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The clinical spectrum of limb girdle muscular dystrophy

A survey in the Netherlands

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Summary

A cross-sectional study was performed in the Netherlands to define the clinical characteristics of the various subtypes within the broad and heterogeneous entity of limb girdle muscular dystrophy (LGMD). An attempt was made to include all known cases of LGMD in the Netherlands. Out of the reported 200 patients, 105 who fulfilled strictly defined criteria were included. Forty-nine patients, mostly suffering from dystrophinopathies and facioscapulohumeral muscular dystrophy, appeared to be misdiagnosed. Thirty-four cases were sporadic, 42 patients came from autosomal recessive and 29 from autosomal dominant families. The estimated prevalence of LGMD in the Netherlands was at least 8.1×10^{-6}. The clinical features of the autosomal recessive and sporadic cases were indistinguishable from those of the autosomal dominant patients, although calf hypertrophy was seen more frequently, and the course of the disease was more severe in autosomal recessive and sporadic cases. The pectoralis, iliopsoas and gluteal muscles, hip adductors and hamstrings were the most affected muscles. Distal muscle involvement occurred late in the course of the disease. Facial weakness was a rare phenomenon. The severity of the clinical picture was correlated with a deteriorating lung function. All autosomal dominantly inherited cases showed a mild course, although in two families life-expectancy was reduced because of concomitant cardiac involvement.

Keywords: limb girdle muscular dystrophy; prevalence; α-sarcoglycan; linkage analysis

Abbreviations: CK = creatine kinase; FSHD = facioscapulohumeral dystrophy; FVC = forced vital capacity; LGMD = limb girdle muscular dystrophy

Introduction

Limb girdle muscular dystrophy has long been a controversial entity (Bradley, 1979; Ten Houten, 1979; Walton and Gardner-Medwin, 1981; Shields, 1994). After careful exclusion of all possible genetically and clinically different diseases which also manifest with limb girdle weakness, a repository of cases remains that fits the diagnosis of LGMD (Bushby, 1994). Recent linkage studies demonstrated an association between LGMD and various chromosomal loci (Beckmann *et al.*, 1991; Ben Othmane *et al.*, 1992; Speer *et al.*, 1992; Bashir *et al.*, 1994; Roberds *et al.*, 1994; Passos-Bueno, 1996) and thereby reaffirmed the existence of LGMD. Partial or complete deficiency of various dystrophin-associated proteins has been demonstrated in patients with autosomal recessive muscular dystrophy, both as a primary feature and as a secondary event (Azibi *et al.*, 1993; Roberds *et al.*, 1994; Bönneman *et al.*, 1995; Lim *et al.*, 1995; Noguchi *et al.*, 1995; Worton, 1995). On a recent workshop, a proposal for a new nomenclature based on gene search results has been made (Bushby and Beckmann, 1995).

LGMD1 designates chromosome 5q-linked autosomal dominant LGMD as described by Gilchrist. Characteristics of this phenotype are late-onset limb girdle weakness, dysarthria and facial weakness (Gilchrist *et al.*, 1988; Speer *et al.*, 1992). Other chromosomal localizations for autosomal dominantly inherited LGMD are as yet unknown. There is mostly a relatively benign course (De Coster *et al.*, 1974; Bethlem and Wijngaarden, 1976; Chutkow *et al.*, 1986; Gilchrist and Leshner, 1986; Marconi *et al.*, 1991; Somer *et al.*, 1991).

LGMD2A denotes the chromosome 15q-linked autosomal recessive disorder encountered in families on Reunion Island (Beckmann *et al.*, 1991). Subsequently several different mutations have been identified in the gene encoding for the proteolytic enzyme calpain 3 (Richard *et al.*, 1995). In the Amish population, linkage to chromosome 15 has also been found in affected families in northern Indiana, first described by Jackson (Jackson and Carey, 1961; Jackson and Strehler, 1968), whereas in the southern Indiana families linkage was excluded (Young *et al.*, 1992; Allamand *et al.*, 1995). The phenotype of the chromosome 15 linked form shows childhood onset progressive limb girdle weakness without calf hypertrophy (Fardeau *et al.*, 1996).

LGMD2B refers to the chromosome 2p13.3-linked subtype (Bashir *et al.*, 1994; Passos-Bueno *et al.*, 1995), that has its onset mostly after the age of 15 years and usually shows slow progression.

