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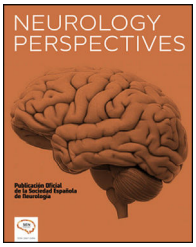

ORIGINAL ARTICLE

Genetic characterization of Limb Girdle Muscular Dystrophies and Pompe Disease in a large Argentine cohort

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KEYWORDS
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Argentina

Abstract
Introduction: The Limb Girdle Muscular Dystrophies (LGMD) are a heterogeneous group of genetically inherited myopathies characterized by progressive weakness of the limb-girdle muscles. Pompe disease (PD) is a treatable lysosomal storage disorder with overlapping clinical features. The prevalence of these muscles disorders in Argentina is unknown.
Aims: To describe the frequency of LGMD and PD and the variants identified in a group of Argentinean patients.
Methods: A retrospective multicenter descriptive study was conducted in patients with muscle weakness investigated by a genetic panel for LGMD and PD through Next Generation Sequencing. The studied genes included: *SGCA, SGCB, SGCG, SGCD, CAPN3, DYSF, TCAP, FKRP, ANO5, HNRPDL, GAA, CAV3*.
Results: Samples from 472 patients were studied (259 males, mean age 39.0 ± 20.1 years old). In 51 patients (10.8%), a genetic disorder was confirmed. The most frequent diagnoses were: LGMD 2A/R1 (*CAPN3*) in 3%, Pompe Disease (*GAA*) in 2.5%, LGMD 2B/R2 (*DYSF*) in 2.1% and LGMD 2I/R9 (*FKRP*) in 0.8%. The main variants identified were *CAPN3*, c.1076C > T (p.P359L); *GAA*,

Abbreviations: ANO5, Anoctamin 5; ACMG, American College of Medical and Genomic Genetics; AD, Autosomal Dominant; AGA/GAA, Acid Alpha-Glucosidase; AR, Autosomal Recessive; CAPN3, Calpain 3; CAV3, Caveolin 3; DBS, Dried Blood Spot Enzyme Assay; DYSF, Dysferlin; FKRP, Fukutin-Related Protein; HNRPDL, Heterogeneous Nuclear Ribonucleoprotein D Like; LGMD, Limb-girdle Muscular Dystrophies; LOPD, Late Onset Pompe Disease; LP, Likely Pathogenic Variant; MLPA, Multiplex Ligation-Dependent Probe Amplification; NGS, Next Generation Sequencing; P, Pathogenic Variant; PD, Pompe Disease; SGCA, Alpha Sarcoglycan; SGCB, Sarcoglycan Beta; SGCD, Sarcoglycan Delta; SGCG, Sarcoglycan Gamma; TCAP, Telethonin; VUS, Variants of Uncertain Significance

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c.-32 13 T > G; *DYSF*, c.5399_5400dupCC (p.F1801fs*24) and *FKRP*, c.826C > A (p.Leu276Ile). In only two of the 12 patients with a definitive diagnosis of PD the panel was carried out for screening purposes (0.4%).
Discussion: This panel confirmed a genetic muscular disorder in 10.8% of the investigated population. LGMD 2A/R1 was the most frequent genetic diagnosis. A definitive molecular diagnosis of Pompe disease was confirmed in 2.5% of the patients however, only 0.4% of the PD cases were new diagnosis.

PALABRAS CLAVE
Distrofias Musculares de Cintura;
Enfermedad de Pompe;
Next-generation sequencing (NGS);
Multiplex ligation-dependent probe amplification (MLPA);
Argentina

Caracterización genética de las Distrofias Musculares de Cinturas y la Enfermedad de Pompe en una larga cohorte Argentina

