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Clinical and genetic features of Calpainopathies in Saudi Arabia – a descriptive cross-sectional study

N. ALHARBI[1], E. SHOSHA[2], H. MURAD[3], I. ALHOMUD[3], A. ALSHEHRI[3], M. ALMUHAIZEA[3], E. CUPLER[4], H. AL-HINDI[5], D. MONIES[6], S. BOHLEGA[3]

[1]Department of Clinical Science, College of Medicine, Princess Nourah Bint Abdulrahman University, Saudi Arabia
[2]Neurology Division, Department of Internal Medicine, McMaster University, Hamilton, Canada
[3]Department of Pathology & laboratory Medicine, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia
[4]Department of Neuroscience, King Faisal Specialist Hospital and Research Center, Jeddah, Saudi Arabia
[5]Department of Pathology & Laboratory Medicine, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia
[6]Department of Genetics, King Faisal Specialist Hospital and Research Center, Riyadh, Saudi Arabia

Abstract. – OBJECTIVE: Limb-girdle muscular dystrophies (LGMD) is a heterogeneous group of genetic disorders characterized by progressive weakness of pelvic and shoulder girdle muscles. The objective is to characterize the phenotypic, pathological, radiological, and genetic findings in LGMD2A phenotype (Calpainopathies).

PATIENTS AND METHODS: The National Saudi Arabian LGMD cohort database was screened for LGMD from January 2000 to January 2021. A descriptive cross-sectional study was done on a total of 112 families with LGMD. Screening for mutation in Calpain (CAPN3) gene was done. Clinical and genetic features of LGMD2A phenotype were the main outcome variables. Epi-info was used for statistical analysis.

RESULTS: 34 subjects from 22 families (19.64%) had the specific LMGD2A phenotype. The mean age of onset was 9.9 ± 4.5 years (Range 4 to 19 years). The major initial symptoms were lower limb weakness, inability to climb stairs, and gait disturbance. Gower's sign occurred on an average of 3.75 to 7.25 years after onset. Loss of ambulation was observed in 55.8%. Two novel mutations in the CAPN3 gene were identified.

CONCLUSIONS: The prevalence of LGMD2A was 19.64% among the national Saudi Arabian LGMD cohort. The clinical presentation was varied and was consistent with other reports from different ethnic groups.

Key Words:
Muscular dystrophies, Limb-girdle muscular dystrophies (LGMD), Phenotype, Genetics, Calpain, CAPN3 gene, Saudi Arabia.

Introduction

Limb-girdle muscular dystrophies (LGMD) comprises a group of muscular disorders which are genetically heterogeneous. They are characterized by progressive weakness of the muscles of the pelvis and shoulder girdle[1,2]. The overall prevalence of LGMD as reported from a meta-analysis is 1.63 per 1,00,000 population with 95% confidence interval of 0.94 to 2.81 per 1,00,000 population[3]. The prevalence in children is 0.48 per 1,00,000 with 95% CI of 0.18 to 1.31. It is the fourth most common muscular dystrophy overall[3]. LGMD Type 2A, also known as »Calpainopathy", results from mutation in the calpain3 (Calpainopathy/CAPN3) gene and autosomal recessive inheritance[4-6]. LGMD Type 2A involves the limb-girdle muscles symmetrically, predominantly in the lower limbs with contracture of joints, early Gower sign, and winging of scapula[1,7]. There is sparing of facial, ocular, and respiratory muscles, usually[1,4,7,8]. The clinical course of this disease is highly variable with age at onset for muscle weakness raging from two to forty years. In the clinical course of LGMD Type 2A, there is a lot of intra and interfamilial clinical variability[7]. The disease is progressive and ultimately results in loss of ambulation, around ten to thirty years after symptom onset[7,9]. Its diagnosis requires comprehensive assessment clinically and detailed biochemical analysis, including screening for calpain3 gene mutation[10]. The important

Corresponding Author: Norah Alharbi, MD; e-mail: Noaalharbi@pnu.edu.sa

diagnostic methods are screening for the CAPN3 gene, protein analysis in the muscle, and functional structure of autolytic activity[11]. Calpain3 is a 94-kDa member of the calpain protease family found abundantly in skeletal muscles[10,12]. It is a heterodimer with a large and a small subunit. It is intracellular calcium modulated non-lysosomal cysteine protease (26 exons) located on chromosomal region 15q15.1[13]. It plays a vital role in modulation of the activity of cytoskeletal proteins. It also has a significant role in the regeneration of muscles[14]. Its deficiency leads to myonuclear apoptosis by producing abnormal sarcomeres that ultimately result in muscle fiber death. The earliest muscles to be affected clinically are the Hip adductors and the Gluteus maximus.

The null mutations result in a more severe phenotype, while compound heterozygote subjects are only the least affected[7]. More than a hundred different types of mutations in the CPAN 3 gene have been known to cause LGMD2A. Hence it is essential to identify the mutation for determining the prognosis. LGMD2A is present in different ethnic groups. It is more common in Caucasians, but its course is more severe in African-Brazilian[15]. Besides a few case reports, there is a lack of high-quality evidence on clinical presentation, mutations, and phenotypes of LGMD2A in Saudi Arabia[8]. Literature is available on different phenotypes, gene mutations, and their outcome in another part of the world[4,7,8,10,16-19]. Hence, the present study aims to describe the prevalence, phenotypic and genetic features of calpainopathy in Saudi Arabia.