Another subset of patients with autosomal recessive inheritance, early onset and rapid progression, known as Duchenne-like severe childhood autosomal recessive muscular dystrophy (SCARMD1), was first reported from Tunisia (Ben Hamida *et al.*, 1983). Linkage analysis has mapped the gene to the pericentromeric region of the long arm of chromosome 13q12 (Ben Othmane *et al.*, 1992). This form is now called LGMD2C. Marked inter- and intrafamilial variability as regards the age of onset and the course of the disease was noted. In some patients secondary α-sarcoglycan deficiency has been demonstrated (Azibi *et al.*, 1993). Recently the gene encoding the dystrophin-associated glycoprotein γ-dystroglycan (35DAG) has been mapped to 13q12, and mutations in this gene were found (Noguchi *et al.*, 1995).

Limb girdle muscular dystrophy with primary α-sarcoglycan deficiency, previously called SCARMD2, has been linked to chromosome 17 and now constitutes LGMD2D (Roberds *et al.*, 1994). A variety of mutations has been found in families with a broad range of clinical presentations and intrafamilial variability, and they may be associated with a range of α-sarcoglycan staining patterns (Matsumura *et al.*, 1992; Fardeau *et al.*, 1993; Roberds *et al.*, 1994; Romero *et al.*, 1994; Zatz *et al.*, 1994; Hayashi *et al.*, 1995; Kawai *et al.*, 1995; Passos-Bueno *et al.*, 1995; Ljunggren *et al.*, 1995; Piccolo *et al.*, 1995).

Limb girdle muscular dystrophy in the old order Amish of southern Indiana is linked to markers on chromosome 4q, and constitutes LGMD2E. The gene for the β-sarcoglycan component of the dystrophin-associated-glycoprotein complex has been mapped to 4q12, and has been found to be defective in these patients, and in one other patient (Bönneman *et al.*, 1995; Lim *et al.*, 1995).

So far, most of our knowledge about LGMD has been derived from large families, that have been suitable for linkage analysis. Detailed genotype–phenotype correlations have not been made as yet, due to extreme variability in severity which could be associated with any of the identified genetic localizations (Jackson and Carey, 1961; Jackson and Strehler, 1968; Ben Hamida *et al.*, 1983; Bashir *et al.*, 1994). At present little is known about prevalence rates, and about the natural history of the disease.

We undertook a study on a large group of LGMD patients collected from all neuromuscular centres in the Netherlands. The aims of this cross-sectional study were (i) to estimate the prevalence of LGMD in the Netherlands, (ii) to make an inventory of the clinical spectrum of LGMD, and (iii) to identify factors associated with the severity of the disease.

Methods

Patients

The survey was performed in 1993 and 1994 on 200 patients in whom a tentative diagnosis of LGMD had been made between 1980 and 1993. They were recruited from the files of all (seven) neuromuscular centres of the university hospitals in the Netherlands, and from our national patients' association ('Vereniging Spierziekten Nederland'). Patients were evaluated in two stages to determine whether they met our inclusion and exclusion criteria. In the first stage the records of all patients were reviewed. After this initial selection, patients who met our criteria and had given informed consent

to participate in the study, were personally examined. The second selection was based on the results of this clinical examination and of the review of muscle biopsy specimens. The inclusion criteria consisted of the following: person of either gender with progressive, more or less symmetric limb girdle weakness, in whom ancillary investigations [including serum creatine kinase (CK) activity, EMG and muscle biopsy] were compatible with a primary myogenic disorder. Patients were excluded if another disorder manifesting with limb girdle weakness was present, including metabolic and inflammatory myopathies, dystrophinopathies, spinal muscular atrophies, congenital myopathies/dystrophies, facioscapulo humeral dystrophy (FSHD), Emery–Dreifuss muscular dystrophy or rigid spine syndrome. Therefore, exclusion criteria were congenital onset, ptosis or weakness of external ocular muscles, skin rash compatible with dermatomyositis, sensory abnormalities, severe facial weakness, distal weakness more severe than proximal; and the presence of florid active denervation, ragged red fibres or numerous extensive cellular infiltrates on muscle biopsy, or glycogen or fat accumulation within the muscle fibres. Abnormalities in dystrophin analysis of muscle biopsy specimens, deletions or duplications within the dystrophin gene (Xp21), the presence of Turner karyotype or X-autosome translocations were additional exclusion criteria.

Seventy-four individuals were excluded during the first evaluation for the following reasons: 11 were deceased; in four insufficient information was available; two lived abroad; three addresses were unknown; 26 refused to enter the study; in 28 another diagnosis was made: 11 were diagnosed as having dystrophinopathy (10 patients with Becker and one manifesting carrier; dystrophin analysis of muscle tissue was carried out in 37 patients), one polymyositis, 11 FSHD, one spinal muscular atrophy, one autosomal dominantly inherited scapulo-ilio-peroneal atrophy with cardiopathy (Jennekens *et al.*, 1975), and three congenital myopathies or muscular dystrophies.