Resumen
Introducción: Las distrofias musculares de cinturas (LGMD, por sus siglas en inglés) son un grupo heterogéneo de miopatías genéticamente heredadas que se caracterizan por una debilidad progresiva de los músculos de las cinturas escapular y pélvica. La enfermedad de Pompe (EP) es un trastorno del almacenamiento lisosomal tratable con características clínicas superpuestas. Se desconoce la prevalencia de estos trastornos musculares en Argentina.
Objetivos: Describir la frecuencia de LGMD y EP y el perfil de variantes identificado en un grupo de pacientes argentinos.
Métodos: Se realizó un estudio descriptivo multicéntrico retrospectivo en pacientes con debilidad muscular investigados mediante un panel genético para LGMD y EP a través de la tecnología Next Generation Sequencing. Los genes estudiados incluyeron: SGCA, SGCB, SGCG, SGCD, CAPN3, DYSF, TCAP, FKRP, ANO5, HNRDPL, GAA, CAV3.
Resultados: Se estudiaron muestras de 472 pacientes (259 varones, edad media 39,0 + 20,1 años). En 51 pacientes (10,8%) se estableció un trastorno genético. Los diagnósticos más frecuentes fueron: LGMD 2A/R1 (CAPN3) en 3%, Enfermedad de Pompe (GAA) en 2,5%, LGMD 2B/R2 (DYSF) en 2,1% y LGMD 2I/R9 (FKRP) en 0,8%. Las principales variantes identificadas fueron: CAPN,: c.1076C > T (p.P359L); GAA, c.-32 13 T > G; DYSF,c.5399_5400dupCC (p.F1801fs*24) y FKRP, c.826C > A (p.Leu276Ile). En solo dos de los 12 pacientes con diagnóstico definitivo de EP, el panel se realizo con fines de cribado (0,4%).
Discusión: Este panel identifico un trastorno muscular genético en el 10.8% de la población estudiada. LGMD 2A/R1 fue el diagnóstico genético más frecuente. Se constituyó un diagnóstico molecular definitivo de enfermedad de Pompe en el 2,5% de los pacientes; sin embargo, solo el 0,4% de los casos de EP fueron diagnósticos nuevos.

Introduction

The Limb girdle muscular dystrophies (LGMD) are a group of genetically inherited muscle diseases that lead to progressive weakness and wasting of the limb-girdle muscles.[1,2] Axial, facial and/or respiratory muscles can be variably involved as well. Although the condition has been well characterized, clinical and genetic heterogeneity is observed in patients with LGMD in terms of age of onset, extent of muscle weakness and prognosis.[1,2] Based on the mode of inheritance the LGMD can be subdivided in two main subtypes, autosomal dominant (LGMD D) or autosomal recessive (LGMD R).[1, 3]

A systematic review, including studies from the USA, Asia, Europe, Africa and Oceania, estimated a combined worldwide combined prevalence of all muscular dystrophies between 3.8 and 26.8 per 100,000 inhabitants and 0.8–5.7 per 100,000 inhabitants for LGMD.[4] LGMD 2A/R1 calpain3-related, LGMD 2B/R2 dysferlin-related and LGMD 2C/R3 γ-sarcoglycan-related are the most frequent LGMD subtypes.[5,6]

There is restricted data concerning the frequency of LGMD in Argentina. In a cohort of patients from Brazil, Argentina, Chile, Ecuador and Colombia with a clinically suspected limb-girdle syndrome studied for LGMD and Pompe Disease (PD), a molecular diagnosis was identified in 16% of the patients. LGMD 2B/R2 dysferlin-related and LGMD 2A/R1 calpain3-related were the most frequent molecular diagnosis in this group.[7]

PD is an autosomal recessive hereditary myopathy, characterized by a deficit of the lysosomal α-glucosidase acid (AGA) enzyme activity,[8] which could be misdiagnosed as

a LGMD. Based on the age of clinical presentation, the PD is classified into an infantile onset or late onset (LOPD), below or above 1 year old respectively.[8] In worldwide multicenteric genetic descriptive studies of LGMD (using next generation sequencing/whole exome sequencing) the frequency of PD ranged from 0.4 to 3%.[7,9–11]

The early recognition, accurate genetic diagnosis and speedy development of population registries for LGMD and PD is of outmost importance since they allow a comprehensive characterization of the disease, adequate family counseling, timely identification of patients and family care needs , appropriate medical follow-up, and access to approved therapies or participation in natural history studies or clinical trials.[12–14]

This study aims to describe the frequency and variant profile of LGMD and PD in a group of Argentinean patients presenting with proximal muscle weakness and studied with a genetic panel for LGMD and PD.

Materials and methods

Patient cohort

The genetic results of the jugal mucosa swab samples from a large group of patients were retrospectively studied. The samples were obtained between 2017 and 2019 from 70 health centers in Argentina and were centrally analyzed in a single center (Genia Laboratory of Molecular Genetics). Inclusion criteria included: patients older than 1 year old referred for a suspected LGMD or presenting with one or more of the following signs/symptoms: 1. proximal, distal, respiratory, facial or paraspinal muscle weakness, 2. proximal or distal limb muscle atrophy, 3. concomitant or isolated hyperckemia, 4. exercise intolerance, cramps or myalgia, 5. dyspnea, 6. winged scapula or 7. calf pseudohypertrophy without a molecular diagnosis or immunohistochemical confirmatory studies. A panel containing the following genes was used: *SGCA* (NM_000023.2), *SGCB* (NM_000232.4), *SGCG* (NM_000231.2), *SGCD* (NM_000337.5), *CAPN3* (NM_000070.2), *DYSF* (NM_003494.3), *TCAP* (NM_003673.3), *FKRP* (NM_024301.4), *ANO5* (NM_213599.2), *HNRPDL* (NM_031372.3), *GAA* (NM_000152.3), *CAV3* (NM_033337.2).

