one from North Africa, no. 15). Among the three families from North Africa, two were inbred (cases 13 and 14), and consanguinity was suspected in one (no. 15). These data suggested an autosomal recessive transmission. No consanguinity was known in the European families.

Age of onset and first clinical signs. The average age of onset of clinical manifestation was 8.5 years, with a large range of 3 to 15 years. Mean age of onset was 8.7 ± 2.9 in males and 8.46 ± 3.8 in females and was earlier in North-African than in European patients (respectively 4.75 ± 1.3 and 9.8 ± 3.1). Fourteen patients presented clinical manifestations before 12 years of age. Three patients (cases 2, 6c, and 6d) were detected at a presymptomatic stage due to an elevation of serum CK at ages 3, 4, and 5 years. In two of these patients (cases 6c and 6d) the serum CK level was estimated because they belonged to an at-risk family with two affected siblings; in patient 2, the CK testing was carried out at the time of a check-up for an intercurrent disease. The three patients (cases 2, 6c, and 6d) developed signs of myopathy respectively at 5, 6, and 9 years. In three cases (cases 8a, 8b, and 12) exercise intolerance was the earliest symptom. In addition, patient 12 had episodes of painful muscle swelling without dark urine. In all other cases, the first complaints were difficulties in climbing stairs and in running. Six patients (cases 4, 9, 10, 13, 15a, and 15b) had a tendency to walk on tip-toe. Proximal upper limb involvement appeared later, but the time elapsed between lower and upper girdle deficiency was difficult to assess because of the less severe functional limitations of the upper limb weakness.

Topography and characteristics of the muscle involvement. All symptomatic patients had weakness of proximal limb muscles predominating in pelvic girdles. The pattern of muscle weakness was roughly similar in all cases, characterized by symmetric and selective involvement of the muscles (figure 1). When examined in the early stages of the disease (stages II/III), patients had waddling gait and slight hyperlordosis. Muscle weakness predominated in glutei and adductors. The psoas were slightly less involved. Femoral muscles were less affected than pelvic muscles; quadriceps and hamstrings were similarly involved. The distal muscle involvement was minimal and predominated in the tibialis anterior. At this stage, even if not considered as functionally limiting, the weakness was also evident in the scapular girdle muscles with scapular winging, limited antepulsion, and abduction of the arms. The weakness of the scapula fixators was diffuse, predominating in serratus magnus, trapezius inferior, latississimus dorsi, rhomboid, and subscapularis. The infraspinatus