At the entry of the study 126 subjects were included. They were examined clinically and their muscle biopsies were reviewed. A tentative subdivision was made into autosomal recessive, sporadic and autosomal dominant cases. Autosomal recessive inheritance was considered likely when consanguinity was proven or when more than one affected sibling, including at least one girl, was present, and the parents were healthy. Sporadic cases might be either autosomal recessive or new mutations of an autosomal dominant form. Autosomal dominant inheritance was considered likely when affected persons in two or more generations were present, and the possibility of X-linked dystrophinopathy was excluded by means of Xp21 screening, and/or dystrophin analysis.

Neurological evaluation

The patients were examined by one of the authors (A.J.K.) using a standardized protocol. Seven patients were seen at home, and all other patients were examined in our hospital. The neurological history included questions about the age of onset, the first symptoms of weakness and progression of the disease to other muscle groups.

Muscle strength was assessed (Kendall *et al.*, 1971), using the British Medical Research Council scale (Medical Research Council, 1943). Thirty-three muscle groups were examined bilaterally. The facial muscles were graded on a three-point scale (0 = severely affected; 1 = mildly affected; 2 = normal). Reflexes were assessed on a five-point scale (0 = absent; 1 = diminished; 2 = normal; 3 = increased; 4 = clonus). The presence of contractures in eight joints on both sides [shoulders, elbows, wrists, hands (fingerflexors), hips, knees and ankles] were graded on a three-point scale (0 = absent; 1 = mild; 2 = severe). The presence of scoliosis, atrophy and hypertrophy were graded on a two-point scale (0 = absent; 1 = present).

Seventy-four healthy family members over the age of 18 years (who also had given their informed consent), were examined as well.

Other investigations

All patients completed a brief questionnaire focusing on respiratory symptoms; i.e. breathlessness, fatigue, sleep-related symptoms, and headache upon waking. Forced vital capacity (FVC) was measured in 84 patients in the sitting position with a hand-held spirometer. It was registered as a percentage of the normal value related to age, height and gender.

All patients were assigned a functional grade based on a scale designed by Vignos *et al.* (1963), and modified by Brooke *et al.* (1981). This scale is subdivided in two parts: one for shoulders and arms, varying from 1 = patients being able to fully abduct their arms, to 6 = loss of useful function of arms and hands; and one for hips and legs, varying from 1 = patients being able to walk and climb stairs without assistance, to 10 = confinement to bed.

Genealogical investigations, extending to five generations of ancestors, were performed in a pilot study that included the first 15 families, excluding those with autosomal dominant inheritance. Genealogical investigations were also carried out in other families in which, on the basis of family history, consanguinity was suspected. Serum CK activity was assessed in all patients and in the majority of their parents and siblings.

Extensive cardiological investigations were performed in all patients. These results will be described in a separate paper.

Muscle biopsy specimens of all patients were re-evaluated by one of the authors (M.de V.) to rule out other causes of limb girdle syndrome as mentioned above. They were classified as either myopathic, dystrophic or neurogenic. Myopathic changes consisted of variation in muscle fibre size, an increase in the number of fibres with internal nuclei and fibre splitting. Dystrophic changes included myopathic changes in combination with degenerating and/or regenerating fibres. Neurogenic changes comprised the presence of small angular

fibres, staining darkly with oxidative enzymes and non-specific esterase, or the presence of fibre-type grouping. Immunohistochemical analysis of α-sarcoglycan was performed in 34 available frozen biopsy specimens.

Obligatory additional ancillary investigations consisted of chromosomal analysis including karyotyping in all sporadic female cases, and Xp21 screening in all sporadic cases. Both immunohistochemical and immunobiochemical analyses of dystrophin in frozen muscle biopsy specimens was performed in all sporadic male cases, in all families in which only males were affected, and in the majority of sporadic female patients. Acid maltase assay and ischaemic forearm test were undertaken when history or (reported) muscle biopsy abnormalities raised suspicion of a metabolic myopathy.

Statistical analysis

We described the relationship between disease duration and patterns of muscle weakness in relative frequencies. Furthermore we assessed the association between FVC and severity of disease in terms of a relative risk with 95% confidence limits. Relative risk expresses how much more likely severe disease is among patients with a marked reduction of pulmonary capacity than among patients with a normal capacity. Cut-off value for the functional leg score was 5, because we considered the loss of independent walking as an important indicator for the progression of the disease. The same applied to the cut-off value for the functional arm score of 2, reflecting the loss of ability to raise the arms above the head. The cut-off point for FVC was set at 70% of the normal value.

