# A Biochemical, Genetic, and Clinical Survey of Autosomal Recessive Limb Girdle Muscular Dystrophies in Turkey

Pervin Dinçer, PhD,* France Leturcq, PhD,† Isabelle Richard, PhD,‡ Federica Piccolo, MS,† Dilek Yalnızoğlu, MD,§ Claudia de Toma, MS,† Zuhal Akçören, MD,‖ Odile Broux, BS,‡ Nathalie Deburgrave, BA,† Lydie Brenguier, BA,‡ Carinne Roudaut, BA,‡ J. Andoni Urtizberea, MD,¶ Daniel Jung, PhD,# Ersin Tan, MD,** Marc Jeanpierre, MD, PhD,† Kevin P. Campbell, PhD,# Jean-Claude Kaplan, MD, PhD,† Jacques S. Beckmann, PhD,‡ and Haluk Topaloğlu, MD§

**Autosomal recessive limb girdle muscular dystrophy (LGMD2) is a clinically and genetically heterogenous group of diseases involving at least six different loci. Five genes have already been identified: calpain-3 at LGMD2A (15q15), and four members of the sarcoglycan (SG) complex, α-SG at LGMD2D (17q21), β-SG at LGMD2E (4q12), γ-SG at LGMD2C (13q12), and δ-SG at LGMD2F (5q33-q34). The gene product at LGMD2B (2p13-p16) is still unknown and at least one other gene is still unmapped. We investigated 20 Turkish families (18 consanguineous) diagnosed as having LGMD2. Most of our patients had onset of symptoms before age 10. The phenotypes varied from severe to benign. We analyzed the SG complex by immunofluorescence and/or western blot. Genotyping was performed using markers defining the six known loci and the suspected genes were screened for mutations. Six of 17 index cases showed deficiency of the SG complex, by immunofluorescence and/or western blot. Seven cases involved one of the known genes of the SG complex (α, 2; β, 1; and γ, 4 cases), and five mutations were documented in the α- and γ-SG genes. After linkage analysis, 10 families were characterized as having LGMD2A (calpain-3 deficiency), and all mutations were eventually identified. One family was classified as having LGMD2B and 1 family that has normal SGs was linked to the chromosome 5q33-q34 locus (LGMD2F). In 1 family there was no linkage to any of the known LGMD2 loci. It appears that in Turkey, there is a broad spectrum of genes and defects involved in LGMD2. It may be possible to correlate genotype to phenotype in LGMD2. All severe cases belonged to the γ-SG–deficiency group. Nine calpain-3–deficient cases had intermediate and 1 had moderate clinical courses. The LGMD2B patient had a moderate clinical expression, whereas the LGMD2F case was truly benign.**

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The limb girdle muscular dystrophies (LGMDs) are a heterogenous group of diseases that are characterized by progressive weakness of the pelvic and shoulder girdle muscles and highly elevated serum creatine kinase [1]. The symptoms usually begin during the first two decades of life, with the disease then gradually worsening, often resulting in loss of walking ability 10 or 20 years after onset [2]. There are several genetically different subgroups within the LGMD nosology. These disorders may be inherited as an autosomal dominant or recessive trait [3]. The accepted nomenclature for autosomal dominant (LGMD1) and recessive forms (LGMD2) was proposed recently at a European Neuromuscular Centre (ENMC)-sponsored workshop on LGMDs [4].

Various autosomal recessive forms were mapped to the following chromosomes: 15q15 (LGMD2A) [5], which is a muscle-specific calpain-3 (CANP3) deficiency [6]; 2p13-p16 (LGMD2B) [7]; 13q12 (LGMD2C) [8–10], 17q12-q21.33 (LGMD2D) [11], 4q12 (LGMD2E) [12, 13], and 5q33-q34 (LGMD2F) [14–17]. The genes at four of these loci have been

From the Departments of *Medical Biology, §Pediatric Neurology, ‖Pediatric Pathology, and **Neurology, Faculty of Medicine, Hacettepe University, Ankara, Turkey; †INSERM 129 and Service de Biochimie and Génétique Moléculaires, Hopital Cochin, Université Paris, Paris, and ‡URA 1922 CNRS, Généthon, and ¶Myobank, AFM, Evry, France; and #Department of Physiology and Biophysics, Howard Hughes Medical Institute, University of Iowa College of Medicine, Iowa City, IA.

