Short Communication

Limb-girdle muscular dystrophy in the Agarwals: Utility of founder mutations in CAPN3 gene

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Abstract

Background and Purpose: Diagnostic evaluation of limb-girdle muscular dystrophy type 2A (LGMD2A) involves specialized studies on muscle biopsy and mutation analysis. Mutation screening is the gold standard for diagnosis but is difficult as the gene is large and multiple mutations are known. This study evaluates the utility of two known founder mutations as a first-line diagnostic test for LGMD2A in the Agarwals. **Materials and Methods:** The Agarwals with limb-girdle muscular dystrophy (LGMD) phenotype were analyzed for two founder alleles (intron 18/exon 19 c.2051-1G>T and exon 22 c.2338G>C). Asymptomatic first-degree relatives of patients with genetically confirmed mutations and desirous of counseling were screened for founder mutations. **Results:** Founder alleles were detected in 26 out of 29 subjects with LGMD phenotype (89%). The most common genotype observed was homozygous for exon 22 c.2338 G>C mutation followed by compound heterozygosity. Single founder allele was identified in two. Single allele was detected in two of the five asymptomatic relatives. **Conclusion:** Eighty-nine percent of the Agarwals having LGMD phenotype have LGMD2A resulting from founder mutations. Founder allele analysis can be utilized as the initial noninvasive diagnostic step for index cases, carrier detection, and counseling.

Key Words

Agarwal, calpainopathy, founder mutation

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Introduction

Limb-girdle muscular dystrophies (LGMDs) are heterogeneous disorders comprising many subtypes having phenotypic overlap. Definitive diagnosis relies on molecular methods and immune assays for specific proteins. Sensitivity and specificity of tests used for qualitative or quantitative determination of sarcolemmal proteins have limitations.[1,2] Demonstration of pathogenic mutations is considered to be confirmative[3,4] but is a formidable task, given the large number of genes involved in LGMDs and the multitude of pathogenic alleles in individual genes.

On this background, the knowledge of founder mutations in populations and communities can be utilized for definitive diagnosis.[5] Allele-specific genetic testing for recurrent mutations has been considered as an efficacious strategy in populations harboring recurrent mutations.[6] Studies on calpainopathies have documented founder mutations in select populations. Yield of specific founder mutation alleles for diagnosis of limb-girdle muscular dystrophy type 2A (LGMD2A) in inbred populations presenting with LGMD phenotype has been previously reported from Brazil and Germany.[7,8] The Agarwals form an Indian example of founder mutations in calpain gene wherein two founder alleles (intron

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18/exon 19 c.2051-1G>T and exon 22 c.2338G>C) (since 2013 nomenclature of mutation intron 18/exon 19 c.2099-1G>T has changed to intron 18/exon 19 c.2051-1G>T) have been described.[9]

Coming initially from northern India, the Agarwals now inhabit most parts of India and also reside in other countries in Asia and the Western Hemisphere. The Agarwals practice intracommunal exogamy. There are 16 divisions (*gotras*). Marriage customs take into account only the paternal sides of the family trees. As this tradition is being followed for many centuries, the Agarwals are known to harbor autosomal recessive diseases such as megalencephelic leukodystrophy, spinocerebellar ataxia-12, and the condition under consideration here, LGMD2A.[9-11]

Yield and impact of analysis of founder mutations in the Agarwals presenting with LGMD phenotype is as yet undetermined. This study was undertaken to evaluate these aspects.

Materials and Methods

This observational study was conducted from January 2010 to April 2015 and was approved by the institutional review board.

Inclusion criteria

1. Patients from the Agarwal community.
2. LGMD phenotype, raised creatine kinase (CK), and electromyography suggestive of myopathy.
3. First-degree relatives of patients with genetically confirmed mutation desirous of counseling.

Exclusion criteria

1. Muscular dystrophies other than LGMD (Duchenne muscular dystrophy, Becker muscular dystrophy, myotonic dystrophy, fascioscapulohumeral dystrophy, congenital muscular dystrophy, etc.).
2. Neurogenic disorders based on detailed neurological examination and electrophysiological studies.
3. Participants not consenting for the study.

History and examination

Age of onset and symptoms were documented and pedigree charts were prepared. Detailed muscle power charting was performed by manual muscle testing scale.[12] Particular attention was given to scapular winging, abdominal hernia, and Achilles tendon contractures. Examination of sensory system and reflexes was performed. Family members were examined when possible.

Investigations

Serum CK was estimated using the dimension method. The patients underwent electromyography. Oxford Synergy Medelec, UK electromyography equipment was used and detailed nerve conduction studies and needle examination were performed using standard protocol.[13]

Genetic analysis

"Agarwal founder mutations" (intron 18/exon 19 c.2051-1G>T and exon 22 c.2338 G>C) studies were conducted.[9] 2 mL of blood sample from each patient was collected in ethylenediamine tetraacetic acid (EDTA) tube. DNA was extracted by Qiagen Blood mini kit, Germany (as per protocol provided). Polymerase chain reaction (PCR) amplification was performed for exons 19 and 22 of *CAPN3* gene followed by Sanger sequencing [Figure 1a and b]. The patient's sequences were then aligned to wild type reference sequences using mutation surveyor alignment software (Softgenetics, Inc., USA) for mutation detection.