Linkage analysis

Most families were too small for linkage analysis. Linkage analysis was performed in two large autosomal dominant families with cardiac involvement to evaluate the possibility of linkage to chromosome 5q.

Results

The study population comprised 126 patients whose records had met our inclusion and exclusion criteria. After clinical examination and reviewing of the muscle biopsy specimens during the second evaluation another 21 patients had to be excluded. In one patient a diagnosis of mitochondrial myopathy seemed more likely on the basis of ptosis and external ophthalmoplegia, six were diagnosed as FSHD because of severe facial weakness or asymmetric scapulo-humeroperoneal weakness (this diagnosis was affirmed by DNA analysis in four and supported in one). Three individuals were thought to have congenital myopathy, one rigid spine syndrome, one Emery–Dreifuss muscular dystrophy, and one Miyoshi (distal) myopathy. Six patients had atypical distribution of weakness, with distal leg muscles more or to the same extent affected as the proximal muscles. Because of neurogenic muscle biopsy changes another two patients were diagnosed as having spinal muscular atrophy and therefore excluded (this diagnosis was affirmed by DNA analysis showing deletions on the spinal muscular atrophy locus on chromosome 5).

Finally, 105 patients, all residents of the Netherlands, entered our study for further analysis. Ninety-seven patients were of Caucasian origin, five came from Morocco, one from Turkey, one from Peru and one was of Indian descent. Forty-two autosomal recessive cases originated from 24 multiplex or consanguineous families; 34 were sporadic patients; 29 individuals came from 10 autosomal dominant families. Nineteen family members (12 in the autosomal recessive families, and seven in the autosomal dominant families) were reported to have the same disorder, but they refused to participate in the study. The prevalence was estimated on January 1, 1993. The number of inhabitants in the Netherlands at that time was 15 239 182. Therefore, the prevalence of all LGMD cases amounted at least to 8.1×10^{-6} [(105+19)/15 239 182] individuals, and of the autosomal recessive and sporadic cases at least to 5.7×10^{-6} [(42+34+12)/15 239 182).

Genealogical investigations were performed in 19 families. In seven of these, in which the family history was suggestive, consanguinity was found. In three other families genealogical investigations were extended because of common surnames in the ancestors in the fifth generation. In all three, consanguinity was confirmed: in the sixth, sixth and 10th generation, respectively. Although two other families of Moroccan origin reported consanguinity as well, no data for genealogical investigations were available.

Clinical characteristics of the patients with LGMD

The bar graph (*see* Fig. 1) shows the distribution of muscle weakness at the time of examination. The autosomal recessive and sporadic cases are considered as one group (*see* Discussion) and are compared with the autosomal dominant patients. The majority of patients had atrophy and weakness of the proximal muscles of upper and lower limbs, particularly when symptoms had been present for >10 years (data not shown). Weakness was most frequently observed in the pectoralis, iliopsoas, gluteal, hip adductor, and the hamstring muscles. Mild facial weakness was seen in only four patients with a mean disease duration of 23.5 years (range 22–27 years). Distal weakness occurred late in the course of the disease. Wrist and finger extensor muscles, tibialis anterior and toe extensor muscles were the most frequently involved distal muscles. No clear distinctions are present between the different subtypes.

Despite severe weakness, no patient lost hand function nor was bedridden. Long leg braces were used by only one patient.

The knee and biceps jerks disappeared first in most patients, whereas the ankle reflexes were often relatively spared. In 13 patients, however, ankle jerks disappeared at an early

