# A homozygous nonsense mutation in *SGCA* is a common cause of LGMD in Assiut, Egypt

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## Abstract

**Introduction**—The genetic causes of limb-girdle muscular dystrophy (LGMD) have been studied in numerous countries, but such investigations have been limited in Egypt.

**Methods**—A cohort of 30 families with suspected LGMD from Assiut, Egypt was studied using immunohistochemistry, homozygosity mapping, Sanger sequencing, and whole exome sequencing.

**Results**—Six families were confirmed to have pathogenic mutations, 4 in *SGCA* and 2 in *DMD*. Of these, 3 families harbored a single nonsense mutation in *SGCA*, suggesting that this may be a common mutation in Assiut, Egypt originating from a founder effect.

**Discussion**—The Assiut region in Egypt appears to share at least several of the common LGMD genes found in other parts of the world. It is notable that 4 of the 6 mutations were ascertained via whole exome sequencing, even though it was the last approach adopted, illustrating the power of this technique for identifying causative mutations for muscular dystrophies.

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## Keywords

Founder effect; Homozygosity mapping; Limb-girdle muscular dystrophy; Sarcoglycanopathy; Whole exome sequencing

## Introduction

Limb girdle muscular dystrophy (LGMD) is a category of a group of rare, heterogeneous, genetically inherited muscle diseases. LGMD causes progressive proximal muscle weakness and a range of histological abnormalities, with variable rates of progression and degrees of severity. Numerous genes have been associated with different forms of LGMD[1]. Inheritance patterns differentiate the two major subtypes: LGMD type 1 (LGMD1) is inherited in a dominant fashion and LGMD type 2 (LGMD2) follows a recessive pattern. To date, 8 dominant forms (LGMD1A-H) and 23 recessive forms (LGMD2A-W) have been described[2,3]. Onset of symptoms may occur at nearly any age, from childhood through adolescence into adulthood; the only exception is infancy, which by definition would indicate the presence of a congenital muscular dystrophy. Mutations in known genes can be found in approximately 45–46% of cases of neuromuscular disorders[4] and LGMD[5]. Clinical genetic testing is not available for some associated genes, especially the less common ones, and access to such testing is often limited, due primarily to the costs involved. This makes it difficult for many affected families to obtain a definitive genetic diagnosis.

In this study, we recruited and analyzed 30 families of Egyptian descent, each of which has one or more individuals with the LGMD phenotype. Immunohistochemistry, homozygosity mapping, Sanger sequencing, and exome sequencing contributed to the identification of causative mutations in known genes for 6 of the families. Four of the families harbored mutations in *SGCA*, while 2 others were found to have cases of dystrophinopathy.

## Materials and Methods

### Patient recruitment and clinical diagnosis

Affected individuals with the clinical diagnosis of LGMD and informative relatives from 30 families were enrolled in an institutionally-approved research protocol at Assiut University, Egypt. The families were numbered 1267–1285, 1357–1364, and 1410–1412. Results of clinical evaluations were available for the study, including medical histories, family histories, physical examination, and serum creatine kinase (CK) levels.

### Muscle histology

Medial gastrocnemius muscle biopsies were performed on selected affected individuals. This muscle was selected as it displayed a lesser degree of weakness in the subjects and would thus be less likely to display excessive fibrosis. The tissue samples were snap frozen and stored at −80°C. Hematoxylin and eosin staining was performed using standard protocols on samples representing families 1268, 1271, 1273, 1275, 1276, 1278, 1280, and 1282. Immunohistochemistry was performed using antibodies to dystrophin (DYS-1, 2, 3), α-, β-, γ- and δ- sarcoglycan, dysferlin, and α- and β-dystroglycan on muscle biopsy samples from

subjects 1271-1, 1273-1, 1275-1, 1276-1, 1278-1, 1280-1, and 1282-1. Staining for DYS-1, 2, 3, and α-, β-, γ- and δ- sarcoglycan was performed on muscle biopsy samples from subjects 12704, 1272-1, 1274-1, 1277-1, 1279-1, 1283-1, and 1285-1. Novocastra NCL-a-SARC antibody was used to stain for α-sarcoglycan.

### DNA extraction

Peripheral blood samples were obtained from affected individuals and informative relatives in the enrolled families. Genomic DNA was extracted from the blood samples using the QIAamp DNA Blood Midi Kit (Qiagen), then quantified using a NanoDrop 1000 Spectrophotometer (Fisher Scientific).