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Address correspondence to Prof Topaloğlu, Department of Pediatric Neurology, Hacettepe Children's Hospital, 06100 Ankara, Turkey.

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identified: They code for γ-sarcoglycan (γ-SG) (35 kd) for LGMD2C [10], α-SG (50 kd) for LGMD2D [11, 18], β-SG (43 kd) for LGMD2E [12, 13], and δ-SG (35 kd) for LGMD2F [16, 17]. These four proteins are tightly associated in the sarcolemma and thus constitute the SG complex within the dystrophin glycoprotein complex (DGC) [19].

To complicate things further, there are adult-onset forms of merosinopathy in which deficiency of the $\alpha_2$ chain of laminin could possibly also lead to an LGMD phenotype [20]. Also, there are some families that do not show linkage to any of the chromosomes cited above [14].

Here, we report the clinical, genetic, and molecular findings of 20 LGMD2 families of Turkish origin.

## Materials and Methods

### *Patients*

Hacettepe University Children's Hospital in Ankara is a major referral center for neuromuscular disorders in Turkey. Between January 1989 and December 1995, we collected a cohort of 20 families with 33 cases fitting the description of LGMD2. Our selection criteria were as follows: (1) a pedigree clearly compatible with an autosomal recessive inheritance; (2) onset after the child walked; (3) progression of muscle weakness of varying severity, showing a limb girdle distribution with sparing of facial muscles; (4) normal intelligence; (5) muscle biopsy compatible with a muscular dystrophy; and (6) demonstration of normal dystrophin immunohistochemistry.

There were 11 multiplex families (14 males and 19 females). Per family, only 1 index case was studied clinically. Age at onset of symptoms was between 2.5 and 24 years. Age at the time of biopsy varied between 2.5 and 42 years. All patients had normal intelligence. The serum creatine kinase levels were at least five times higher (usually 10–20 times) than normal. The follow-up duration was between 1 and 7 years.

For practical purposes, the following functional stages of dystrophy were graded, taking into consideration the indicated parameters: (1) *severe,* if onset was in childhood and the disability was Duchenne muscular dystrophy (DMD)-like; (2) *intermediate,* if onset was in childhood whereas the progression or disability was like Becker muscular dystrophy (BMD); (3) *moderate,* if onset was in adulthood and the patient showed physical disability of any grade; and (4) *benign,* if onset was in adulthood and the patient did not have any disability (able to run freely, but may be weak, ie, less than grade I [21]). The classifications of DMD and BMD were taken from Dubowitz [22]; that is, a child who becomes off-feet before 13 years of age is registered as DMD-like, and one who is still ambulant after age 16 is registered as BMD-like.

### *Cardiac Evaluation*

Chest x-ray and routine electrocardiographic recordings were obtained twice yearly. Cardiac echograms, performed in 14 index cases, were normal.

### *Muscle Biopsy*

After informed consent, muscle biopsies were performed by using standard open biopsy techniques under local anesthesia, by two of us (D.Y. and H.T.). In four instances, either the blocks were not available to process or the family refused muscle biopsy. Samples were snap-frozen in isopentane, cooled, and stored in liquid nitrogen until processed. Frozen blocks were also saved for western blot.

Serial 6-μm sections, cut by using a cryostat, were stained for routine histology including hematoxylin–eosin and modified Gomori trichrome staining and a battery of histochemical reactions. Immunohistochemical studies were done for the following: spectrin (SPEC1, Novacastra); dystrophin (DYS1, DYS2, and DYS3, Novacastra); α-SG and β-dystroglycan (50 DAG and 43 DAG, Novacastra); and laminin $\alpha_1$ (laminin A) and $\alpha_2$ (merosin) chains (Chemicon).