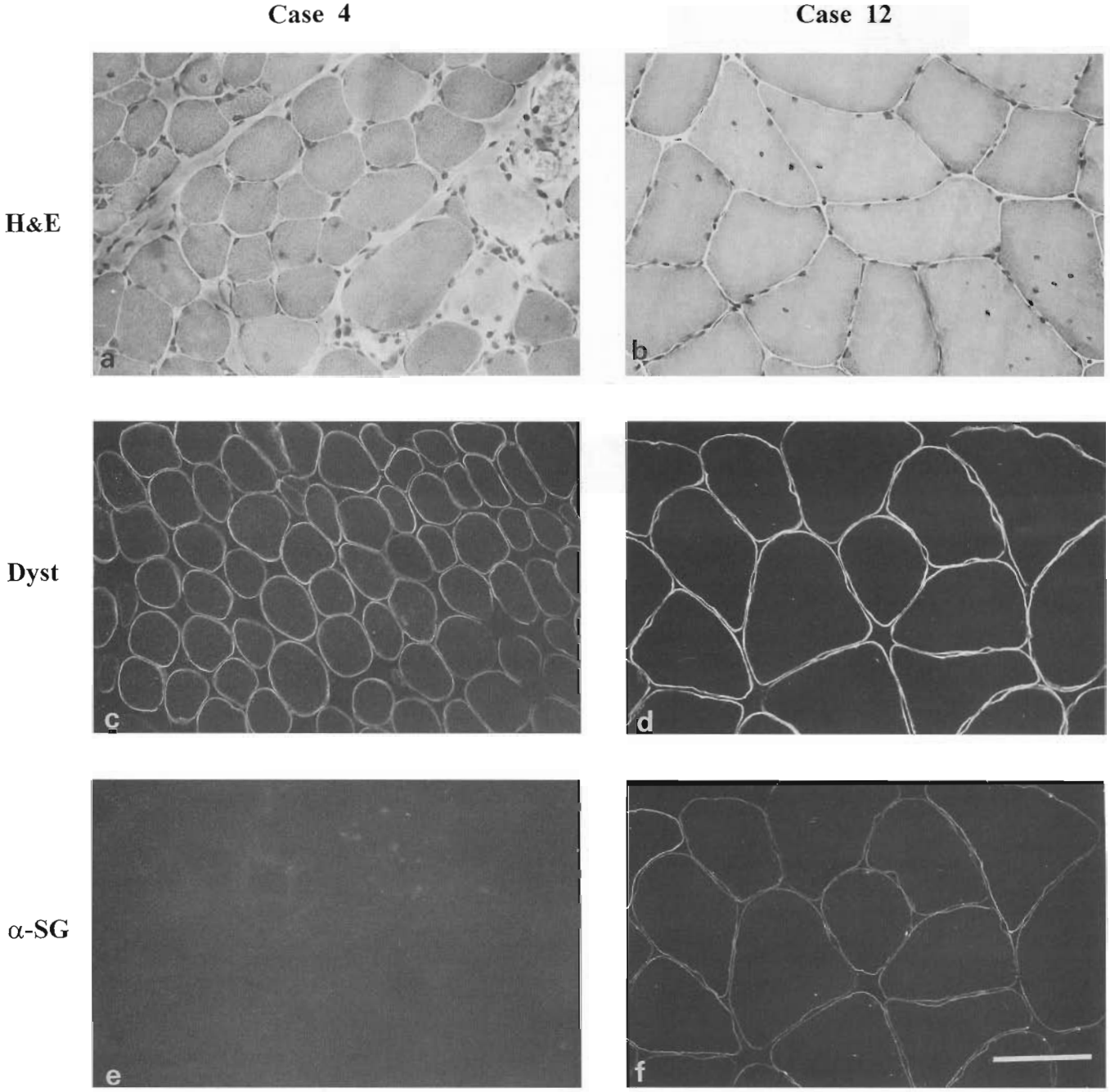


Figure 2. Histologic and immunocytochemical study of muscle biopsy specimens showed variable changes. Hematoxylin-eosin (H&E) staining of cryostat sections showing a necrotic-regenerating pattern in case 4, biopsied at 8 years of age (A) and minor changes, consisting mainly in variation in muscle fiber diameter and internal nuclei, in case 12, biopsied at 28 years of age (B). Immunocytochemical analysis of serial sections did not reveal significant changes of dystrophin expression in these cases: case 4 (c), case 12 (d), while α-sarcoglycan was absent in case 4 (e) or moderately reduced in case 12 (f). Bar = 100 μm.

muscle was clearly more affected than supraspinatus. The involvement of deltoid was early and marked. The biceps brachii muscle involvement was also present, but less pronounced. Triceps brachii, pronator, and supinator radii were minimally and infrequently involved. Other upper limb muscles were spared. Trunk extensors were more affected than the abdominal; neck muscles were relatively spared. At more advanced stages of the disease (V to VII), the distribution of the muscle weakness remained identical, extending in the upper limbs to the triceps brachii, pronator, and supinator radii and to a lesser degree to palmaris and radialis and in the lower limbs to the posterior tibialis and peroneus. The selectivity of muscle involvement was still present, the severe weakness and atrophy of biceps brachii contrasting with partial respect of triceps. Hand muscles and triceps surae were relatively spared even at late stages. Calf hypertrophy was present in all but three patients (nos. 7, 8a, and 8b). In addition, hypertrophy of the lower part of thigh muscles was found in three mildly affected cases: 8a, 8b, and 12. In the other