Genetic analysis

Next Generation Sequencing (NGS) methodology: The extraction and purification of genomic DNA was performed using a DNeasy® Blood & Tissue Kit from Quiagen®. Gene amplification was carried out using a primer pool designed with AmpliSeq™ technology which allowed the amplification of 95% of the coding region and flanking intronic regions. It also included the sequencing of the deep intronic region (intron 14) of the *CAPN3* gene to investigate the pathogenic variant c.1782 + 1072G > C. Sequencing of the amplified regions using Post-LightTM Ion Semiconductor Sequencing (NGS) on the Personal Genome Machine® System platform was performed. The identification or variant calling according to the human reference genome hg19 (Assembly GRCh37) was performed using the Ion Torrent Variant Caller software. The Ingenuity Variant Analysis™ Software was used to record the identified variants and to filter and interpret their clinical significance. The detected variants were researched in international databases, including the Human Gene Mutation Database and available bibliography. Variants were classified according to the guidelines of the American College of Medical and Genomic Genetics: Pathogenic (P), Likely Pathogenic (LP), Variants of Uncertain Significance (VUS), Probably Benign and Benign. Benign variants were not reported.[15] Variants of uncertain significance and probably benign variants were reported only if they were exonic or intronic close to the exon (distance less than or equal to 13 base pairs).

If a variant was not identified in the *GAA* gene through the genetic panel, a Multiplex Ligation-dependent Probe Amplification (MLPA) methodology was performed. The MRC-Holland® Commercial Kit- P453-A1 for the *GAA* gene was used and analysis of the fragments obtained was done by automatic sequencer. Study of the genomic rearrangements, duplications and deletions in the coding regions of the *GAA* gene was done with specific probes for exons 1, 3–10, 12–20 besides to reference probes. The analysis was performed using the Coffalyser software. The percentage of variants detected with this technique is greater than 97%.

A definitive molecular diagnosis was confirmed when two pathogenic (P), two likely pathogenic (LP) or a P and a LP variant were presented according to an autosomal recessive mode of inheritance, except for P/LP variants in the *CAV3* gene.

Written informed consent from the patients, parents and/or legal guardians and attending physician were obtained. The study was approved by the Institutional Review Board of the Hospital Británico de Buenos Aires.

Data are expressed as number and percentage for categorical variables and as mean ± SD for quantitative variables. For descriptive statistical analyses the statistics program SPSS for Windows version 23.0 was used.

Results

The samples from 472 patients were studied (258 males, mean age 39.0 ± 20.1 years old, range 1–81). The 82% of the population were older than 18 years old (Table 1).

Out of 472 patients, 272 (57.6%) had a genetic variant identified through the forementioned panel. One hundred and twenty three (123/472, 26.1%) patients showed at least one P/LP variant and 149 (149/472, 31.5%) at least one Variant of Uncertain Significance (VUS). A total of 176 P/LP variants and 179 VUS were identified (Table 1, Table 2 and Table 3). Overall, *DYSF* was the gene in which more genetic variants were identified (Fig. 1a).*CAPN3* represented the gene with the highest frequency of P/LP variants and *DYSF* the one with the highest frequency of VUS (Fig. 1b and c). No novel variants were identified.

Of the 123 patients having at least one P/LP variant, a definitive molecular diagnosis of a recessive genetic muscular disease was confirmed in 51 (41.4%) which represented the 10.8% of the total population (Tables 1 and 4). Eighty-four percent (43/51) of the patients were compound heterozygous variants and 16% (8/51) were homozygous. The three most common identified disorders were LGMD 2A/R1 (*CAPN3*) in 3%, Pompe Disease (*GAA*) in 2.5% and LGMD 2B/R2 (*DYSF*) in 2.1% (Table 4). The 72 remaining patients

Table 1 Demographic and genetic data.