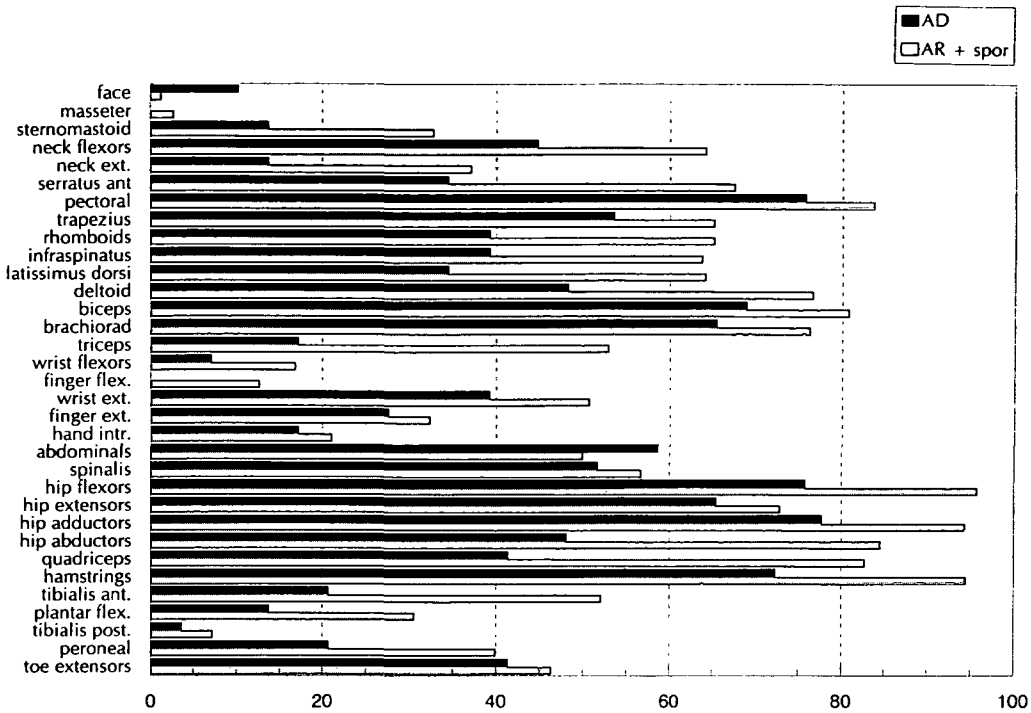

Fig. 1 Muscle involvement on 105 LGMD patients. The bar graph shows the distribution of weakness at the time of examination. The autosomal recessive and sporadic cases are considered as one group and compared with the autosomal dominant patients. AD = autosomal dominant; AR = autosomal recessive; spor = sporadic.

stage of the disease. Achilles tendon contractures were observed in 59% of patients with a median disease duration of 23 years (range 0–54 years). Scoliosis was present in 26.4% of patients with a median disease duration of 22 years (range 4–54 years).

Eighty-four patients had no respiratory complaints, 11 had slight breathlessness, three experienced continuous breathlessness, three had sleep-related problems and four required assisted ventilation during the night. The median FVC for all 84 patients in whom it was measured was 95.0% (range 16.9–158). The median FVC for wheelchair-bound patients was 68.0% (range 16.9–123.8). Patients with a pronounced reduction of FVC were more likely to have severely impaired functional arms scores (relative risk = 4.4; 95% confidence limits: 2.0/8.4) and leg scores (relative risk = 5.9; 95% confidence limits: 2.6/13.4).

Subdivision into autosomal recessive, sporadic, autosomal dominant cases (see also Table 1)

Autosomal recessive LGMD

Consanguinity was present in three families with sporadic patients and in eight of the 21 multiplex families.

The symptoms started in the proximal leg muscles in 36 patients. Weakness of the shoulder girdle and upper arm muscles occurred in 20 patients after a median disease duration of 10 years (range 1–35 years). In six patients, muscle weakness became manifest simultaneously in upper and lower limbs. Thirty-three patients developed weakness of the distal leg muscles in the course of the disease. In five patients the only affected distal leg muscles were the calves. Slight facial weakness was seen in one 22-year-old patient, who had been wheelchair-dependent for 11 years and required assisted ventilation during the night.

Dystrophic changes were seen in all muscle biopsy specimens.

Three patients out of 13 in whom α-sarcoglycan screening was performed showed a partial deficiency. One was a 19-year-old wheelchair-dependent Moroccan girl, the other a 46-year-old Dutch woman who walked until the age of 44 years, and the third a 55-year-old man, who lost the ability to walk at the age of 46 years.

Sporadic cases

In 32 of the 34 cases, weakness was first evident in the proximal leg muscles. In 20 of those patients, weakness of the shoulder girdle muscles became evident after a median disease duration of 10 years (range 1–43 years). In two patients, muscle weakness became manifest simultaneously in upper and lower limbs. Late distal leg involvement was present in 20 patients. In one patient the calf muscles were the only affected distal leg muscles. Facial weakness was not seen. Two patients required assisted ventilation during the night.