Patients and Methods

A descriptive observational cross-sectional study was carried out on a total of 112 families with LGMD in Saudi Arabia at a major tertiary care teaching hospital. The LGMD families were identified by screening the national Saudi Arabian LGMD cohort database from January 2000 to January 2021. The database was screened using the following inclusion criteria: Clinical manifestations showing limb-girdle muscular weakness and serum Creatinine Kinase (CK) level > 500U/L, also known as HyperCKemia. The Ethical Board approved the study at King Faisal Specialist Hospital and Research Centre (KFSH&RC), Riyadh, Saudi Arabia (RAC#2070005). 112 Families with LGMD identified by the database contributed to the study population. Universal sampling was used. All the subjects belonging to the LGMD cohort were included in the study after getting informed consent. Hence the sample size was 112 families. All subjects (and their parents if aged below 18) were included in the study only after obtaining informed consent before enrolment. Those subjects not giving consent were excluded from the study.

Clinical Course and Examination

All the study subjects underwent a detailed clinical examination during the screening visit. Clinical data, such as age, gender, medical history ranging from first symptoms and age at its onset to age at diagnosis, walking ability, use of walking aids, use of wheelchair use, and age at first use were collected. Detailed family history was also taken. Other family members were also assessed for the possibility of the disease by performing a neurological examination and testing their blood Creatinine Kinase (CK) levels.

Muscle Strength and Functional Status Assessment

Muscle strength was assessed using Modified Medical Research Council (MRC) grading (0-5)[20], and functional status was evaluated by the Gardner-Medwin and Walton scales[21,22]. Follow-up visits were regularly scheduled twice per year for all patients. All measurements were performed for each patient by one of the authors throughout the study.

Biochemical Investigations, Cardiac Investigations, Spirometry, Electromyography, and Muscle Imaging

Workup including Cardiac investigation, mainly electrocardiogram (ECG) and echocardiography with measurement of left ventricular ejection fraction (LVEF), were obtained. The respiratory evaluation was performed using standardized spirometry. Forced vital capacity (FVC) and forced expiratory volume in 1 sec (FEV1) were assessed and expressed in percentage of predicted values for age, gender, weight, and height. Electromyography (EMG) study was performed in 17 out of 34 subjects (50%). Serum creatine kinase levels were measured in all patients' multiple times during the disease. Muscle magnetic resonance imaging (MRI) was performed in a sample of 12 (35.3%) patients in axial and coronal planes of the lower limbs using the following sequences: T1-weighted(T1w), T2-weighted (T2w), proton-density weight-

ed (PDW), and short Tau Inversion Recovery (STIR). Multiple slices of the thigh, as well as the calf muscle, were analyzed.

Pathological Assessment

Muscle specimens were obtained from nineteen out of thirty-four subjects, taken from the vastus lateralis, semimembranosus, or the biceps brachii. Frozen sections from muscle specimens were prepared and processed according to standard protocol, and stained with hematoxylin and eosin (H&E), modified Gomori trichrome, periodic acid-Schiff (PAS), oil red O, as well as a battery of enzyme histochemical stains, including reduced nicotinamide adenine dinucleotide tetrazolium reductase (NADH-TR), succinic dehydrogenase (SDH), cytochrome oxidase (COX), acid phosphatase, alkaline phosphatase, nonspecific esterase and adenosine triphosphatase at pH 4.3, 4.6 and 9.4. Apart from the above routine studies, immunohistochemical stains for some cytoskeletal proteins were performed. There is no available antibody to calpain-3. The slides were available for review by one of the authors (HAH) for 15 biopsies. Pathological data were extracted from the pathology reports for the remaining 4 cases.

Genetic Analysis

For the Genetic analysis we followed the same steps done by Mones et al[23].

Results

Table I shows the baseline characteristics of the subjects with LGMD2A phenotype. Twenty-two families (34 patients) were diagnosed with LGMD2A. 18 (52.9%) out of 34 subjects were females. 76.5% of subjects had a consanguineous marriage, while 70.5% had a positive family history for LGMD2A. The first-degree consanguinity was observed in fifteen (68.18%) families. Multiple affected individuals in the same family were seen in 12 (54.5%) families. Most of the patients originated from the South and Southwest region of Saudi Arabia with the same percentage, 32.4%. 54.5% of the families were Bedouin (Nomads) in origin. Of special interest, 75% of the Nomads family were a product of consanguine marriage, and 83.33% of them had a positive family history. The age at disease onset ranged between 2 to 19 years (mean 9.6± 4.4, median 10). Most of the patients (85.29%) except five patients had their disease onset below the age of fifteen.

Table I. Baseline characteristics of the study population (n = 34).