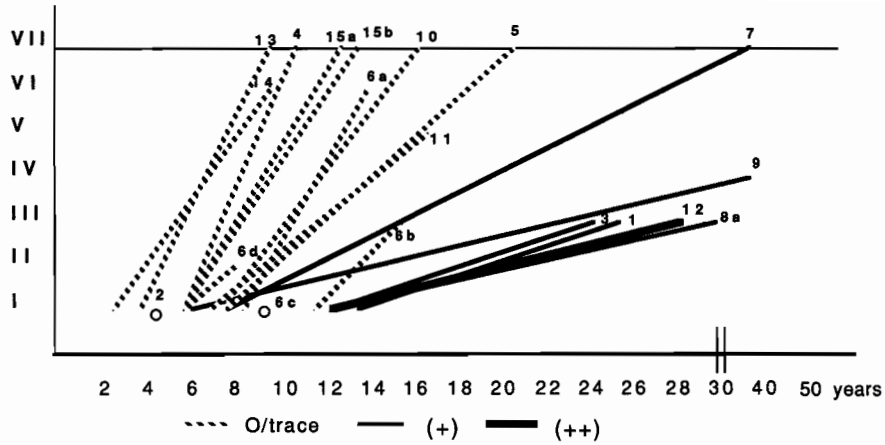


Figure 3. Correlation between clinical severity and the α-sarcoglycan expression tested by immunofluorescence in 19 patients. Functional stages graded from I to VII according to the Gardner-Medwin and Walton scale (1974).[25] Patients 13, 14, 15a, and 15b are originated from North Africa. The numbers close to the lines indicate the number of the case in the table. Intensity of immunostaining was visually estimated (normal signal, scored +++). Hatched lines correspond to complete absence or trace of α-sarcoglycan; thin and thick continuous lines correspond to a partial α-sarcoglycan deficiency, respectively scored + and ++. Patients 2 and 6c, represented by open circles, had absence of α-sarcoglycan. The numbers close to the lines indicate the number of the case in the table.

cases, there was a clear evidence of thigh atrophy. Atrophy was also present in scapular girdle muscles and in biceps brachii. Enlarged tongue was observed in only one case (no. 10). In the early stages and mild cases, contractures were absent or moderate, limited to the calf muscles, and sometimes associated with contractures around the knee. In the severely affected patients, marked contractures developed around the ankles, hips, and knees. Heel cord tenotomy was performed in two cases (nos. 13 and 15b). Kyphoscoliosis was found in six cases, requiring surgical correction in only one case (no. 6a). There was never any clinical involvement of facial, ocular, and velopharyngeal muscles. Intellectual impairment was never observed.

CT. The CT examination revealed the selective involvement of muscles. The pelvic muscles presented the more severe degree of involvement, particularly the glutei. Among the femoral muscles, those of the posterior and the deep anterior compartments were similarly involved. In contrast, the internal compartment muscles were spared in the mildly affected patients. Hypertrophy of rectus femoris, sartorius, and gracilis was observed in a mildly affected patient (case 12). The changes were more severe in the upper part of thigh than in lower one. Distal muscles were preserved except in the severe cases, in which there was some involvement of the tibialis anterior.

Ancillary investigations. Electromyographic examination showed abnormalities compatible with a myopathic process. Serum CK levels were constantly elevated, ranging from four to 90 times the normal value. There was no correlation between CK levels and age or functional stage. The respiratory function was normal or moderately reduced even at stage VII. Ventilatory assistance was never required. Echocardiographic examination performed in 17 patients did not reveal any significant abnormality. Electrocardiography showed negative T waves in one patient (case 6a).

Evolution and severity. There was a marked heterogeneity of severity. Eight patients became wheelchair bound: six of them before 15 years of age (cases 4, 6a, 10, 13, 15a, and 15b), one at age 21 (case 5), and one at age 32 (case 7). In our series, all patients of North African origin had severe myopathy. The clinical course was particularly mild in five European patients (cases 1, 8a, 8b, 9, and 12), with onset between 12 and 14 years of age, and a moderate weakness in adulthood. Generally, the earlier the onset, the faster the progression.

There was a variability of the clinical severity and age of onset in one French family (case 6) with four affected members: in one patient (case 6a), the muscle weakness began early and progressed rapidly while in another (case

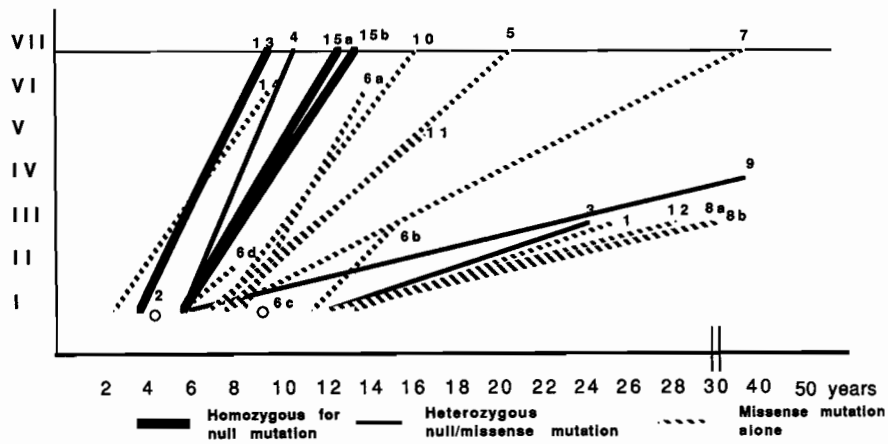


Figure 4. Correlation between clinical severity and the gene mutations. Thick continuous lines correspond to null mutations in homozygous conditions; thin continuous lines correspond to patients heterozygous, null/missense mutation; hatched lines correspond to patients with a double missense mutations, generally in heterozygous conditions. Patients 2 and 6c, represented by open circles, had double missence mutations. The numbers close to the lines indicate the number of the case in the table.