Total population	472
Male/Female, n (%)	258 (55%)/214 (45%)
Age (yeras) [mean, SD, minimum, maximum}	38.9 + 20 (1–81)
Patients between 1–18 years old, n (%)	83 (18.0%)
Patients above 18 years old, n (%)	389 (82.0%)
Patients with genetic variants, n (%)	272 (57.6%)
Patients with at least one P/LP variant, n (%)	123 (26%)
Patients with a definitive molecular diagnosis, n (%)	51 (10.8%)
Patients with at least one VUS, n (%)	149 (31.5%)
Patients with negative results	200 (42.3%)
Frequency of variants identified by the panel	355
Frequency of P/LP variants	176 (49.6%)
Frequency of VUS	179 (50.4%)

SD: standard deviation. P: Pathogenic Variants. LP: Likely Pathogenic Variants. VUS: Variants of Uncertain Significance.

presented heterozygous variants for autosomal recessive diseases and were confirmed as carriers.

Twenty-one patients presented at least one P/LP variant in the *GAA* gene. In 12 of them a definitive molecular diagnosis of PD was confirmed, being all compounds heterozygous. We further obtained a detailed medical history of each of the confirmed patients with PD. In 10 patients with PD a low blood enzymatic activity was already known by the primary clinician by the time of the genetic panel referral. Thus, in these 10 patients with PD the panel was requested to genetically confirm the diagnosis rather than for screening purposes. In the remaining two patients with PD, the suspected diagnosis was a LGMD and enzymatic studies were not requested before performing the genetic panel.

The remaining nine patients were heterozygous for a P/LP variant in the *GAA* gene. Seven of them had a normal MLPA and in the other two patients the study was not performed. Among the patients with a normal MLPA, the enzymatic activity was low in only two patients. The first patient was a 22-year-old man presenting with myalgia and carrying the variant c.-32-13 T > G. The second one, was a 21-year-old man presenting with hiperckemia and mild tongue and neck flexor weakness and carrying the variant c.2237G > A (p. Trp746Ter). Both patients had a first degree relative with a genetic and clinical diagnosis of PD, a maternal uncle in the former and the father of the patient in the latter.

Discussion

This paper represents the first genetic multicenter study of LGMD and PD performed in Argentina and evaluated by a centralized single reference laboratory.

Next Generation Sequencing technology enabled a definitive molecular diagnosis of a LGMD in 10.8% of the population. This result was similar to those reported in Canada (15%,[16] 17%[10]) and in Latin American (16%).[7] However, it was lower than the diagnostic yield reported in the USA (21%),[9] Italy (23%[17]), Germany (33%)[18] and in a multicenter study including 9 countries (24%).[19] Differences among studies are most likely due to reasons such as: variations in the number of genes included in the panels,

Table 2 Pathogenic/likely pathogenic variants with a frequency > 2 (n = 176).

Gene	DNA variant	Protein variant	Frequency	Status	Location
GAA	c.-32-13 T > G	Unknown	15	P	Intronic
CAPN3	c.1076C > T	p.Pro359Leu	8	P	Exonic
ANO5	c.692G > T	p.Gly231Val	6	LP	Exonic
CAPN3	c.328C > T	p.Arg110Ter	5	P	Exonic
CAPN3	c.1468C > T	p.Arg490Trp	5	P	Exonic
CAPN3	c.223dup	p.Tyr75LeuTer5	5	P	Exonic
CAPN3	c.2362_2363delinsTCATCT	p.Arg788SerTer14	5	P	Exonic
DYSF	c.5399_5400dupCC	p.Phe1801ProfsTer24	5	P	Exonic
SGCA	c.229C > T	p.Arg77Cys	5	LP	Exonic
TCAP	c.157C > T	p.Gln53Ter	4	P	Exonic
FKRP	c.826C > A	p.Leu276Ile	3	P	Exonic
ANO5	c.172C > T	p.Arg58Trp	3	LP	Exonic
SGCG	c.525delT	p.Phe175Leufs	3	P	Exonic
GAA	c.1076-1G > A	unknown	2	P	Intronic
DYSF	c.1402C > T	p.Arg468Cys	2	LP	Exonic
FKRP	c.1486 T > A	p.Ter496Arg	2	LP	Exonic
GAA	c.2237G > A	p.Trp746Ter	2	P	Exonic
GAA	c.2608C > T	p.Arg870Ter	2	P	Exonic
DYSF	c.4513 T > A	p.Tyr1505Asn	2	LP	Exonic
DYSF	c.5785-7G > A	Unknown	2	LP	Intronic
CAPN3	c.802-9G > A	Unknown	2	LP	Intronic
SGCA	c.850C > T	p.Arg284Cys	2	LP	Exonic

GAA: Acid Alpha-Glucosidase. CAPN3: Calpain 3. ANO5: Anoctamin 5. DYSF: Dysferlin. SGCA: Sarcoglycan Alpha. TCAP: Telethonin. FKRP: Fukutin-Related Protein. SGCG: Sarcoglycan Gamma. SGCB: Sarcoglycan Beta. P: Pathogenic. LP: Likely Pathogenic.