### *Immunoblots*

Immunoblot analyses were performed in 15 patients as described by Piccolo and colleagues [18]. The primary antibodies used were against, respectively, dystrophin (DYS1 and DYS2, Novacastra), α-SG (50DAG, Novacastra), and β-SG and γ-SG [19].

### *Genotyping and Linkage Studies*

Highly polymorphic markers of chromosomes 2p13-p16, 4q12, 5q33-q34, 6q2, 13q12, 15q15.1-q15.3, and 17q12-q21.33 were used for analyzing the 20 LGMD families. $(CA)_n$ microsatellite markers D2S327, D2S292, D2S291, D4S1536, D4S2971, D4S2996, D5S436, D5S2014, D5S673, D5S2012, D5S1978, D5S410, D5S422, D5S400, D5S429, D6S262, D6S403, D13S115, D13S232, D13S292, D13S1294, D17S941, and D17S791 were provided from the Genethon human genetic linkage map [23, 24]. Polymerase chain reaction (PCR) conditions and primer sequences were as described therein. The $(CA)_n$ repeat in intron 6 of the α-SG gene (at 17q12-q21.33) [11, 25] and D15S514, D15S779, D15S782, D15S780, and D15S778 were used as described [26, 27]. Linkage analysis was performed using the LINKAGE software programs [28]. For consanguineous families, linkage was ascertained by homozygosity by descent (HBD) [29].

### *Analyses of Mutations*

NUCLEIC ACIDS. Genomic DNA was extracted by using conventional phenol/chloroform methodology. Total RNA was isolated from skeletal muscle using the RNAzol method (Bioprobe systems) or Fast Prep (Bio 101). Reverse transcription was performed under standard conditions using 1 μg of RNA in a total volume of 30 μl containing 20 units of RNAsin (Promega) and 200 units of superscript reverse transcriptase (Bethesda Research Laboratory). Sequencing was performed using an ABI 377 (Perkin-Elmer) automated sequencer.

α-SG. The first eight exons and flanking intronic sequences of genomic DNA were PCR amplified and analyzed by denaturating gradient gel electrophoresis [30]. PCR-amplified fragments showing an abnormal pattern after denaturating gradient gel electrophoresis were analyzed by direct sequenc-

ing. Exons 9 and 10 were analyzed by direct sequencing of cDNA [30]. Mutations creating or destroying a restriction site were checked by enzyme digestion of genomic DNA for each propositus and his or her relatives.

β-SG. Six exons of genomic DNA were amplified [12] and directly sequenced.

γ-SG. Four overlapping fragments of muscle cDNA, amplified according to Noguchi and co-workers [10], were directly sequenced.

CALPAIN-3. To detect the CANP3 mutations, four overlapping muscle cDNA fragments spanning the entire CANP3 coding sequence were amplified. A second amplification was done using nested primers, and PCR amplification products were sequenced directly. In families for which no mutations were identified on analysis of the cDNA, the 24 exons were systematically studied by heteroduplex analyses or direct sequencing of exons [6]. Heteroduplex analyses in ethidium bromide–stained Hydrolink mutation detection enhancer gels (AT Biochem, USA) were performed on PCR products essentially as shown previously [6, 31]. Because all these families were consanguineous, theoretically the parents were carriers of the same mutation. Parents were thus also investigated by heteroduplex analyses and all were found to be heterozygous carriers.

Mutation nomenclature was established according to the Ad Hoc Committee on Mutation Nomenclature [32] and Beutler and associates [33].

## Results

Following the combined data of protein analyses and chromosomal linkage, we classified our LGMD2 families into the following groups (Table): Groups 1, 2, and 3, sarcoglycanopathies (LGMD2D, LGMD2C, and LGMD2E); Group 4, LGMD2F; Group 5, CANP3 deficiency (LGMD2A); Group 6, LGMD2B; and Group 7, unlinked to any of the above.

### *Groups 1, 2, and 3*

There are 8 families in this group.