### Homozygosity mapping

DNA samples from affected individuals in families known or suspected to be consanguineous (1267–1269, 1271–1275, 1277, 1280, 1284, 1357, 1358, 1360–1363) were selected for genotyping at 10,204 single nucleotide polymorphisms (SNPs) using the GeneChip Human Mapping 10K 2.0 Array (Affymetrix), and homozygosity mapping was performed as previously described[6].

### Whole exome sequencing

The Genomics Platform at the Broad Institute was used to perform whole exome sequencing of DNA samples representing selected subjects for whom immunohistochemistry and homozygosity mapping did not suggest the associated gene harboring the causative mutations. The Agilent Sure-Select Human All Exon v2.0, 44Mb baited target and the Broad's in-solution hybrid selection process were used to target exons in genomic DNA. At least 250 ng of DNA with concentrations of at least 2ng/μl were submitted for each sample. The exome-sequencing pipeline included sample plating, library preparation (2- plexing of samples per hybridization), hybrid capture, sequencing (76bp paired reads), sample identification quality control check, and data storage. The hybrid selection libraries cover >80% of targets at 20× or more, with a mean target coverage of >80×. The exome sequencing data was de-multiplexed and each sample's sequence data were aggregated into a single Picard BAM file. Exome sequencing data was processed through a pipeline based on Picard (https://github.com/broadinstitute/picard), using base quality score recalibration and local realignment at known insertions and deletions. The BWA aligner (https://github.com/lh3/bwa) mapped reads to the human genome build 37 (hg19) reference sequence. Single nucleotide polymorphisms (SNPs) and insertions/deletions (indels) were jointly called across all samples using the Genome Analysis Toolkit (GATK) HaplotypeCaller package version 3.3. Default filters were applied to SNP and indel calls using the GATK's Variant Quality Score Recalibration (VQSR) approach. The variants were annotated using Variant Effect Predictor. The variant call set was uploaded on to xBrowse (https://atgu.mgh.harvard.edu/xbrowse/) and an analysis limited to the candidate gene list was performed using the various inheritance patterns. The main report contains variants restricted to nonsense, frameshift, essential splice site and missense variants and filtered on variant site and genotype quality. The appendix listing each gene contains all variants discovered regardless of annotation and quality. Species conservation and predicted

pathogenicity of these variants was examined using SIFT[7], PolyPhen-2[8], Mutation taster[9], and FATHMM[10].

### Sanger sequencing

Sanger sequencing was performed for a number of candidate genes and mutations ascertained via muscle immunohistochemistry, homozygosity mapping, and whole exome sequencing. Sequencing was first performed in an affected family member for confirmation and then specific mutations were sequenced in other family members to track cosegregation patterns. Coding regions of *SGCA, SGCB, SGCG, SGCD*, and *FKRP* were amplified via PCR using standard oligonucleotide primers[11]. Amplicons were assessed via agarose gel electrophoresis, then purified by treating 5 μl of PCR product with 2μl of Exonuclease and Shrimp Alkaline Phosphatase mix (Exo-SAP-IT; Affymetrix) and submitted to the Molecular Genetics Core Facility at Boston Children's Hospital and the Interdisciplinary Center for Biotechnology Research (ICBR) at the University of Florida for sequencing. Sequence data were analyzed using Sequencher v.5.2.3 (GeneCodes Corporation, Ann Arbor, Michigan).

### Cloning of PCR products

The *SGCA* exon harboring the compound heterozygous NM_000023.2 c.739G>A and c. 746T>C mutations (see Results Section below) was amplified from genomic DNA samples representing family 1280, then cloned into the pGEM®-T Easy Vector (Promega) following the manufacturer's protocol. Five colonies from each sample were picked and multiplied. Plasmid DNA was extracted from these colonies and sequenced using universal M13 forward and reverse primers.