Table 3 Variants of unknown significance identified (n = 179).

Gene	DNA variant	Protein variant	Location	Effect
SGCA	c.-90C > A	Unknown	Intronic	Synonymous
DYSF	c.5999G > A	p.Arg2000Gln	Exonic	Missense
DYSF	c.2902A > T	p.Met986Leu	Exonic	Missense
CAPN3	c.1842G > C	p.Glu614Asp	Exonic	Missense
SGCA	c.466C > T	p.Arg156Cys	Exonic	Missense
SGCG	c.469G > C	p.Glu157Gln	Exonic	Missense
GAA	c.506 T > C	p.Leu169Pro	Exonic	Missense
ANO5	c.1333-9A > G	Unknown	Intronic	Splice site mutation
GAA	c.1437 + 8G > A	Unknown	Intronic	Splice site mutation
ANO5	c.155A > G	p.Asn52Ser	Exonic	Missense
DYSF	c.1744C > T	p.Arg582Trp	Exonic	Missense
CAPN3	c.202 T > C	p.Cys68Arg	Exonic	Missense
FKRP	c.235G > A	p.Val79Met	Exonic	Missense
DYSF	c.2731A > G	p.Ile911Val	Exonic	Missense
DYSF	c.2948A > C	p.Lys983Thr	Exonic	Missense
SGCD	c.451 T > G	p.Ser151Ala	Exonic	Missense
CAPN3	c.469G > A	p.Glu157Lys	Exonic	Missense
DYSF	c.551C > T	p.Thr184Ile	Exonic	Missense
SGCG	c.8G > A	p.Arg3His	Exonic	Missense
TCAP	c.32C > T	p.Ser11Leu	Exonic	Missense
TCAP	c.113G > T	p.Cys38Phe	Exonic	Missense
Gen	ADN Variant	Protein	Location	Effect
CAPN3	c.1154C > T	p.Ala385Val	Exonic	Missense
FKRP	c.11C > G	p.Thr4Ser	Exonic	Missense
DYSF	c.1351A > G	p.Met451Val	Exonic	Missense
ANO5	c.1417G > A	p.Val473Ile	Exonic	Missense
CAPN3	c.1521C > T	p.Tyr507=	Exonic	Splice site mutation
CAPN3	c.1525-91C > T	Unknown	Intronic	Not specified
ANO5	c.155A > G	Unknown	Exonic	Missense
DYSF	c.1657C > T	p.Arg553Cys	Exonic	Missense
DYSF	c.1754A > G	p.Lys585Arg	Exonic	Missense
ANO5	c.2141C > G	p.Thr714Ser	Exonic	Missense
GAA	c.2155G > T	p.Ala719Ser	Exonic	Missense
SGCG	c.223A > G	p.Lys75Glu	Exonic	Missense
GAA	c.264C > G	p.Ser88Arg	Exonic	Missense
GAA	c.268 T > A	p.Phe90Ile	Exonic	Missense
GAA	c.2735C > T	p.Ala912Val	Exonic	Missense
DYSF	c.3071C > T	p.Pro1024Leu	Exonic	Missense
DYSF	c.3076G > A	p.Asp1026Asn	Exonic	Missense
DYSF	c.3541G > A	p.Asp1181Asn	Exonic	Missense
CAV3	c.401C > T	p.Ala134Val	Exonic	Missense
DYSF	c.3771G > C	p.Trp1257Cys	Exonic	Missense
CAV3	c.401C > T	p.Ala134Val	Exonic	Missense
SGCA	c.421C > A	p.Arg141Ser	Exonic	Missense
DYSF	c.5216C > A	p.Pro1739Gln	Exonic	Missense
DYSF	c.5246G > A	p.Arg1749His	Exonic	Missense
SGCG	c.539A > T	p.Glu180Val	Exonic	Missense
DYSF	c.551C > T	p.Thr184Ile	Exonic	Missense
DYSF	c.703G > A	p.Val235Met	Exonic	Missense
SGCB	c.754-5A > G	Unknown	Intronic	Not specified
DYSF	c.776G > A	p.Ser259Asn	Exonic	Missense
SGCB	c.892G > A	p.Val298Met	Exonic	Missense
SGCA	c.984-10G > A	Unknown	Intronic	Splice site mutation
ANO5	c.2168A > C	p.Lys723Thr	Exonic	Missense

ANO5: Anoctamin. GAA: Acid Alpha-Glucosidase. CAPN3: Calpain 3. CAV3: Caveolin 3. DYSF: Dysferlin. SGCA: Sarcoglycan Alpha. SGCB: Sarcoglycan Beta. SGCD: Sarcoglycan Delta. SGCG: Sarcoglycan Gamma.