GROUP 1: α-SARCOGLYCANOPATHY. Family 1 is 1 of the 3 nonconsanguineous families in our cohort. The index case has onset at age 7 years, corresponding to a moderate/intermediate clinical course. α-SG is only mildly reduced on immunofluorescence (see Table). On denaturating gradient gel electrophoresis screening of α-SG exons, heteroduplex bands were detected in exons 3 and 7. When PCR products were sequenced, a CGT → CCT mutation on codon 284 in exon 7, encoding cysteine instead of arginine, was detected. In this compound heterozygote patient, a CTC → CCT mutation on codon 89 in exon 3, encoding proline instead of leucine, was also found.

Family 2 is also compatible with linkage as judged by HBD. The patient clearly demonstrates a moderate

*Table. Biochemical, Genetic, and Molecular Findings*

| Groups | α-SG IF | α-SG WB | β-SG WB | γ-SG WB | Linkage Chr 17 (LGMD2D) | Linkage Chr 13 (LGMD2C) | Linkage Chr 4 (LGMD2E) | Linkage Chr 15 (LGMD2A) | Linkage Chr 2 (LGMD2B) | Linkage Chr 5 (LGMD2F) | Linkage Chr 6 (CMD) | Mutation | Clinical Progression |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group 1 | | | | | | | | | | | | | |
| α-Sarcoglycanopathies | | | | | | | | | | | | | |
| Family 1 | +++ | | | | L | | | | | | | R284C/L89P | Moderate/int[a] |
| Family 2 | ++ | + | | ++/+++ | L | NL | NL | | | | | | Moderate |
| Group 2 | | | | | | | | | | | | | |
| γ-Sarcoglycanopathies | | | | | | | | | | | | | |
| Family 3 | ++ | ++/+++ | | 0 | | L | | | | | | 923-924 del TG | Severe |
| Family 4 | 0 | 0 | | 0 | NL | L | NL | | | | | | Severe |
| Family 5 | ++ | + | | 0 | | L | | | | | | 923-924 del TG | Intermediate |
| Family 6 | ++ | + | | 0 | NL | L | NL | | | | | 510 del 120 | Severe |
| Group 3 | | | | | | | | | | | | | |
| β-Sarcoglycanopathies | | | | | | | | | | | | | |
| Family 7 | | NM | | | NL | NL | L | | NL | | | | Severe |
| Group 4 | | | | | | | | | | | | | |
| Chr. 5 family | | | | | | | | | | | | | |
| Family 8 | N | N | N | N | NL | NL | NL | NL | NL | L | | | Benign |
| Group 5 | | | | | | | | | | | | | |
| Calpain deficiency | | | | | | | | | | | | | |
| Family 9 | N | N | N | N | | | | L | | | | 551 del A | Intermediate[b] |
| Family 10 | N | N | N | N | | | | L | | | | 19-23 del | Intermediate |
| Family 11 | N | N | N | N | | | | L | | | | 551 del A | Intermediate[b] |
| Family 12 | N | N | N | N | | | | L | | | | Y336N/R490Q | Intermediate |
| Family 13 | N | N | N | N | | | | L | | | | A702V | Intermediate[b] |
| Family 14 | N | N | N | N | | | | L | | | | R748Q | Intermediate |
| Family 15 | N | N | N | N | | | | L | | | | 551 del A | Intermediate |
| Family 16 | N | N | N | N | | | | L | | | | 551 del A | Moderate |
| Family 17 | N | | | | | | | L | | | | Y537X | Intermediate |
| Family 18 | | | | | | | | L | | | | 551 del A | Intermediate |
| Group 6 | | | | | | | | | | | | | |
| Chr. 2 family | | | | | | | | | | | | | |
| Family 19 | N | N | N | N | NL | NL | | NL | L | | | | Moderate |
| Group 7 | | | | | | | | | | | | | |
| Family 20 | | NM | | | NL | NL | NL | NL | NL | NL | NL | | Intermediate |

[a]Onset in childhood, progression very slow.
[b]Onset at 10 to 12 years, follow-up duration too short to tell.