## Results

### Clinical features

All affected individuals were determined to have a clinical diagnosis consistent with LGMD. However, due to the lack of availability of local clinical genetic testing, the potential diagnosis of dystrophinopathy could not be excluded in all families prior to enrollment, especially those who did not have affected females and whose inheritance patterns did not indicate male-to-male transmission. Detailed clinical features of the probands for whom causative mutations were identified are described in Supplemental Table 1

### Muscle histology

Hematoxylin and eosin staining confirmed dystrophic pathology in muscle samples from the selected affected individuals. The most notable immunohistochemical finding was absent α-sarcoglycan staining in an affected individual from family 1276 (Figure 1), prompting Sanger sequencing of the entire *SGCA* gene in the probands of this family. Of note, α-sarcoglycan staining was present albeit patchy in 1280-1, thus Sanger sequencing for *SGCA* was not performed prior to exome sequencing (Figure 1). Other immunohistochemical abnormalities were observed but Sanger sequencing did not confirm primary causative mutations for these other findings (data not shown).

### Homozygosity mapping

Due to the selection of only one affected individual for genotyping in each family, numerous candidate causative genes were identified on homozygosity mapping. Among these, *SGCA* was found to harbor pathogenic mutations on Sanger sequencing for family 1269 (see below). Sequencing of candidate genes for other families did not yield causative mutations (data not shown).

### Whole exome sequencing

Affected individuals from families 1273–1275, 1277–1278, 1280–1284 were selected for whole exome sequencing based on a lack of plausible candidate causative mutations after the analyses noted above. This analysis identified likely mutations in 4 families: a homozygous NM_000023.2 c.574C>T; NP_000014.1 p.Arg192Ter nonsense mutation in *SGCA* for family 1274, a hemizygous NM_4006.2 c.8333G>A; NP_003997.1 p.Arg2778His missense mutation in the dystrophin (*DMD*) gene for family 1277, compound heterozygous NM_000023.2 c.739G>A; NP_000014.1 p.Val247Met and NM_000023.2 c.746T>C; NP_000014.1 p.Leu249Pro missense mutations in *SGCA* for family 1280, and a hemizygous NM_4006.2 c.7523delC; NP_003997.1 p.Glu2508Gly fs Ter2 frameshift mutation in *DMD* for family 1281.

### Sanger sequencing and cloning

Sanger sequencing confirmed the homozygous *SGCA* NM_000023.2 c.574C>T; NP_000014.1 p.Arg192Ter nonsense mutation in family 1274 that was identified on whole exome sequencing, and identified the same homozygous mutation in families 1269 and 1276 (Supplemental Figure 1). Sanger sequencing also confirmed the mutations in families 1277, 1280, and 1281 found on whole exome sequencing (Supplemental Figure 1). Co-segregation was confirmed in all 6 families with identified mutations (Figures 2 and Supplemental Figure 1). Screening for the *SGCA* NM_000023.2 c.574C>T; NP_000014.1 p.Arg192Ter mutation in all remaining families demonstrated no additional affected kindreds (Supplemental Figure 2). Cloning of SGCA exon 6 amplified from the genomic DNA of 1280-1, 1280-2, and 1280-3 confirmed that the two mutations were *in trans* (Supplemental Figure 1).

## Discussion

The analysis of these families spanned the years 2010–2015, a period of rapid advances in genomic technologies. As a result, several techniques helped identify the associated genes in various families, including muscle immunohistochemistry, homozygosity mapping, and whole exome sequencing. This hybrid approach illustrates how yields can be optimized by relying on complementary genetic approaches. It is notable that whole exome sequencing ascertained confirmed mutations in 4 of the 6 families in this cohort, including the compound heterozygous *SGCA* mutations in the setting of intact, albeit patchy α-staining for family 1280. Residual α-sarcoglycan staining in the setting of *SGCA* mutations has been described previously[12,13]. Other candidate genes that were sequenced due to results of immunohistochemistry and homozygosity mapping did not yield causative mutations, illustrating the power of whole exome sequencing in this setting. Given that all mutations

identified were in previously described muscular dystrophy genes, the equivalent results could have been obtained via broad spectrum Sanger sequencing. However, the rapidly declining cost of exome sequencing suggests that this newer technology will play an increasingly important role in screening such cohorts. Analysis continues for the remaining 24 families for whom causative mutations have not been identified to date. One potential strategy for further analysis is comparative genomic hybridization, but that is beyond the scope of the current study.