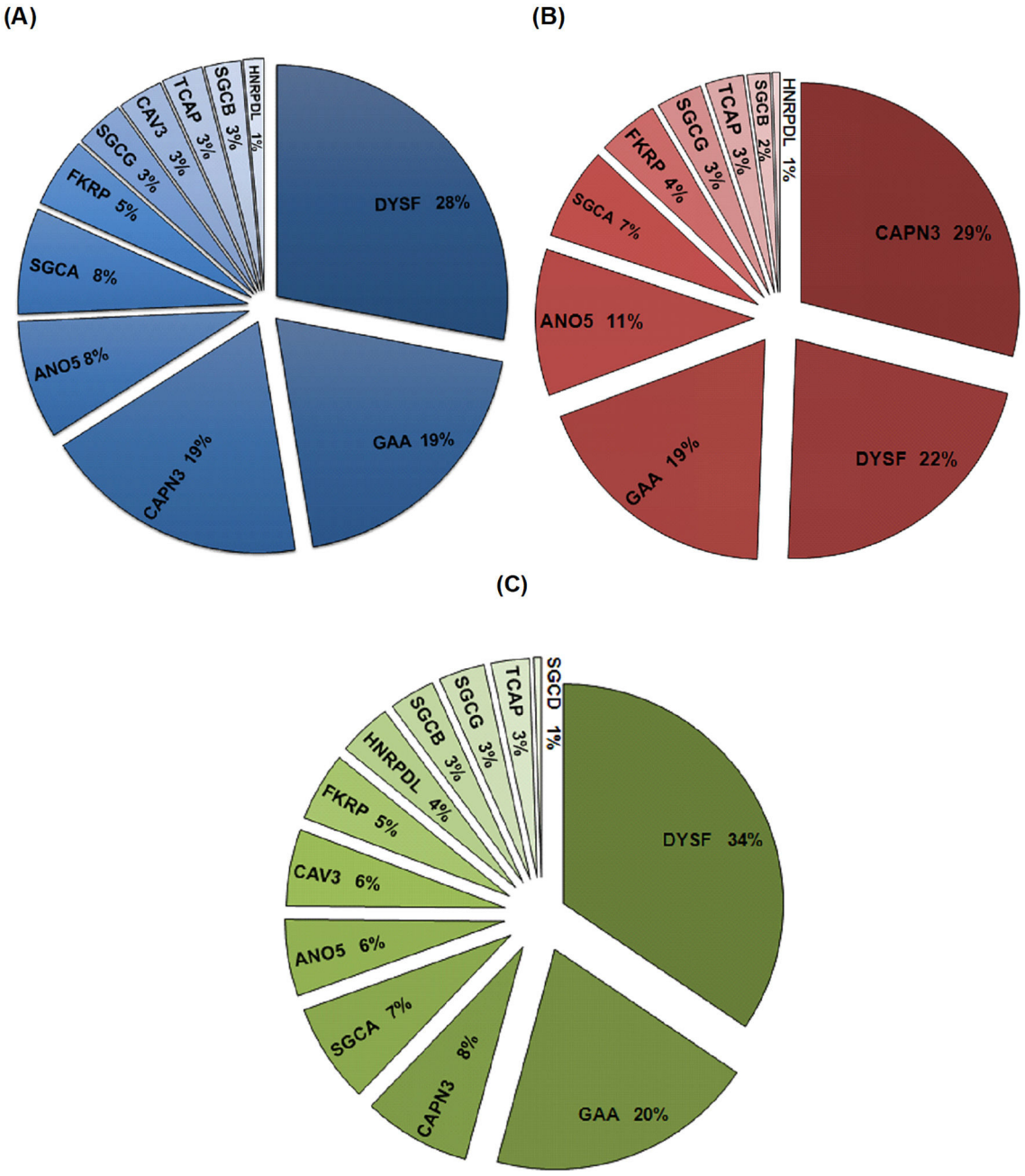

Fig 1 Frequency of variants identified. Pie chart **A** shows the distribution by gene of all the variants (n = 355) identified in the population studied. Pie chart **B** shows the distribution by gene of the pathogenic/likely pathogenic (n = 176) variants identified in the population studied. Pie chart **C** shows the distribution by gene of the Variants of Unknown Significance identified in the population studied (n = 179). AGA/GAA: Acid Alpha-Glucosidase, ANO5: Anoctamin 5, CAPN3: Calpain 3, CAV3: Caveolin 3, DYSF: Dysferlin, FKRP: Fukutin-Related Protein, HNRPDL: Heterogeneous Nuclear Ribonucleoprotein D Like, SGCA: Alpha Sarcoglycan, SGCB: Sarcoglycan Beta, SGCD: Sarcoglycan Delta, SGCG: Sarcoglycan Gamma, TCAP: Telethonin.

ranging from 12 genes in our study to 169 in others,[19,25] as well as different inclusion criteria and ethnic backgrounds.