SG = sarcoglycan; IF = immunofluorescence; WB = western blotting; Chr = chromosome; CMD = congenital muscular dystrophy; N = normal; L = linked; NL = not linked; NM = no muscle; int = intermediate; +++ = mild; ++ = moderate; + = severe; 0 = no expression.

phenotype. α-SG is significantly reduced compared with γ-SG in muscle (see Table). No mutation was found in the α-SG gene, with the exception of an isosemantic C → T polymorphism in exon 7 [8] and a C → T transition at nucleotide 1,233 in the 3′-untranslated region [30]. Denaturating gradient gel electrophoresis has an overall sensitivity of 95% for detecting heteroduplex and homoduplex bands. For this purpose, all exon PCR products were sequenced directly and still no mutation was found. After this, to spot a possible splicing mutation, mRNA analyses were performed on muscle biopsies from the patient from this consanguineous family. No mutation was found after amplification of α-SG cDNA and subsequent direct sequence analysis. Final demonstration of the primary α-sarcoglycanopathy in this patient awaits the identification of the pathogenic mutation.

GROUP 2: γ-SARCOGLYCANOPATHY. There are 4 families (6 cases) within this group, based on protein data and chromosomal linkage analysis (HBD). Clinically, 3 families (Families 3, 4, and 6) showed severe courses. One had intermediate severity (Family 5). Western blot analysis showed total absence of γ-SG in all families whose muscle tissues were available, whereas α-SG levels were only reduced in 3 (Families 3, 5, and 6) and were absent in 1 (Family 4) (see Table). In Families 3 and 5, not known to be related but who live in the same geographic area, direct sequencing of the γ-SG cDNA revealed a homozygous TG deletion (923-924delTG) in a (TG)4 repeat in the 3′ coding area of the γ-SG gene, which is equivalent to a null mutation. This mutation segregated in the 2 families in a mendelian fashion. The disease is more severe in Family 3 than in Family 5, although both families share the same mutation. In Family 6, an in-frame deletion of 120 bp (510del120) has been identified. No mutation was detected in the γ-SG gene of Family 4.

GROUP 3: β-SARCOGLYCANOPATHY. Based on HBD, 1 family belongs to this group. We have no muscle sample from the index case, which was a 20-year-old girl. The onset was at 12 years, and she demonstrated a severe phenotype. Two more affected siblings were lost, at ages 20 and 22 years. Further studies by mutation screening were suggestive of deletion (data not shown). We have not yet characterized the mutation.