S. No	Variable	Frequency/Mean ± S.D./Median	Percentage/Range
1.	Sex		
	Male	16	47.1%
	Female	18	52.9%
2.	Consanguineous Marriage		
	Yes	26	76.5%
	No	8	23.5%
3.	Family history		
	Yes	24	70.6%
	No	10	29.4%
4.	Geographical Region		
	South	11	32.4%
	West	11	32.4%
	Central	7	20.6%
	East	4	11.8%
	North	1	2.9%
5.	Gower siagn		
	Positive	26	76.5%
	Negative	8	23.5%
6.	Ambulation		
	Lost	19	55.8%
	Not lost	15	44.1%
7.	Age of onset in years (n=34)	9.65 ± 4.49	2 to 19
8.	Age of positive Gower sign (n=26)	15.27 ± 5.25	3 to 30
9.	Age of lost ambulation (n=19)	21.6 ± 5.59	2 to 32
10.	Mean CK (in IU/L) (n=34)	3138 ± 2797.5	858 to 13,496
11.	Median CK (in IU/L) (n=34)	2090.5	858 to 13,496

Gower's sign occurred on an average of 3.75 to 7.25 years after onset and was noted in 76.5% of patients. Initial symptoms were lower limb weakness, inability to climb stairs, and gait disturbance in most patients. Loss of ambulation was observed in 55.8% of subjects within 5-19 years after onset. All patients had normal early motor development. Then, at around the age of 9.6 years (range 2-19), they started to have difficulty in climbing the stairs (as the initial symptom in most patients), difficulty in running and getting up from chairs, which progressed over few years to involve shoulder girdle, as well as in almost all the patients. One patient was asymptomatic, and he was accidentally diagnosed with hyperCKemia at age 4. The muscle weakness was more severe in the lower limbs, mainly the hip extensor and knee flexor. At the time of the study, most of the patients have limb-girdle distribution weakness of MRC 2-4/5 in the hip and 3-4/5 in the shoulder, with weak-or absent reflexes. Interestingly, patient #32 A had asymmetrical involvement of the muscle; the power of the left upper and lower limb was 2/5, and in the right upper and lower limb 4/5. Patient# 29 was weaker distally more than proximally. Moreover, the power in his distal lower limb was 1/5 comparing to 4/5 proximally, resulting in a wrong initial diagnosis of Miyoshi distal myopathy. Positive Gower sign is seen in 26 patients (76.5%). 20 (55.8%) of the patients become wheelchair bounded during ten years of follow-up. 15 (44.1%) of the patients remained ambulant at the study time. Seven of them already have Gower (4 female and 3 male). Seven patients (20.5%) had spine abnormalities (five scoliosis, two lordosis). Three patients had calves hypertrophy. Joint contracture, mainly at the ankle, was observed in 5 (14.7%) patients. Serum CK levels during the course of the disease were elevated 4-50 times the normal range in all the patients. It ranged widely between 858-13496 IU/L (normally less than 195 IU\/\L). The cardiac screening was done in 19 (55.8%) patients. It was normal on both electrocardiography (ECG) and Echo except for two patients (10.5%). one patient had dextrocardia, and the other had right ventricular hypertrophy, which did not signify cardiac muscle involvement. Neither of them had any cardiac complaints. Respiratory function was normal in all patients except one patient who had restrictive lung function (FVC<60). Electromyogram (EMG) was performed in 17 (50%) patients. It showed myopathic changes in all except for one subject. There was a lot of inter and intra familiar variability, including the age of onset and disease severity.

Table II shows that the time between the age of onset of disease and the age of Gower's sign, the time between the age of onset and loss of ambulation was significantly higher in males compared to females.

Table III shows the genetic mutations seen in the LGMD2A cohort. Two novel mutations in the CAPN3 gene were identified. Eighteen distinct mutations were found.

Figure 1 shows the comparison of the clinical course of the disease across gender. The age at onset of disease, age at positive Gower's sign, and age at loss of ambulation was significantly higher in males compared to females. There was a significant rapid progression in LGMD2A in this cohort in females compared to males.

Table II. Comparison of characteristics of the clinical course of the disease across the gender.

Variable	Sex	N	Median	Interquartile Range	Mann-Whitney U	Z-value	*p*-value
Time between age of onset and age of positive Gower sign in years	Male	11	7	(5.00-8.00)	44	-2.019	0.044*
	Female	15	4	(3.00-6.00)			
Time between age of onset and loss of ambulation in years	Male	9	14	(10.00-16.00)	16.5	-2.345	0.019*
	Female	10	9	(7.75-10.75)			
Time between age of positive Gower sign and loss of ambulation in years	Male	8	8	(4.75-11.75)	13.5	-2.407	0.016*
	Female	10	4	(2.75-5.50)			

*Statistically significant if $p < 0.05$.

Table III. Mutations identified in Saudi Arabian LGMD2A cohort: (n = 18).