Calpain-3 (14/472 patients, 3%) was the most frequent LGMD identified in this study similarly to others.[5,9,20,26] Nevertheless, a Latin American study,[7] which included 50% of patients from Brazil, reported that the most frequent AR LGMD in this region was due to mutations in the *DYSF* gene. Likewise, two additional Brazilian studies identified dysferlin mutations as the most common cause of LGMD[21,22] in that country. The difference in the LGMD distribution between neighbour countries might be due to historical reason since Argentina was an Spanish colony and had a remarkable immigration from mainly Italy and Spain at the end of the 19th century and the first decades of the 20th century,[23] whereas Brazil had an important Portuguese immigration background. LGMD2B was identified as the most frequent LGMD in a Portuguese study[24] as well.

Table 4 Genetic muscular diseases diagnosed in the study.

Gene	Disease	Molecular diagnosis frequency within the total population n (%) (n = 472)	Molecular diagnosis frequency within the patients with a definitive molecular diagnosis % (n = 51)	P/LP variant frequency % (n = 176)	Main variant identified/ Presentation	Frequency of P/LP variants identified by gene
CAPN3	LGMD 2A (R1 calpain3)	14 (3%)	27%	51 (29%)	c.1076C > T (p.Pro359Leu) Homozygous	22
GAA	LGMD 2 V (Pompe disease)	12 (2,5%)	24%	33 (19%)	c.-32-13 T > G Compound Heterozygous	13
DYSF	LGMD 2B (R2 dysferlin)	10 (2,1%)	20%	38 (21.5%)	c.5399_5400dupCC (p. F1801fs*24) Homozygous	24
FKRP	LGMD 2I (R9 FKRP)	4 (0,8%)	8%	8 (4.5%)	c.826C > A (p.Leu276Ile) Compound Heterozygous	5
SGCA	LGMD 2D (R3 α-sarcoglycan)	3 (0,6%)	6%	12 (7.0%)	c.850C > T (p.Arg284Cys) Homozygous/Compound Heterozygous	3
ANO5	LGMD 2L (R12 anoctamin 5)	2 (0,4%)	4%	19 (11%)	4 mutations Compound heterozygous	11
TCAP	LGMD 2G (R7 telethonin)	2 (0,4%)	4%	5 (3.0%)	c.157C > T (p.Gln53Ter) Homozygous	1
SGCG	LGMD 2C (R5 γ-sarcoglycan)	2 (0,4%)	4%	5 (3.0%)	c.525del p.(Phe175Leufs) Homozygous/Compound heterozygous	2
HNDPRL	LGMD 1G (D3 HNRNPDL)	1 (0,2%)	2%	1 (0.5%)	c.1132G > C (p.Asp378His) Heterozygous	1
SGCB	LGMD 2E (R4 β-sarcoglycan)	1 (0,2%)	2%	2 (1.0%)	c.551A > G (p.Tyr184Cys) c.33 + 1G > C Compound heterozygous	2

P: Pathogenic. LP: Likely Pathogenic. CAPN3: Calpain 3. LGMD: Limb-girdle Muscular Dystrophies. GAA: Acid Alpha-Glucosidase. DYSF: Dysferlin. FKRP: Fukutin-Related Protein. SGCA: Sarcoglycan Alpha. ANO5: Anoctamin 5. TCAP: Telethonin. SGCG: Sarcoglycan Gamma. HNRPDL: Heterogeneous Nuclear Ribonucleoprotein D Like. SGCB: Sarcoglycan Beta.