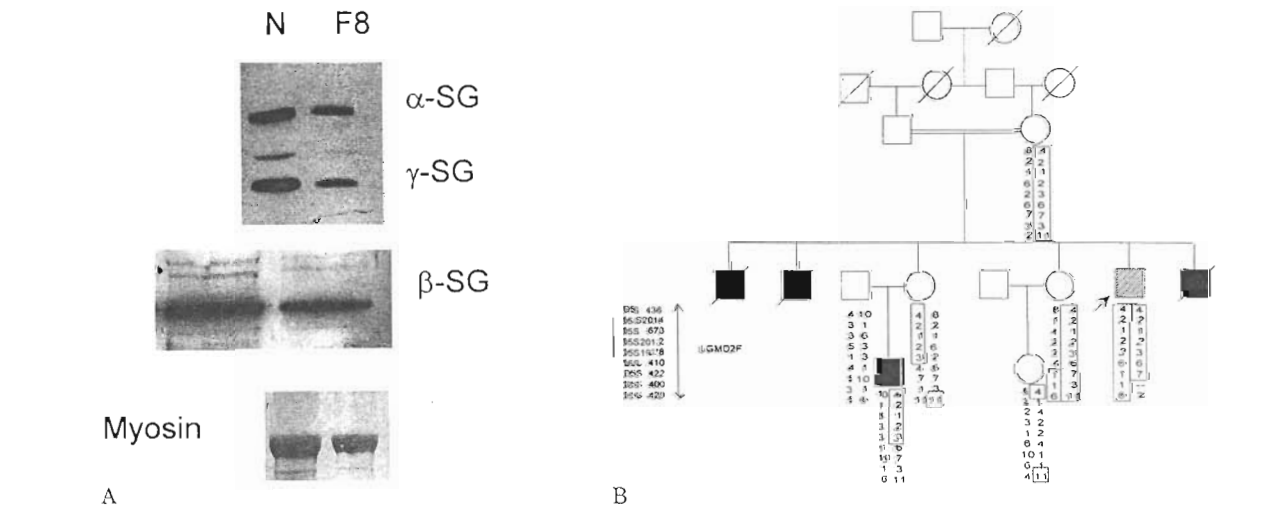
*Group 4: LGMD2F (Sarcoglycans Normal)*

The index case of this interesting family has already been reported elsewhere [34]. The patient is now 32 years of age with a fully benign clinical course that had onset at age 13. He is fully ambulatory, with a minimal physical handicap that does not interfere with his daily life (ie, less than grade I described by Gardner-Medwin and Walton [21]) and, although all other known LGMD2 loci were excluded (see Table), the haplotype data were compatible with a chromosome 5q33-q34 assignment. The α-, β-, and γ-SGs were, however, preserved (Fig). We have not tested this individual for the most recent identified SG protein, the δ [15–17]. His nephew, who is affected with DMD and carries an out-of-frame (exons 45–52) deletion, also shows a carrier haplotype for this autosomal locus.

*Group 5: Calpain-3 Deficiency*

There are 10 families in this group, based on HBD. The age of onset in members of this group varies between 2.5 and 14 years, and they invariably show either an intermediate or a moderate clinical picture.

*Fig. (A) Western blot analysis of α-, β-, and γ-sarcoglycans (γ-SGs) in Family 8. Lower lane is myosin control. N = normal; F8 = Family 8. (B) Chromosome 5 haplotype data of Family 8. The* arrow *indicates the proband.* ■ *= Duchenne muscular dystrophy.*



The oldest patient is 23 years old, and none of the patients within this group is nonambulatory so far. Heteroduplex analysis and direct sequencing of cDNA fragments or exons allowed the identification of all the mutations present in these families, which were four missense (Y336N, R490Q, A702V, and R748Q), two frameshift (19-23delGCATC and 551delA), and one nonsense (Y537X). Among them, all but Y336N and R490Q have been subjected to another report [35]. It is interesting that the 551delA mutation was found in 5 unrelated families. All of them but 1 (Family 16) shared the same haplotype, if we admit that the discrepancies at D15S782 could be due to the high mutability of this microsatellite [35]. As expected, the patients were all homozygous for mutations, except for Family 12. Despite the consanguinity in Family 12, patients from one branch were shown to be compound heterozygotes.

*Group 6: LGMD2B*

The family has 3 affected siblings who had shown initial manifestations in their early 20s and were running moderate courses. The oldest sibling is 46 years of age and can walk 30 m unaided. The index case is 44 and she is ambulatory with the aid of a cane. The youngest brother is 36, is fully ambulatory, and drives a car specially equipped for handicapped drivers. The patient shows normal immunohistochemical staining and immunoblotting for SGs (see Table). Two-point linkage analysis between the LGMD2B locus and D2S327/D2S292/D2S291 haplotype provided a positive lod score for Family 19 ($Z_{max}$; 1.33 at $\theta$; 0.00 cM).

*Group 7: Nonlinked to Any of the Above*

Of the 20 families, 1 failed to show linkage by HBD to any of the LGMD2 regions identified. The index case here had onset of symptoms at age 3. Her creatine kinase level was elevated, at 1,328 U/L. We have been following her for 6 years, and she shows an intermediate course; at age 9 she has scapular winging, she walks on her tiptoes and raises from the floor with a Gower's maneuver, and she cannot run. In addition, we performed linkage analysis for chromosome 6q2 markers [36], taking into consideration that merosinopathy can be seen in childhood-onset autosomal recessive muscular dystrophy cases [20]. Again, there was no evidence for linkage. This family was not studied for a possible linkage to syntrophin loci (ie, 8q23-q24, 16q23, and 20q12).

## Discussion

This study is a first attempt for a systematic clinical, genetic, and molecular investigation of the autosomal recessive LGMDs in Turkey. It appears that in this country, there is a broad spectrum of genes and mutations involved in LGMD2, not including another yet-unidentified LGMD2 locus as suggested by Passos Bueno and colleagues [14].