Mutation	Protein change	Mutation type	Effect	Evidence of pathogenicity
c.1076C>T	Pro359Leu	Missense	Likely Pathogenic	PM1, PM2, PP2, PP3
c.2242C>T	Arg748Ter	Nonsense	Pathogenic	PVS1, PM2, PP3, PP5
c.801+1G>A		Splicing	Pathogenic	PVS1, PM2, PP3, PP5
c.334A>G	Ile112Val	Missense	Likely pathogenic	PM1, PM2, PP2, PP3
c.146G>A	Arg49His	Missense	Pathogenic	PM1, PM2, PM5, PP2, PP3, PP5
c.163G>A	Gly55Arg	Missense	Unknown significance	PM2, PM5, PP2
c.310G>T	Glu104Ter	Nonsense	Pathogenic	PVS1, PM2, PP3, PP5
c.118-1G>A	Arg49His	Missense	Pathogenic	PM1, PM2, PP2, PP3
c.G163A	Gly55Arg	Missense	Unknown significance	PM2, PM5, PP2
c.520G>T	Val174Leu	Missense	Likely pathogenic	PM1, PM2, PP2, PP3
c.940C>T:p.Q314X	Gly55Arg	Misense	Pathogenic	PM2, PM5, PP2
c.145C>T:	Arg49Cys	Missense	Pathogenic	PM1, PM2, PM5, PP2, PP3, PP5
c.2334_2335?1200?		Insertion	Insertion	
c.G163A:p.G55R	Gly55Arg	Missense	Unknown significance	PM2, PM5, PP2
c.1699G>A;p.G567R	Gly567Amg	missense	Likely pathogenic	PM2, PM3, PP2, PP3, PP4
c.2381-1G>A		Splicing	Pathogenic	PVS1, PM2, PM3, PM4, PP1-M, PP1-S, PP1, PP3, PP4
g.IV55+1 G>A		Splicing	Pathogenic	PVS1, PM2, PP3, PP5
c.1350_1351del:pS450fs	Arg49His	Frameshift	Pathogenic	PVS1, PM2, PP3, PP5

Figure 2 shows the MRI findings of the lower limb muscles in LGMD Type 2A. MRI of the thigh, hip, and calf muscles was done for 12(35.3%) patients and showed diffuse involvement in the dorsal compartments of the hip and thigh, such as the glutei adductor Magnus and semimembranosus muscles. The soleus and the medial heads of the gastrocnemius in the posterior compartments were prominently affected about the calf muscles.

Figure 3 shows selected light microscopic images from some cases of the current cohort.

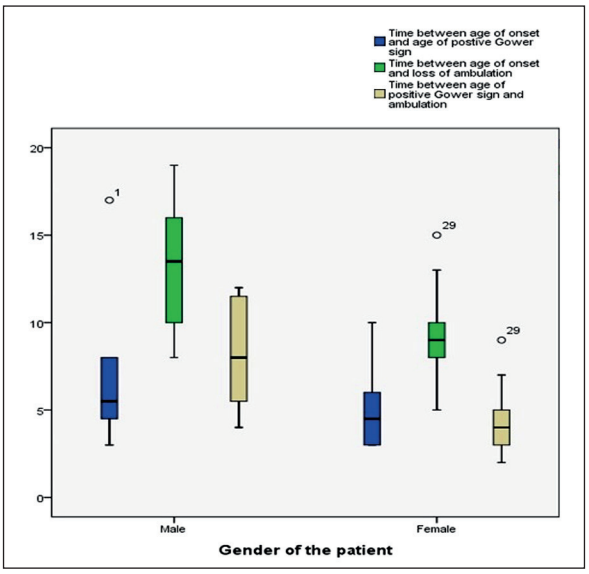

Figure 1. Comparison of characteristics of the clinical course of the disease across the gender. Box and whisker plot for median time between age of onset of disease and age of positive Gower sign, age of onset of disease and age at loss of ambulation, age of positive Gower sign and age at loss of ambulation respectively are shown. The median (Q_2) is represented with the InterQuartile Range (Q_1-Q_3). There was a significant rapid progression in LGMD2A in this cohort in females compared to males.