Dystrophic changes were seen in the muscle biopsies of

Table 1 *The clinical characteristics of the patients with LGMD (n = 105)*

	Autosomal recessive total	Sporadic total	Autosomal dominant		
			Total (M + F)	Cardiac involvement	
				Absent	Present
Number of patients (M;F)	42 (17;25)	34 (12;22)	29 (12;17)	14 (4;10)	15 (8;7)
Median age in years (range)	36 (8–66)	31 (3–65)	39 (30–69)	48 (33–69)	33 (30–61)
Median age of onset in years (range)	12 (1–57)	8 (1–33)	12 (3–52)	12 (3–52)	10 (5–38)
Median disease duration in years (range)	20 (2–52)	18 (1–58)	23 (0–50)	23 (10–43)	22 (0–50)
Number of wheelchair dependent patients	15	12	3	3	0
Median age of losing ambulation in years (range)	26 (8–50)	16 (11–69)	45 (33–47)	45 (33–47)	–
Calf hypertrophy (number of patients)	23	14	5	3	2
Achilles tendon contractures (number of patients)	27	20	14	9	5
Scoliosis (number of patients)	8	11	5	3	2
Median serum CK activity (times normal value)	23.6 (1–720)	21.8 (1–116.3)	5.4 (1–34)	10 (2.6–34)	1.3 (1–8.3)
Median FVC* (% of the normal value)	96.6 (29–158)	89.7 (16.9–126.7)	96.5 (48.7–142.1)	102.1 (48.7–142.1)	96.1 (59.4–113.6)

*Measured in 84 patients.

29 patients, one showed an end stage myopathy, and in four, myopathic changes were present.

In three of the 21 muscle biopsies stained for α-sarcoglycan, this protein was found to be completely absent. One girl, aged 17 years, had been wheelchair-dependent for 3 years, and the other two, aged 10 and 27 years, were barely able to walk independently.

Autosomal dominant LGMD

On the basis of the results of a cardiological investigation, a clear distinction between autosomal dominant families with and without cardiac involvement emerged.

Autosomal dominant LGMD without cardiac involvement. This group consisted of 14 individuals from eight families. Weakness started in the proximal muscles of the legs in 13 patients, the shoulder girdle and proximal upper limb muscles being affected in 10 patients after a median disease duration of 13 years (range 5–36 years). In one patient, the shoulder and pelvic girdle muscles were simultaneously involved. Seven patients showed distal leg muscle weakness. Slight facial muscle weakness was seen in one patient.

Dystrophic muscle biopsies were seen in 12 patients, and two patients showed myopathic changes.

Autosomal dominant LGMD with cardiac involvement. This group consisted of 15 patients from two families. Symptoms at onset were due to muscle weakness of the proximal legs. In three patients, shoulder and proximal arm muscles became affected as well after a median disease duration of 21 years (range 10–25 years). Distal leg weakness was observed in seven patients. Slight facial muscle weakness was seen in two. Seven patients had very mild contractures of their elbows.

Four patients showed dystrophic muscle biopsy changes.

Cardiological abnormalities included a variety of dysrhythmias and conduction disturbances, such as atrioventricular block and bradycardia. In some, cardiological involvement gave rise to syncopal attacks and even sudden cardiac death.

Congestive cardiomyopathy was present in two individuals.

Chromosomal linkage studies excluded the locus on chromosome 5q (these data will be the subject of a separate paper).

Family members

In the autosomal recessive families 20 additional family members, and 33 family members of the sporadic cases were examined, but no muscle weakness was found. Serum CK activity, estimated in 36 individuals, was normal in 31. Five had a slightly raised serum CK activity ranging from 1.2 to 1.8 times the upper limit.

Examination of 21 additional family members of the autosomal dominant cases yielded three individuals who were presumed to have the disorder because they complained of decreased muscle strength, and because of the presence of a slightly elevated serum CK activity (1.2 to 2.5 times the upper limit), although there was no muscle weakness. The serum CK activity of the other 18 family members was normal, except for one individual who had a slightly elevated serum CK activity (1.2 times the upper limit).

Discussion

In this cross-sectional study we have attempted to include all known cases of LGMD in the Netherlands. Initially, 200 patients were reported with a tentative diagnosis of LGMD. After subjecting the records of all patients to clearly defined inclusion and exclusion criteria, 126 patients remained. These individuals were examined clinically, and their muscle biopsy specimens were reviewed. After this second screening,

another 21 patients had to be excluded, leaving only 105 patients for further analysis. Therefore, we stress the importance of thorough investigations to rule out other diseases before accepting a diagnosis of LGMD. The introduction of new techniques such as dystrophin analysis, and Xp21, chromosome 4q and chromosome 5 screening are extremely helpful to exclude dystrophinopathies, FSHD and spinal muscular atrophy, respectively. A total of 28 patients suffering from these conditions were initially misdiagnosed as LGMD.