In agreement with Beckmann and Bushby [37], to discriminate between different LGMD2 entities, both immunofluorescence and western blot are helpful for the initial screening. α-SG immunofluorescence can be taken as a first step to screen all cases for sarcoglycanopathy and to separate these cases from the others.

Linkage analysis correlates clearly with protein studies, because in cases where α-, β-, and γ-SGs were normal, the linkage was always in favor of other chromosomes, excluding any of the sarcoglycanopathies except LGMD2F.

The clinical severity of primary α-sarcoglycanopathies also varies strikingly. The most severe course has been observed in patients in whom α-SG was completely absent and in those homozygous for null mutations [18]. Missense mutations, causing a pronounced but variable decrease in the amount of α-SG, were usually observed in milder forms of variable severity [18, 38].

The patient with ascertained primary α-SG in the present series (Family 1) was compound heterozygous for two missense mutations, L89P and R284C in the α-SG gene. She exhibits a moderate/intermediate course. This is consistent with the previous observation that the R284C mutation gives a benign or moderate phenotype [30].

No mutation was found in Family 2. It should be remembered that family haplotype data are consistent but do not prove linkage in small families with close consanguinity; that is, the affected child could be homozygous for this chromosomal region by chance. This issue will thus be solved on identification of the mutation.

The 4 patients eligible for primary γ-sarcoglycanopathies on the basis of linkage analysis had the most severe clinical courses of the series. In all cases, γ-SG was not detectable on western blots, in contrast with α-SG, which was much less reduced except in the patient belonging to Family 4, for whom no mutation has been identified as yet. It is interesting that this patient, who had a total lack of these proteins, also manifests a severe clinical course. In 2 cases, a 2-bp frameshifting deletion (923-924delTG) was found in the γ-SG mRNA, leading to a severe phenotype in 1 case (Family 3) and to an intermediate phenotype in another case (Family 5). In Family 6, an in-frame deletion of 120 bp (510del120) was observed in the γ-SG mRNA. In the case of McNally and colleagues [39], γ-SG was also absent from muscle whereas there was some preservation of α-SG, the specific mutation at the carboxyl terminus.

Genotype–phenotype correlations have not yet been made in primary γ-sarcoglycanopathies. It is tempting to speculate that the differential impact of a given pri-

mary sarcoglycanopathy on the other members of the complex (secondary sarcoglycanopathies) may modulate the severity of the disease. However, this hypothesis is not consistent with our findings in Families 3 and 5, carrying the same presumed null-type allele where the clinical status does not appear to correlate positively with the amount of residual α-SG, because the most severely affected patient also shows the highest amount of residual α-SG (see Table). Similarly, intrafamilial clinical heterogeneity was recently reported by McNally and colleagues [40]. These phenotypic differences for genoidentical siblings could be due to the presence of a modifier gene or environmental factors.

Our chromosome 4q12–linked family (Family 7) runs a severe course. The case reported by Bönnemann and co-workers [13] had a truncated mutation on both alleles, thereby causing a severe phenotype. The presence of a missense rather than a nonsense mutation in the southern Indiana Amish population could explain the long survivability and slow disease progression in affected individuals. However, recently, Bönnemann and co-workers [41] found three missense mutations in 3 severe LGMD2E families. We do not know the mutation in Family 7; yet it is likely that it also involves a null-type mutation.

We have 10 families within the LGMD2A group. Nine of them run an intermediate course, and 1 (Family 16) runs a moderate course although it shares the same mutation but a different haplotype as 4 other LGMD2A families. Also noteworthy, from a clinical viewpoint is the earlier onset in our cases compared with those reported in the literature [4, 42]. Two of our cases (Families 10 and 14) had disease onset at 3 and 2.5 years, respectively, whereas the lowest figure reported in the literature has generally been 8 years. One other peculiarity of this group is that 3 index cases (Families 13, 16, and 18) had scapular winging. The index case of Family 12 has two different missense (Y336N and R490Q) mutations in exon 7 and exon 11 and is showing an intermediate phenotype. Whereas 2 other members of this specific family, having homozygous mutations (R490Q), display a more severe clinical course, it is interesting that the patients that show HBD for chromosome 15q15.1-q15.3 markers are not from a known consanguineous marriage. Yet in the second branch, which is the result of a consanguineous mating, the patients are compound heterozygous, for both the chromosome 15 haplotype and the LGMD2A mutation.