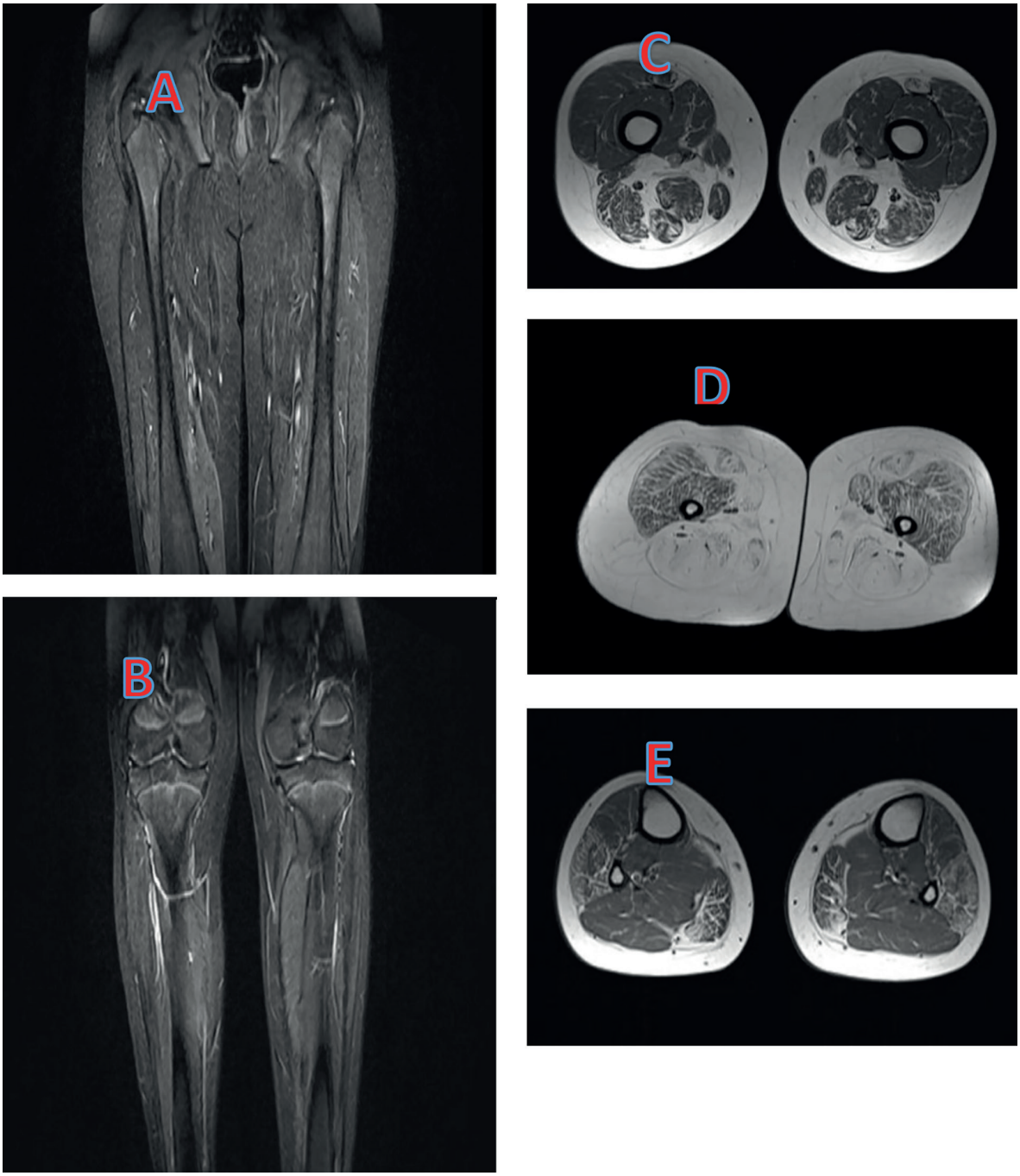

Figure 2. Muscle MRI findings in calpainopathy. **A-B,** Coronal STIR MRI of the thigh **(A)** and leg **(B)** muscle demonstrating bilateral symmetrical increase signal intensity of the medial compartment of the thigh and posterior compartment of the leg muscle consistence with fatty changes and edema **(C)** axial T1 MRI of the upper thigh muscle showed atrophic change mainly the adductor magnus, hamstring muscle , vastus medialis and vastus intermedius ,noticed different stage of the disease picture. **D,** Showed advance atrophy (patient #8A) **(E)** axial T1 MRI of the upper leg muscle showed atrophic changes in the gastrocnemius and soleus muscle in symmetrical fashion.

Pathological Findings

Muscle biopsy was obtained from 19 (56%) patients. The tissue was procured and processed according to standard procedures. All biopsies showed variable degrees of myopathic features, and overt dystrophic changes were seen in 13 biopsies (68% of patients). Myofiber necrosis and/or regeneration were seen in 16 (84%) biopsies.