In a world survey of population frequencies (Emery, 1991), the prevalence and incidence rates for mainly adult onset cases of LGMD were estimated at $20–40\times10^{-6}$. In a study by Yates and Emery (1985) the prevalence of adult onset cases was 7×10^{-6} at the most. Because of our well-defined diagnostic criteria and the demographic advantages of the Netherlands for ascertaining the majority of LGMD cases, probably a more reliable prevalence could be established. In our study the prevalence of cases with childhood and adult onset was at least 8.1×10^{-6}, which is lower than previously reported. We presume that the previously mentioned prevalence rates have been artificially inflated by a number of neurogenic and myopathic disorders with a limb girdle distribution of weakness mimicking LGMD. On the other hand, our prevalence rate may be an underestimate because our study comprised a larger number of females than males, and presumably a number of male LGMD patients in the files of the neuromuscular centres, in whom no dystrophin analysis has been performed, have been misclassified as Duchenne or Becker muscular dystrophy.

Although it has been reported that in LGMD weakness may begin in either upper or lower limb muscles (Walton, 1993; Bushby, 1994), in our patients weakness started either in the proximal leg muscles or simultaneously in upper and lower limbs. All patients reported to have onset of weakness in the shoulder girdle muscles were diagnosed as FSHD, because they had either facial weakness, asymmetric weakness, only slightly elevated serum CK activities, or a family history consistent with autosomal dominant inheritance. In two of our patients with scapular onset limb girdle weakness, in whom a diagnosis of FSHD was proven by DNA analysis, only a trace of facial weakness was demonstrable, occurring in a late stage of the disease. The muscle disorder in these patients resembles a recently described scapular onset muscular dystrophy without apparent facial involvement that had possible allelism with FSHD (Jardine *et al.*, 1994).

Forty-two patients came from autosomal recessive families, 34 cases were sporadic and 29 were autosomal dominant cases. The clinical features of the different genotypes, including the distribution of weakness, the presence of contractures and reflexes, were indistinguishable. Only calf hypertrophy was more frequently seen in autosomal recessive and sporadic cases. Likewise, the clinical picture was more severe as compared with the autosomal dominant cases. Serum CK activities in our autosomal dominant families (median 5.4 times the upper limit of normal) were lower than those of the autosomal recessive cases (median value 23.6 times the upper limit of normal), which is line with the literature (Bushby, 1994).

Awaiting the results of direct mutation analysis and further immunostaining of the available muscle biopsies in our patients, we tried to make a distinction between the different phenotypes.

Sixteen patients of our autosomal recessive group strongly resembled the Tunisian patients in whom linkage to chromosome 13 was found (LGMD2C). They all showed the features described by Ben Hamida *et al.* (1983), i.e. onset of their disease before the age of 13 years, severe progression leading to wheelchair-dependency before the age of 31 years, marked rise of serum CK activity, almost consistent hypertrophy of the calves and dystrophic muscle biopsy changes. Five out of 16 originated from consanguineous Moroccan marriages.

Eighteen patients might match the description of the chromosome 15 linked LGMD2A (Jackson and Carey, 1961; Jackson and Strehler, 1968; Beckmann *et al.*, 1991; Young *et al.*, 1992; Fardeau *et al.*, 1996), in which the following characteristics were found: age of onset between 3 and 30 years of age (predominantly childhood onset) with quite a variable age of confinement to a wheelchair, dystrophic changes on muscle biopsy and gross abnormalities of serum enzymes particularly in the early stages. Since shoulder and pelvic girdle muscles were reported to be affected to the same extent (Jackson and Carey, 1961; Jackson and Strehler, 1968, Fardeau *et al.*, 1996), especially the patients in our study, who had had simultaneous onset of weakness in shoulder and hip girdle muscles, might fit into this category.

The distinction between LGMD2A and LGMD2C, however, is difficult to make because interfamilial variability is frequent, and there is considerable overlap.

The clinical picture of the two families with onset in the late teens and relatively slow progression, in which recently linkage to chromosome 2 was found (LGMD2B) (Bashir *et al.*, 1994), has not been described in detail yet. Distal (Miyoshi) myopathy, with onset of muscle weakness in the gastrocnemius and soleus muscles, has been reported to be linked to the same locus (Bejaoui *et al.*, 1995). Interestingly, CT scanning of LGMD2B patients showed early involvement of the soleus, along with the abductors and the hamstring muscles (Bushby and Beckmann, 1995). Five of our autosomal recessive patients had limb girdle weakness and a selective decrease in strength and atrophy of the calf muscles. This is a remarkable finding since calf muscles are usually among the most preserved muscles in limb girdle muscular dystrophy (Walton and Gardner-Medwin, 1981).