Families showing linkage to regions other than 4q12, 13q12, and 17q12-q21.33 all demonstrated normal appearance of SGs in muscle tissue. All 3 siblings linked to chromosome 2 manifest a late onset, ie, in the second decade, and a relatively silent course. Our index case in this group was a physical education teacher until age 23. In parallel to this, our candidate for a chromosome 5q33-q34–linked case had a late onset of 13 years of age and he is the only benign case in our series. In contrast to cases reported by Passos-Bueno and colleagues [14], which showed SG abnormality and had severe clinical outcome, our case has normal SGs by immunofluorescence and western blot. It is interesting that his sister, her affected child with DMD, and his mother are carriers of both DMD and this autosomal locus. It is thus crucial to demonstrate whether this is an authentic LGMD2F with mutation in the δ-SG, because it would be the first sarcoglycanopathy devoid of any secondary sarcoglycanopathy documented, and it could give valuable biochemical insights as well as dramatically impact the diagnostic procedure [15–17].

Family 20 did not show linkage to the above chromosomes. This suggests the possible involvement of a new locus for LGMD2, in agreement with Passos-Bueno and colleagues [14] who also suggested the existence of a new chromosomal locus.

A few additional clinical findings are noteworthy. First, cardiomyopathy is not a health problem in these LGMD2 patients, as none of 14 tested index cases initially had or later developed cardiomyopathy. Second, prominent calves may well be encountered in several forms of LGMD2. We have noticed this in our α-, β-, and γ-SG–deficient groups and also in those with CANP3 deficiency. However, enlargement of the calves was never to the extent as that seen in patients with DMD. Third, the most severe cases belonged to the LGMD2C group, as 3 of 4 cases from this group became off-feet within a few years after onset. Also, in this group, 3 cases had delayed walking, which was occasionally seen in other LGMD2s, as well. Fourth, as in patients with DMD and BMD, creatine kinase levels usually correlate inversely with the stage of disease and thus with the muscle bulk of the patient [22], rather than the phenotype, and likewise in our series, high creatine kinase levels were frequently encountered even in milder cases. Fifth, scapular winging along with a relatively milder course may be suggestive of CANP3 deficiency. Last, the common mutation of 551delA for LGMD2A, which was seen in 5 of 10 patients, does not correlate strictly with the clinical phenotype in individual cases. Four cases within the latter group showed an intermediate phenotype (Families 9, 11, 15, and 18), whereas 1 had a moderate course (Family 16). In addition, scapular winging was observed in 2 of these (Families 16 and 18) but not in the others. Moreover, despite fitting into the intermediate group by definition, clinical severity differed between these individual cases. This is not surprising, because at least some clinical variation exists between brothers with DMD, and thus sharing the same mutation is a well-known phenomenon. However, because the 551delA

mutation is relatively frequent, this knowledge may be used for a diagnostic scheme in our population.

Adult-onset merosinopathy should now also be considered within the differential diagnosis of LGMD2. In patients with this autosomal recessive condition, the onset may be delayed to 12 years of age [20]. In this condition, the 300-kd portion of the laminin $\alpha_2$ chain is missing or abnormal. Clinically, these patients demonstrate a mild form of muscular dystrophy, along with the magnetic resonance imaging abnormalities typically seen in classic early-onset congenital muscular dystrophy [43]. We have checked our Family 20 for the laminin $\alpha_2$ chain locus, but this locus is excluded.

In conclusion, 19 of our 20 Turkish LGMD2 families have a defined locus in six different chromosomes, demonstrating the genetic variation for LGMD2 in Turkey. This richness in the genetic background may well be accepted as a result of numerous migrational events in and out of Anotolia throughout the centuries.

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