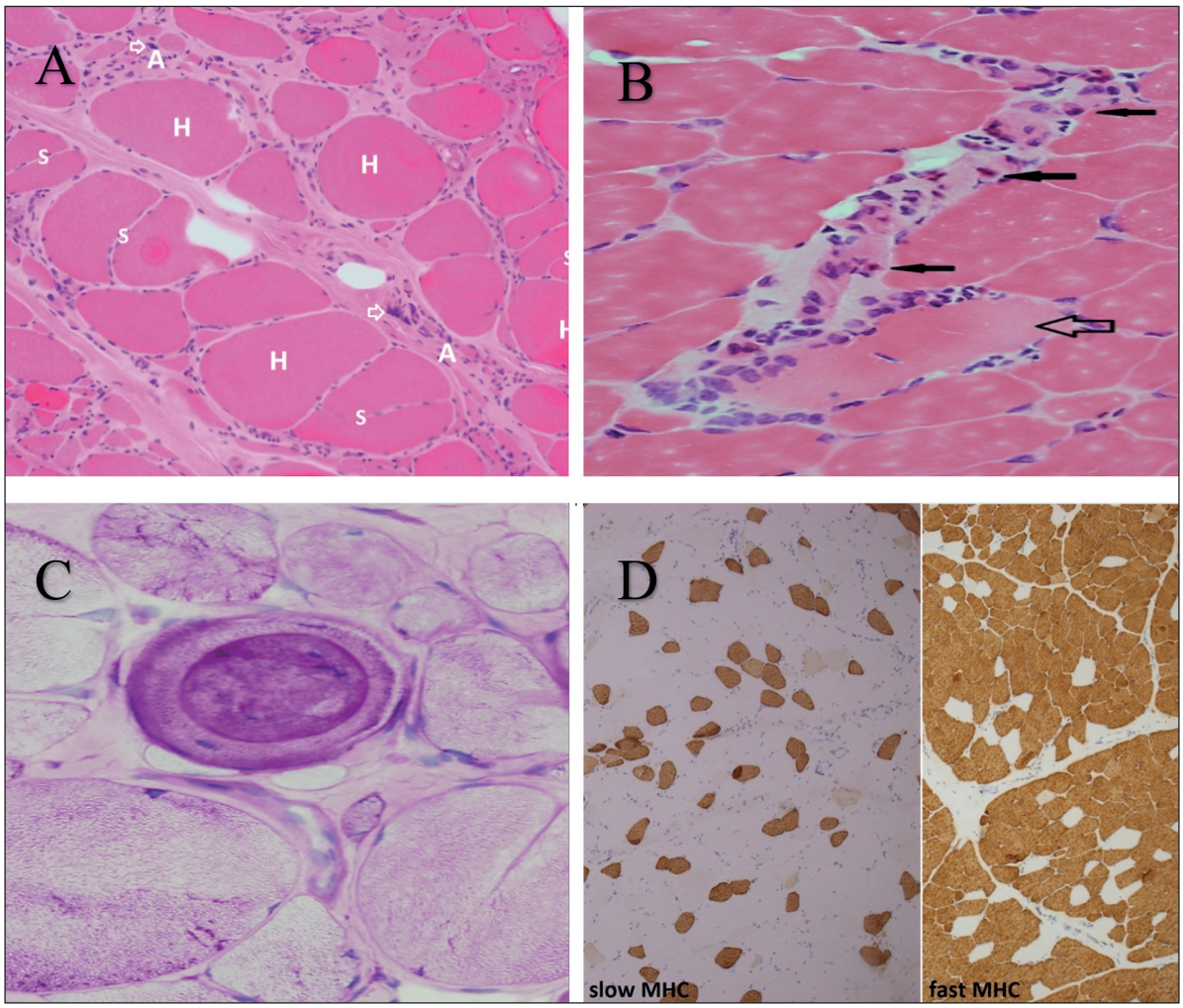

Figure 3. Selected light microscopic images from some cases of the current cohort. Panel (**A**; case #34) depicts the cardinal dystrophic features that are common to almost all cases of LGMD. There is a wide variation in myofiber size with groups of atrophic fibers **(A)** and scattered hypertrophic fibers (H) are noted some of which have splits (s). Moderate endomysial fibrosis is evident with pyknotic nuclear clumps (open arrows). Panel (**B**; case 28) is from case #28 shows endomysial inflammatory infiltrate that is rich in eosinophils (solid arrows). A necrotic fiber (open arrow) is also noted. A ring/whorled fiber is depicted in panel (**C**; case #30). Type 2 predominance is observed in case #28 (panels D). Panels **A** and **B**, H&E stain, original magnification 200×. Panel **C**, PAS stain. Panel **D**, immunohistochemical stains of the slow and fast isoforms of myosin heavy chain.

Endomysial fibrosis was absent in 3 biopsies (17%), mild in 6, and moderate in the remaining cases. A neurogenic component was noted in 4 biopsies (21%). In one biopsy (patient #23), the neurogenic component was prominent, and the biopsy was interpreted initially as chronic denervation with secondary myopathic changes. In that biopsy, there was moderate endomysial fibrosis and a rare, rimmed vacuole. A mild inflammatory infiltrate was observed in 5 biopsies (26%), 2 of which included eosinophil leukocytes. Other nonspecific changes included mitochondrial proliferation (NADH-TR and SDH positive) in 8 (42%) biopsies and lobulated fibers in 2 biopsies by NADH-TR. In one patient (#29), mitochondrial proliferation was marked. There was a predominance of type 2 myofibers in 3 cases and of type 1 myofibers in 2 cases. One case (patient #32) showed type 2 atrophy without neurogenic changes.

Immunohistochemical Findings

Dystrophin expression was mildly reduced in one out of 18 cases that were stained with anti-dystrophin antibodies; dysferlin expression was absent in one case and mildly reduced in 3

out of 13 cases that were stained with anti-dysferlin antibodies, and the expression of sarcoglycans was variably reduced or focally lost in 7 biopsies. Interestingly, there was a selective loss of the expression of γ-sarcoglycan in one case (patient #9) and of dysferlin in another (patient #29); therefore, these cases were labeled initially γ-sarcoglycanopathy and dysferlinopathy, respectively.