6b) the disease manifested later and had a slower evolution (see table). On the other hand, no intrafamilial variability was observed in two other families, one from France (no. 8) and another from North Africa (no. 15), each one with two affected members. Several patients were previously diagnosed as having: DMD, BMD (cases 3, 5, 10, 12), DMD "in girls" (cases 1 and 10); only one patient (case 11) was considered as having limb-girdle dystrophy. A metabolic myopathy was suspected in two sisters (cases 8a and 8b) who complained of a painful fatigability from 15 years of age and had a mild and almost stable proximal weakness.

Histopathologic data. Light microscopic study of transverse sections of muscle biopsy specimens showed variation in muscle fiber diameter without group atrophy and increased number of internal nuclei. Necrosis and regeneration was intense in several patients, particularly in young, severely affected patients, and moderate or absent in a few others (figure 2). There was also a marked variation in the intensity of the fibrosis. Type I predominance was common. These findings were compatible with a primary muscular dystrophic process.

Immunocytochemistry and immunoblotting data. Adhalin (α-sarcoglycan) deficiency assessed by immunofluorescence was complete in 10 out of 19 patients (53%), marked (trace) in three cases (16%), less pronounced (scored + or ++) in six cases (31%) (see figure 2). The immunoblot analysis for α-sarcoglycan showed on the whole similar results (see table). There was a fairly clear correlation between α-sarcoglycan deficiency and clinical severity (figure 3). Among the five more slightly affected patients, (stage III or IV after 25 years of age), none had a complete defect: α-sarcoglycan expression was scored + in four cases (nos. 1, 3, 8a, and 9) and scored ++ in one case (no. 12). The six more severely affected patients (wheelchair-bound before 15 years) had a very marked α-sarcoglycan deficiency. The β- and γ-sarcoglycan expression studied in eight and 14 cases respectively was always decreased (see table). However these proteins were always more highly expressed than α-sarcoglycan; they were present in muscle biopsy specimens from patients with a complete absence of α-sarcoglycan.

Adhalin (α-sarcoglycan) gene mutations. In the present series of 20 patients, 15 different mutations of the α-sarcoglycan gene were found: 10 missense and five null (see table). All the missense mutations resided in the extracellular domain. Six patients from five sibships were homozygous for α-sarcoglycan gene mutation, including the three consanguineous North African families and two European families without evidence of consanguinity (nos. 2 and 11). Twelve patients from nine European families were compound heterozygotes. In two patients from one European family (no. 8), only one mutation could be detected. Among the null mutations, three were of aberrant splicing type and two were nonsense. Double missense mutations were found in twelve patients, from nine families. The arginine-cysteine substitution at amino acid 77 was the most frequent mutation, affecting a cohort of six patients. The correlation between the type of mutation and the clinical severity of myopathy is shown in figure 4. The three patients homozygous for a null mutation (cases 13, 15a, and 15b) were early wheel-chair bound and had a complete α-sarcoglycan deficiency. Among the three patients having both a null and missense mutation, two were mildly affected and in them the expression of α-sarcoglycan was moderately reduced (cases 3 and 9), while the third patient (case 4) had a severe myopathy and absence of α-sarcoglycan. The patients with double missense mutations could have a mild (cases 1, 6b, and 12) or a severe myopathy (cases 5, 6a, 10, and 14). The same combination of missense mutations Arg 77 Cyst/Arg 284 Cyst was found in two patients from different sibships with different severity (case 7, stage VII at 32, case 12, stage III at 29 years of age). On the whole, these data indicate that genotype and phenotype correlate only in part.