Most of the sporadic cases will probably be autosomal recessive cases, because nearly all patients had serum CK activities that were elevated more than 10 times the normal value, and calf hypertrophy was frequently observed. Genealogical investigations in these particular patients do not seem very useful, because in our pilot study consanguinity

could only be confirmed in families in which it was already suspected. The same considerations concerning classification as have been made for the autosomal recessive cases can be applied to the sporadic group. Six of the 34 patients had atypical features. One patient, who became wheelchair-dependent at the age of 16 years, had a slightly elevated serum CK activity throughout all stages of the disease. His muscle biopsy specimen revealed an end stage myopathy. This patient might resemble the patients of the large Sudanese kindred described by Salih *et al.* (1983) who had been suffering from a severe, progressive form of muscular dystrophy with onset between the ages of 3 and 5 years, with dystrophic features on muscle biopsy, and a mildly elevated (up to five times the normal value) serum CK activity. Four patients had an only slightly elevated serum CK activity and sole myopathic changes, without degenerating or regenerating fibres, on muscle biopsy. Another patient showed dystrophic changes, but had an only four times elevated serum CK activity. The latter five may be *de novo* mutations of autosomal dominant LGMD.

α-Sarcoglycan deficiency in three autosomal recessive cases and absence in three sporadic cases, indicate that primary and secondary α-sarcoglycan deficiencies are encountered in the Netherlands as well. The clinical pictures of these patients showed great variation, as is also the case in the reported patients (Matsumura *et al.*, 1992; Azibi *et al.*, 1993; Fardeau *et al.*, 1993; Roberds *et al.*, 1994; Romero *et al.*, 1994; Zatz *et al.*, 1994; Hayashi *et al.*, 1995; Ljunggren *et al.*, 1995; Passos-Bueno *et al.*, 1995; Piccolo *et al.*, 1995).

Autosomal dominantly inherited LGMD was believed to be rare (Walton and Nattrass, 1954; Jerusalem and Sieb, 1992). As yet, relatively few families have been described with autosomal dominant LGMD, showing a wide range of clinical features and histological findings (Schneidermann *et al.*, 1969; Bacon and Smith, 1971; De Coster *et al.*, 1974; Bethlem and Wijngaarden, 1976; Hastings *et al.*, 1980; Chutkow *et al.*, 1986; Gilchrist *et al.*, 1988; Marconi *et al.*, 1991; Somer *et al.*, 1991; Miller *et al.*, 1992). In our study, the autosomal dominantly inherited LGMD cases encompass 28% (29 out of 105) of the LGMD patients. Most of the reported autosomal dominant forms had a relatively late onset, i.e. in the third or fourth decade (Bacon and Smith, 1971; De Coster *et al.*, 1974; Aguilar *et al.*, 1978; Chutkow *et al.*, 1986; Gilchrist and Leshner, 1986; Marconi *et al.*, 1991), although symptoms sometimes occurred in childhood (Schneidermann *et al.*, 1969; Bethlem and Wijngaarden, 1976; Fenichel *et al.*, 1982; Miller *et al.*, 1985; Panegyres *et al.*, 1990; Somer *et al.*, 1991). In our families, the mean age of onset was 23.4 years. In 15 patients the onset of symptoms was before the age of 13 years. Calf enlargement was observed in some cases (Aguilar *et al.*, 1978; Panegyres *et al.*, 1990; Hastings *et al.*, 1980), and, indeed, in our families it was found in five of the 29 patients. Dysarthria and facial weakness were distinctive features of LGMD1, but these were not seen in our families. Linkage to chromosome 5 was excluded in our two large autosomal dominant families.

In keeping with the literature, progression of the disease was usually mild in our patients. Only three became wheelchair-bound after the age of 30 years. However, in two of our autosomal dominant families, severe cardiac involvement shortened the life-expectancy. To an extent these families resemble autosomal dominant Emery–Dreifuss muscular dystrophy, but the absence of early contractures and of spinal rigidity clearly distinguishes between these disorders. Therefore, the disorder has been recognized as a new entity (Van der Kooi *et al.*, 1996).

Forced vital capacity decreased with deterioration of the clinical picture. Life expectancy is probably determined by pulmonary function. This implies that, especially in the more severely disabled patients, doctors should be aware of the development of respiratory symptoms. Notably appearance of sleep-related problems and continuous breathlessness necessitate further investigations in order to institute timely artificial ventilation.

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