Collectively, the sarcoglycanopathies (LGMD2C, LGMD2D, LGMD2E, LGMD2F), caused by mutations in SGCG[14,15], *SGCA*[16], *SGCB*[17], and *SGCD*[18] Respectively, comprise some of the more common subtypes of LGMD. They are especially common in Brazil, where they account for as much as 68% of cases of LGMD2[19]. Other common forms of LGMD include: LGMD2A, caused by mutations in *CAPN3*[20]; LGMD2B, a dysferlinopathy caused by mutations in *DYSF*[21,22]; LGMD2I, caused by mutations in *FKRP*[23,24]; and LGMD2L, caused by mutations in ANO5[25,26]. A recent review mapped the geographical distribution and prevalence of LGMD throughout the world, based on currently published data, and did not report data from Egypt[27]. In the same year, dysferlinopathy was reported to be a common cause of LGMD in Egypt[28]. As the current cohort does not appear to harbor pathogenic mutations in *DYSF*, further studies on a broader geographic scale will be needed to help delineate the genetic epidemiology of LGMD in Egypt.

The tetrameric sarcoglycan complex is composed of the 4 sarcoglycans expressed in muscle (α- sarcoglycan, β-sarcoglycan, γ-sarcoglycan, and δ-sarcoglycan) and is in turn part of a larger macromolecular complex of proteins called the dystrophin-associated protein complex (DAPC). The DAPC contributes to the stability of the plasma membrane cytoskeleton, maintains sarcolemmal integrity, and ensures transduction during muscle contraction[29]. Mutations in *SGCG* were reported as early as 1995 in Northern Africa, though not including Egypt[14]. LGMD2D is caused by mutations in *SGCA*. *SGCA* c.229C>T, p.R77C in exon 3 is a founder mutation from the Magdalen Islands in the Gulf of St. Lawrence. It is the most common *SGCA* mutation reported internationally[30]. The same mutation was also reported as a founder mutation in several LGMD2D patients from Finland[31] and also from Europe and Brazil[27]. A homozygous *SGCA* c. 157G>A mutation in exon 2 was found in two Tunisian siblings[32]. The current study identified a homozygous *SGCA* NM_000023.2 c.574C>T; NP_000014.1 p.Arg192Ter nonsense mutation in 3 unrelated families among the 30 that were screened. The frequency of this mutation suggests a possible founder effect. More than 70 pathogenic *SGCA* mutations have been described to date[33]. The *SGCA* c.574 C>T mutation has been reported previously, in a sporadic family with two affected siblings from Albania[34], and appears to be the only previously published report of this mutation. Genetic diagnosis in LGMD along with other genetic disorders will become increasingly important in the coming years as new molecular therapies will target specific gene defects and even specific mutation types. For example, several studies suggest that LGMD2D may be amenable to gene therapy approaches[35–37].

There is considerable phenotypic overlap between LGMDs and the dystrophinopathies, the latter caused by mutations in *DMD*[38,39]. As more LGMD genes were discovered, patients who had previously been diagnosed with Duchenne muscular dystrophy were sometimes

found to have LGMD instead[40]. Sarcoglycanopathies may cause either a severe Duchenne-like phenotype or a milder Becker-like phenotype. The two may often be distinguished by the inheritance pattern, though this may be difficult to discern in a sporadic male case. Cardiac complications are essentially universal in Duchenne muscular dystrophy, whereas they are less consistently present in the sarcoglycanopathies; as the cardiac complications arise in the later stages of disease, this clinical feature may not help distinguish between the two in the early stages of disease[41]. The two families in the current report found to have *DMD* mutations had clinical features that resembled those of LGMD, and have milder phenotypes, suggesting that they are most likely to have Becker muscular dystrophy (1277-1 had positive dystrophin staining on muscle biopsy and was able to father children, while 1281-1 has a normal CK and remains ambulatory). A study of Duchenne muscular dystrophy in Egypt using multiplex PCR found that 78 (51.3%) of 152 subjects showed out-of-frame deletions in the *DMD* gene[42]. The *DMD* NM_4006.2 c.8333G>A mutation found in family 1277 does not appear to have been published before, but is reported online for a patient with a Becker muscular dystrophy phenotype from Valentia, Spain (http://dmd.nl). The arginine amino acid residue at this locus is very well conserved among several species, and the *DMD* c.8333G>A mutation co-segregated with the phenotype in family 1277. The *DMD* NM_4006.2 c.7523delA mutation in family 1281 also co-segregates with the phenotype among the other family members. This mutation does not appear to have been published previously.