Discussion

The present study is one of its kind in describing the prevalence, phenotypic, and genotypic Calpainopathy features of 22 LGMD Type 2A families in Saudi Arabia. LGMD2A is characterized by a slowly progressive myopathy with variability depending on the type of mutation and gender[7]. In the present study, 34 subjects from 22 out of 112 families (19.64%) were identified with calpain 3 gene mutation. LGMD2A is the most common autosomal recessive LGMD[4,24-26]. In regions like the Basque region of Spain, LGMD2A accounts for almost 80% of all cases of LGMD. The prevalence of LGMD2A in India[4] was 47% 4, in Czech[27] 32.6% .32 and 37% in Italy[26]. A study from the United States reported a prevalence ofLGMD2A as 9.2% among LGMD subjects[28]. The prevalence in the present study was 19.64%, greater than that reported in the United States and lesser compared to European countries. This difference in the proportion of LGMD2A could be due to various factors such as consanguinity, demographic and social factors determining their access to diagnosis besides the factors inherent to the studies or surveys such as sample size. Previously only one family has been reported from Saudi Arabia with LGMD2A[8].

Most of the subjects in the present study shared the same classic features as reported originally in patients from Reunion Island (limb-girdle pattern of muscle involvement with joint contracture, sparing the face, and ocular muscles)[7]. The majority of our families were of Bedouin (Normans) origin with a high rate of first-degree consanguinity. Consanguineous marriages are common in the Saudi community, which in turn reduces the genetic variation and increases the chances of recessive disease[29]. Another study[30] compared the consanguinity between the parent (mothers) and their daughters. It showed there was a significantly higher rate of the first-cousin consanguinity among the daughters in comparison with the parents (37.9% *vs*. 29.7%). In the current study, most of the ascertained families were consanguineous (15 out of 22, 68.18%). Among the consanguineous family group, 9 (60%) had a positive family history of the affected member. On the other hand, half of the non-consanguineous group 7 (31.8 %) had a positive family history. There was an equal distribution of male (47.1%) and female subjects (52.9%) in this cohort. In the Female gender, the mean age at onset of disease (8.17 years) was relatively less compared to males (10 years) but without statistical significance. Similar observations have been documented[31].

The onset of the disease is usually in the first or during the second decade of life. In the present study, the mean age of disease onset was 9.65± 4.49 years, while the median age was 10 years. The age of disease onset ranged from as low as 2 years to as high as 19 years. The mean age at onset was higher in the study of Reunion Island at 13.5 ± 7 years[7]. Also, there was greater variability in disease onset age in their study compared to the present study (3-33 years *vs*. 2-19 in the present study). There may be reduced variability in clinical features in isolated populations with increased consanguinity[25].

The most typical presentation in LGMD2A is a symmetrical scapular-humeral-pelvic weakness with sparing of facial muscles. The pattern of clinical course in the present study was the involvement of limb-girdle muscles followed by Gower sign, 3.75 to 7.25 years later. Regarding the Gower sign, about 76.5% (n=26) of the patient developed the Gower sign within 1-17 years from the disease onset. Females developed Gower signs at an earlier age (4.3 years from disease onset) compared to males (5.3 years from disease onset). Also, a higher proportion of women developed Gower sign compared to men (57.6 % *vs*. 42.3%). So, females usually have a more severe disease course compared to males, as shown in Figure 1.

Loss of ambulation in LGMD2A patients occurs about 10-30 years after the onset of the disease. From the current data, about 19 (55.8 %) of the patients became non-ambulant within twenty years of the disease onset, 47.3% of them lost their ability to ambulate with less than 10 years. In the study by Urtasun et al[26] similar to the present study, the subjects became wheelchair-bound 11-28 years after onset. The percentage of males who lost ambulation (28.12%) did not differ significantly from the percentage of the females (31.25%) in the present study. But Females were confined to a wheelchair at an

earlier age (5-15 years from disease onset, mean 8.7 years) compared to males (7- 19 years from disease onset, mean 12.7 years). This observation suggests a more rapid progression in LGMD2A affected females than in males. Results reported from Brazilian, Bulgarian, and France families with regards to differences in disease progression based on gender were inconsistent compared to the present study findings[7,15]. Only 15 (44.1%) patients remained ambulant at the study time. Six of them already have a Gower sign and started to use one walking device. Compared to other studies, there was a higher proportion of loss of ambulation in the present study[7,26]. In the present study, loss of ambulation occurred at an earlier age compared to other studies reported from countries like Italy, Japan, and Mexico[26,32,33].

Examination and investigations in the present study identified marked inter and intrafamilial heterogeneity in the features and severity of the clinical course in LGMD2A muscular dystrophy, which is in line with what was reported previously[7,15,34]. The age at onset in this study differs mostly within the families. In family #10, the diagnosis of LGMD2A was first made in the index case at the age of 10 years, hyperkalemia was noted at the age of 4 in her brother. However, at age 16, he has only minor weakness of the hip and shoulder girdle muscles. Further, in another family, the disease started at the age of 2, while all the other sibs had the disease onset between the age of 4-19.