The second most common identified myopathy in our study was PD (12/472, 2,5%). This frequency is similar to that reported on previous studies.[10,17] After the genetic results, we were able to obtain clinical information from all PD patients identified and it became clear that 10/12 patients already had low GAA enzymatic levels which were not reported at the time of the genetic testing. Therefore, only 2/472 (0.4%) patients with no previous suspected diagnosis of PD were identified through this screening panel as was previously reported in Latin America (0.4%).[7] This represented 3.9% (2/51) of all patients with a confirmed myopathy. Screening genetic studies searching for PD should carefully exclude patients with known low GAA enzymatic levels in order to inform a more accurate PD frequency (Table 5).[5,8,11,14,15,19,20] In addition, two types of screening studies of PD are reported in the literature. In the first one, patients with a LGMD phenotype, hyperckemia or an unspecified myopathy were initially studied with enzymatic assays and the results were lately confirmed with a genetic technique. The reported PD frequency using this approach was between 1.7% and 4%.[27–31] The second approach consists of prospective or retrospective genetic studies of PD in patients with variable inclusion criteria (Table 5) in the majority of which PD was identified in less than 1%. The inclusion of patients with family history or previous abnormal enzymatic levels could be opened to selection bias. In this study, as well as in the USA and Europe, the most common variant identified in the *GAA* gene was the c.-32-13 T > G intronic variant different from those reported in Asia-Pacific (c.2238G > C) and in the Middle East (c.1064 T > C).[32,33]

Seventy-two patients carrying a heterozygous pathogenic variant for an AR LGMD were identified. Nine patients had an heterozygous pathogenic variant in the *GAA* gene, among whom two had a low enzymatic essay despite the fact that a second variant in the *GAA* gene could not be identified through MLPA. This finding could be due to limitations of the study methodology used to detect intronic or cryptic gene variants. Although this finding did not modify the frequency of patients with a definitive diagnosis of PD in this study, as the molecular diagnostic criteria was not fulfilled by none of these two patients, they both showed mild phenotypes of PD. Patients with similar clinical and molecular findings were reported to respond to enzyme replacement therapy[34] and should prompt further investigations to identify a second variant in the *GAA* gene that could explain the patients clinical features.

Limitations of this work include its retrospective nature with lack and a non-homogeneous collection of detailed

Table 5 Pompe disease frequency reported in the literature.

Study	Study methodology/Inclusion of patients with 1° degree family history/previous abnormal GAA enzymatic levels	Patients diagnosed of PD/Total study population	Patients diagnosed of PD/Total LGMD cases
Savarese et al[17] Italy 2016	Prospective multicenter Yes/Not specified	10/504 = 1.9%	10/115 = 8.6%
Lévesque et al[10] Canada 2016	Retrospective multicenter Yes/Not specified	1/34 = 2.9%	1/17 = 6%
Babi Ramesh Reddy Nallamilli et al[9] USA 2018	Prospective multicenter No/Not specified	28/4656 = 0.6%	28/1003 = 2.8%
Bevilacqua et al[7] Latin America 2020	Retrospective multicenter Not specified/No	9/2103 = 0.4%	9/335 = 2.7%
Topf[19] Europe and Middle East 2020	Prospective multicenter Yes/Not specified	10/1001 = 0.9%	10/240 = 4%
Thuriot et al[16] Canada 2020	Prospective multicenter Not specified/Not specified	8/1236 = 0.6%	8/187 = 4.2%
Our study	Retrospective multicenter Yes/Yes	12/472 = 2.5%	12/51 = 23%
		PD patients without previous DBS: 2/472 = 0.4%	PD patients without previous DBS: 2/51 = 3.9%

GAA: Acid Alpha-Glucosidase. PD: Pompe Disease. LGMD: Limb-girdle Muscular Dystrophies.

clinical information of the majority of the patients, the use of a bounded genetic panel and the lack of family segregation analysis. Furthermore, patients below 1 year old were not included in the analysis. Finally, whole exome sequencing,[11] whole genome sequencing and RNA-seq-based transcriptome analysis[35] were not feasible. All these factors might have affected the frequency of the LGMD and PD identified in this study.

In conclusion, this study represents the first genetic characterization of LGMD and PD in Argentina. We identified a genetic muscular disorder in 10.8% of the investigated population. CAPN3 was the main LGMD, and a definitive molecular diagnosis of new PD cases was confirmed in 0.4% of the patients. Molecular tests are useful tools for a prompt diagnosis of muscular genetic disorders, allowing their early recognition, consideration for treatment and genetic counseling. Results of molecular studies need to be integrated with clinical and complementary assessments to reach a final diagnosis. Inclusion criteria for genetic screening evaluations of muscular disorders could critically affect the results, so, information regarding family history, previous abnormal muscle pathology or abnormal enzymatic assays for PD should be carefully excluded since they may lead to frequency inaccuracies. Further researches are needed to expand the characterization of the LGMD and PD in Argentina.

Conflict of interest

The authors and members of the Argentine Muscular Dystrophy Consortium have no conflicts of interest to declare.

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Data availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request.

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