It is notable that both 1277 and 1281 families are consanguineous, but harbor causal mutations in *DMD*, which is X-linked. This is an interesting example of how consanguinity may be misleading in genetic analyses. Similarly, family 1280, also consanguineous, harbors *SGCA* mutations that are compound heterozygous. Thus, it is important to remember that genetic diseases in consanguineous families may not always be caused by homozygous recessive mutations.

This study shows that mutations in *SGCA* are a common cause of LGMD in Assiut, Egypt, along with the *DYSF* mutations reported previously. The moderate size of this clinic-based cohort makes it premature to draw more general conclusions about the overall genetic epidemiology of LGMD in Egypt; however, the data presented here provide useful information that will complement future studies. The *SGCA* NM_000023.2 c.574C>T; NP_000014.1 p.Arg192Ter mutation is likely due to a founder effect, and is expected to be present in other affected families. These results also illustrate the difficulty in distinguishing dystrophinopathies from LGMDs without access to genetic testing or muscle pathology resources.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

## Abbreviations

| | |
|---|---|
| **CK** | creatine kinase |
| ***DMD*** | dystrophin |
| **LGMD** | limb-girdle muscular dystrophy |
| ***SGCA*** | α-sarcoglycan |
| ***SGCB*** | β-sarcoglycan |
| ***SGCD*** | δ-sarcoglycan |
| ***SGCG*** | γ-sarcoglycan |