In all the patients, the disease started with lower limb-girdle muscle involvement, e.g., tip-toe walking and inability to climb the stair. As noted previously, the hip extensors and adductors are selectively more affected. In the upper limb, Shoulder abductors and adductors and elbow flexors were the most impaired muscles. Calves muscle pseudohypertrophy was detected only in three (8.8%) patients. Contractures present in 5(14.7%) patients its mild and predominate in the Achilles tendons. The percentage of both muscle hypertrophy and joint contracture was less in the present study compared to results from other parts of the world[7,15]. Literature data suggest that joint contractures are typical for LGMD2A and may be common even in early disease stages.6 but notably, we did not observe this. Scoliosis was seen in 5 (14.7) patients; in two of them, it's started earlier during the disease course.

Heart involvement is generally rare in LGMD2A, although there are some cases that had cardiomyopathy and cardiac arrest[35,36]. In the present study, only one patient, had right ventricle hypertrophy, which is likely to be a coincidence. Another incidental finding was dextrocardia which was discovered in another patient. There was no respiratory muscle involvement in all patients in this cohort except one (Patient #3) who had restrictive respiratory function ended using BiPAP after twelve-year years from the disease onset. The respiratory muscles involvement was less frequent than a report from another ethnic group[4,26].

Electromyogram studies showed myopathic changes in all the patients apart from one patient, #14A, who had neurogenic changes, which were also seen in his muscle biopsy. Neurogenic damages have been sporadically reported in Calpainopathy patients[37].

The MRI finding was in accordance with the clinical findings as to the hip extensors and adductors, and knee flexors were the most affected muscles (Figure 3). It has been reported that the pathology seems to start in the adductor Magnus muscle and spreads to the semitendinosus and thereafter to all the hamstring muscles, which is similar to our findings[38].

Pathological examination of the muscle shows a variable degree of nonspecific myopathic and dystrophic changes. These changes include a variation of myofiber size (atrophy and hypertrophy); necrosis and regeneration, internalized nuclei and split fibers; and endomysial fibrosis and fatty infiltration. In 5 cases, there was mild inflammatory cellular infiltrate. In two of them it showed eosinophils infiltration (Figure 3B) Eosinophils can serve as a histological clue to Calpinopathy in muscle biopsy. Inflammation with eosinophils has been observed[6,39]. It is assumed that eosinophilic infiltration might be an early pathological stage of calpainopathy[39,40]. Nevertheless, the relationship between inflammation and the Calpainopathy has not yet been established. Two of our cases had lobulated fibers in their biopsies; a feature also has been previously reported[41,42]. Secondary mitochondrial proliferation was observed in 8 cases in our cohort and was marked in one of them (case #29). Case# 23 (family 14) was unique as the patient muscle biopsy & EMG showed neurogenic changes. At the begging patient was thought to have spinal muscular atrophy, but the genetic testing was negative. The patient subsequently underwent whole-exome sequencing, which revealed homozygous missense in calpain 3 deficiency. There was a report of few cases of patients who pre-

viously were thought to have anterior horn cell disease and ended by diagnosed with calpain 3 deficiency[17,43]. This pattern is thought to be secondary to the reinnervation process after nerve degeneration or fiber group necrosis, although neither nerve degeneration nor groups of necrotic fibers were observed on this muscle biopsy. The exact mechanism of neurogenic changes remains elusive. Mild neurogenic changes were also observed in 3 other patients.

Genetically, two novel mutations in the CAPN3 gene were identified in the present study, and they add to the spectrum of CAPN reported mutations all over the world. 18 distinct mutations were found: there were eleven types of missense pathogenic changes, one insertion, and one frameshift mutation were found. In the present study, the mutations were concentrated in exons 2 and 5. Splice mutation on exon 5 c.801+1G>A g.IV55+1 G>A was noted in two families (#8,19). This mutation was also reported in a study done in Japan[38]. The previously reported Calpain-3 mutations showed that they are distributed along with the 24 exons, with a slight mutational 'hot spot' in exon 21[44-46]. Only one mutation in this exon was found in the present study. The findings from the present study indicate that screening of exons: 2 and 5 would allow us to identify most of the distinct mutations.

Conclusions

This is the first report on the LGMD2A features among native Saudi Arabs. Calpainopathy represents 19.64%of all LMGD cases, second to dysferlinopathy. The disease course seems to be more aggressive than other ethnic reports with female predominance. Eosinophilic cellular infiltration and mitochondrial proliferation were infrequently encountered. In contrast to other ethnic groups, the identified mutation was concentrated in exon 2 and 5.our finding expands the known phenotype and genotype of this disorder.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Limitation

The study was a descriptive cross-sectional study. It was a record-based study. Hence Recall bias could have affected the clinical course of the disease, such as the age of onset of symptoms, etc. The sampling frame was from the national cohort database for LGMD. So, there is a problem of generalizability of the present study findings to the community as it is an autosomal recessive condition. Also, there are possibilities for LGMD subjects with mild clinical courses not to come to the attention of the health care system.

Ethical Approval

The study was approved by King Faisal Specialist Hospital & Research Center institutional review board RAC#2070005.

Patients Consent

Consent was obtained from all patients and their caregivers if their age below 18 years).

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