Discussion. The 20 patients reported in this study belonged to 15 families, originating from Europe and from North Africa. Several authors have reported[12,14,21] patients with adhalin deficiency from other geographic origins (Japanese, Brazilian, Afro-American), which confirms the universal distribution of the disease. The identification of primary adhalin (α-sarcoglycan) deficiency in North Africa shows that SCARMD is genetically heterogeneous in these countries, where primary α-sarcoglycanopathy coexists with γ-sarcoglycanopathy, linked to chromosome 13q12.[16,17,24,26] Our patients had the following common clinical features: (1) autosomal recessive inheritance pattern when there was a family history of this disease, (2) involvement of both girdles in a symmetric fashion, with a selective and peculiar distribution, (3) noninvolvement of facial, ocular, and velopharyngeal muscles, (4) calf hypertrophy, (5) absence of cardiac involvement. The clinical pattern of primary adhalinopathies has many features in common with that of the secondary adhalinopathies either linked to 13q12 (γ-sarcoglycanopathy, mainly found in the Arabian community[24,26]) or linked to 4q12 (β-sarcoglycanopathy, described in the Amish community of Southern Indiana[22]). In secondary adhalinopathies, both girdle involvement and calf hypertrophy are also often present.

Calf hypertrophy and marked quadriceps femoris wasting, contrasting with spared thigh internal compartment muscles, are similarly observed in Duchenne/Becker dystrophy and in adhalinopathy, and explain why in the European patients, the disease was often initially misdiagnosed as DMD in males and symptomatic DMD heterozygotes in females. However, adhalinopathy differs from dystrophinopathies by other main features: autosomal recessive inheritance, early and marked involvement of scapular fixator muscles, and absence of cardiomyopathy.

The clinical pattern of adhalinopathy is also different from that of the limb-girdle muscular dystrophy associated to calpain 3 gene mutations, first reported in Reunion Island.[27] In the latter disease, for instance, in the upper limbs there is a striking preservation of supra- and infraspinatus muscles and deltoid muscle is involved later than in the primary adhalinopathy. The lower limb distribution of muscle wasting is also clearly different in these two dis-

eases, with a selective and predominant involvement of biceps femoris and triceps surae in the calpainopathy, whereas quadriceps and tibialis anterior muscles are more and earlier involved in the adhalinopathy.

As shown in previous reports,[12,14,20] there was marked heterogeneity in severity in primary adhalinopathy. Six patients of the present series were wheelchair-bound before 15 years of age and on the whole the myopathy was severe in about 50% of patients. In contrast the myopathy was particularly mild in five patients, with onset between age 12 and 14, and preserved ambulation in adulthood. Four patients from this "benign" group had painful muscle episodes, clearly induced by exercise in two cases, and leading initially to the diagnosis of metabolic myopathy. This particular clinical presentation of primary adhalinopathy is similar to the pseudometabolic forms of dystrophinopathy.[28] An intrafamilial variability may exist in primary adhalinopathy,[12] but in the present study it occurred only in one of the three sibships.

The muscle expression of α-sarcoglycan varied and there was a clear correlation between the severity of the myopathy and the α-sarcoglycan expression: the amount of the protein was moderately decreased in all mildly affected patients but the protein was absent or very deficient in wheel-chair bound patients. The expression of β- and γ-sarcoglycans was also decreased, at a lesser degree than α-sarcoglycan, indicating that the different components of sarcoglycan are tightly associated and that a primary loss of α-sarcoglycan could lead to the disruption of other components of the sarcoglycan complex. In patients affected by β- or γ-sarcoglycanopathies the expression of all sarcoglycan proteins is also decreased.[22-24,26]

There were 15 different mutations in the present series of patients. This broad spectrum of α-sarcoglycan gene mutations excludes a founder effect. A potentially severe mutation (aberrant splicing or nonsense mutations) occurred in a minority of patients whereas missense mutations were more common. The Arg77Cys missense mutation was the most frequent, as in other series.[12,14] The genotype and phenotype correlated only in part: the three patients homozygous for null mutations were early wheel-chair bound and had a complete α-sarcoglycan deficiency, but some patients with either mild or severe myopathy and varying decrease of α-sarcoglycan expression had double missense mutations. The pathogenic effect of the missense mutation of α-sarcoglycan could depend on its localization. However this hypothesis does not explain the variation in severity in patients having two identical mutations, as we found in the same or in different families. Other still unknown genetic factors could modulate the cellular or the molecular consequences of the primary gene defect. The consequence of localization of mutations on the function and stability of α-sarcoglycan should be studied further.

Note added in proof. After submission of this article the following paper, which focuses on the molecular pathology of α-sarcoglycan, has been accepted for publication in the *Journal of Medical Genetics*: Carrié A, Leturcq F, Piccolo F, et al. Mutational diversity and hot spots in the α-sarcoglycan gene in autosomal recessive muscular dystrophy (LGMD2D).

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