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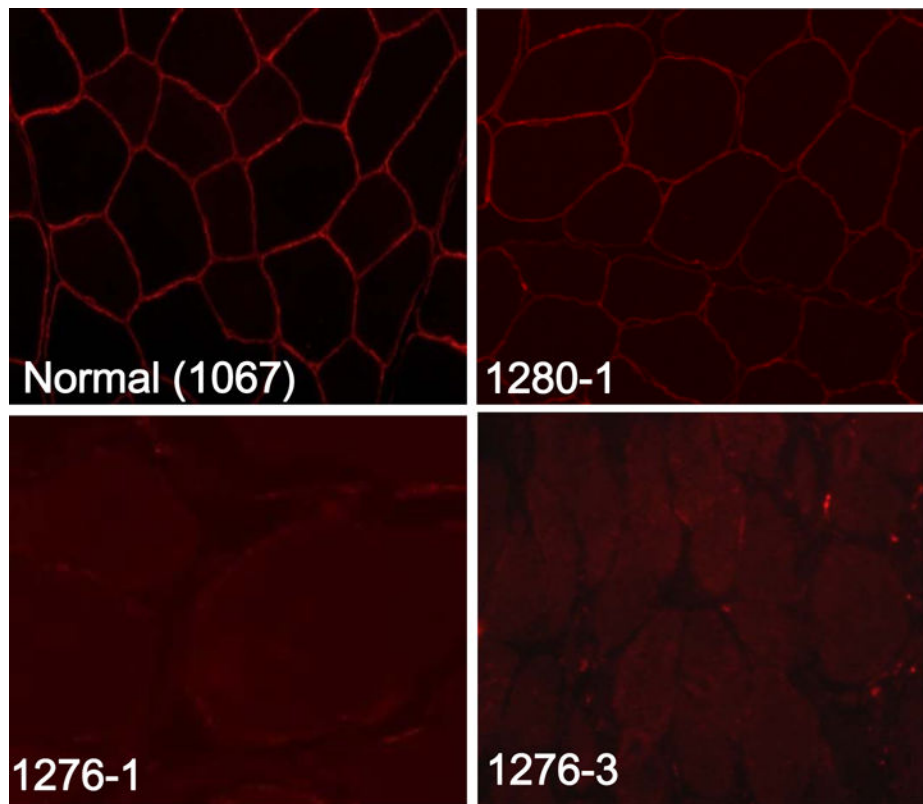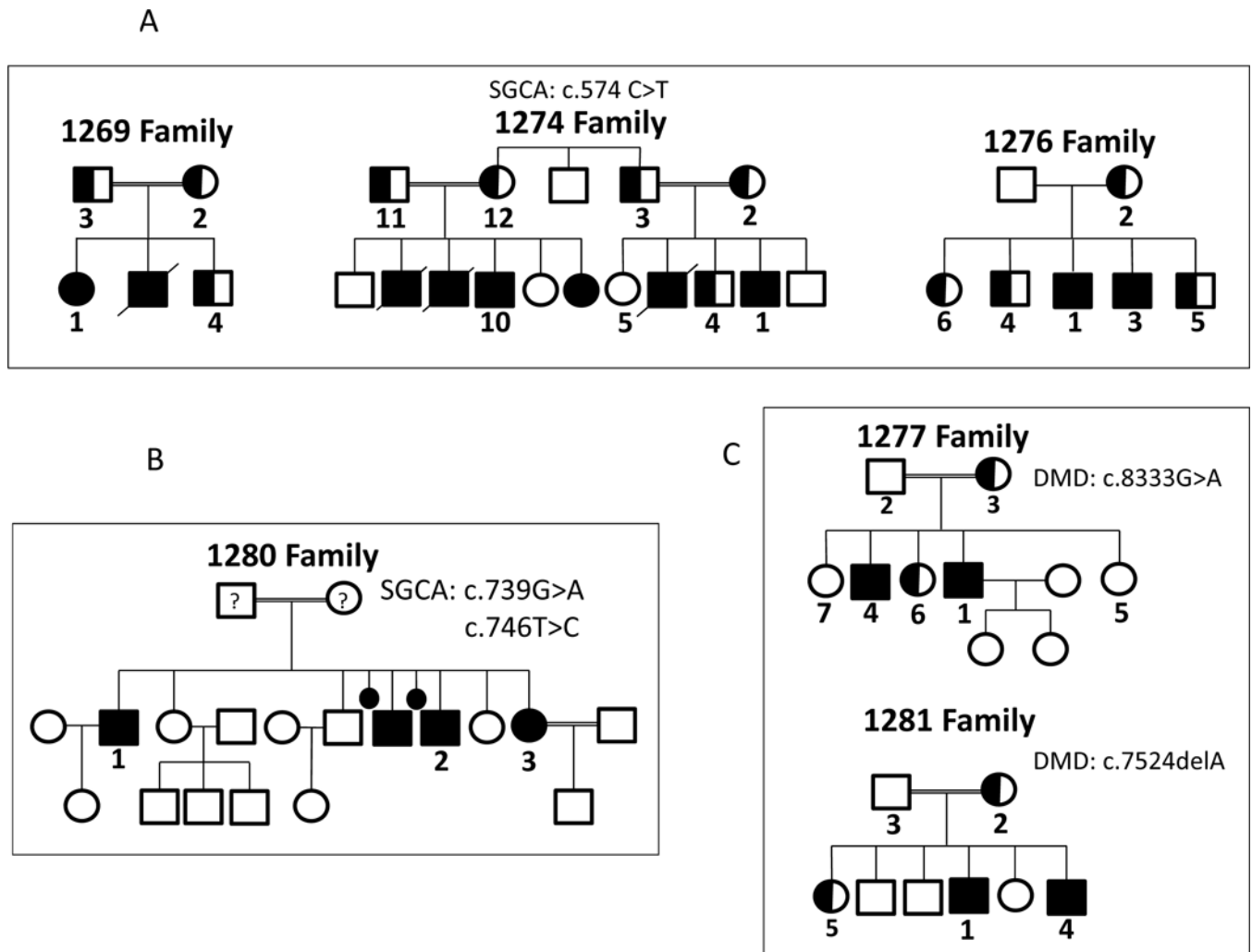
**Figure 1.**
Immunohistochemistry with α–sarcoglycan antibodies (NCL-a-SARC, Novocastra) revealed the presence of α–sarcoglycan protein in control muscle sections (1067), but not in muscle sections from affected individuals 1276-1 or 1276-3; patchy staining was noted in 1280–1.

**Figure 2.**
(A) Pedigrees for the three families who harbor the *SGCA* c.574C>T mutation. (B) Pedigree of family 1280 showed 3 affected siblings 1280-1, 1280-2, and 1280-3 who have compound heterozygous *SGCA* c.739G>A and c.746T>C mutations. Genotypes of parents of proband (1280-1) are unknown and hence represented with '?'. (C) Pedigree of family 1277 with two affected brothers harboring a hemizygous c.8333G>A *DMD* mutation, and pedigree of family 1281 with two affected brothers harboring a hemizygous c.7523delA DMD mutation. Filled circles squares represent affected individuals confirmed to have homozygous, compound heterozygous, or hemizygous mutations. Half-filled circles and squares represent heterozygous carriers. Unfilled circles and squares represent unaffected individuals.