Symptomatic individuals testing negative for the mutations were studied further. Monocyte assay/ western blot to detect the expression of dysferlin protein for LGMD2B (funded by Jain Foundation USA, India project) followed by available genetic evaluation as applicable was performed.

Results

Preliminary data

Thirty-four subjects fulfilled inclusion criteria (29 LGMD patients and 5 asymptomatic relatives of index cases with known mutation in the founder alleles). There were 13 males and 21 females. Age at presentation ranged 18-48 years and the onset of the symptoms was between 11 years and 30 years. The most common phenotype was the pelvifemoral type (24/29) followed by scapuloheumeral type (5/29). 21/29 patients had scapular winging [Figure 2a], 25/29 had Achilles tendon contractures [Figure 2b], and abdominal herniae were seen in two patients. Average CK value was 3430.

Analysis of founder mutations

Founder alleles were detected in 26 out of 29 index cases, resulting in a sensitivity of 89% [Table 1]. The most common genotype observed was homozygous state for exon 22 c.2338 G>C mutation followed by compound heterozygosity. Single

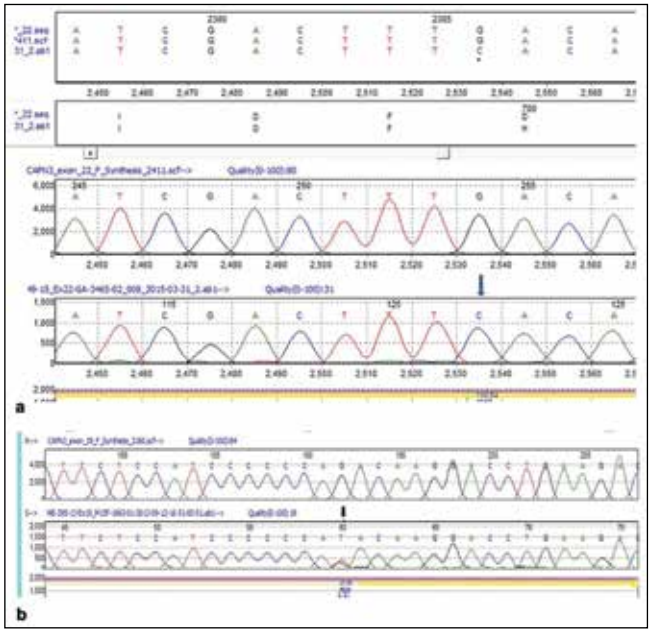

Figure 1: (a) Sangers sequencing showing CAPN3-Ex 22 homozygous mutation c.2338 G>C in patient sample (arrow) (b) Sangers sequencing showing CAPN3-Intron 18/Ex 19 heterozygous mutation c.2051-1G>T in patient sample (arrow)

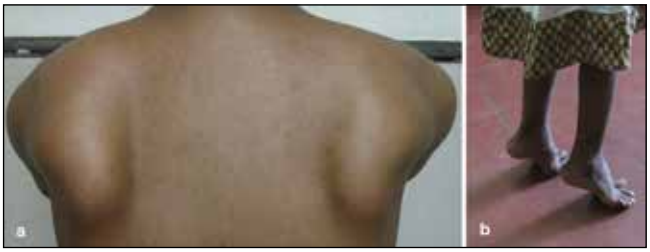

Figure 2: (a) Showing scapular winging (b) Showing toe walking due to ankle contractures

founder allele was identified in two patients. In two of the three index cases who did not show the founder mutation in CAPN3 gene, monocyte western blot assay showed absent dysferlin protein and on further evaluation, using new generation sequencing for LGMD panel showed homozygous mutation in the dysferlin gene (c.5713 C>T), confirming LGMD2B in these two siblings. In the remaining single index case, molecular diagnosis could not be reached.

Analysis of family members

Five asymptomatic relatives sought help for the founder allele analysis. Among them, two were offsprings of an affected homozygous mother planning marriage and the remaining three were spouses of index cases having mutation in founder alleles [Table 2].

Discussion

In the Agarwals presenting with LGMD phenotype, the frequency of finding founder alleles was very high (89%). This high frequency of detection underscores two important facts. First, LGMD2A is the most frequent LGMD in the Agarwals and second, it is a result of mutations in the two founder alleles. Hence, studying these two alleles can give substantial detection rates. Thus, the present study demonstrates the utility of private mutation analysis in the Agarwals. Only three out of 29 patients did not have mutations in the founder alleles. Two of these, on further investigations were detected to have mutations in the dysferlin gene (c.5713 C>T) and in one, molecular diagnosis was not achieved. The present study did not find a predilection toward any of the *gotras*.

Detection of private mutations has been successfully used in LGMD2A in inbred populations. High frequency of five founder mutations in the calpain gene has been reported from inbred families in Réunion Island, France (19/20 patients)[14] and a very high frequency of single founder allele from a single large family of 17 patients from the Mocheni population of the Alps.[15] Two other founder mutations have been known in the calpain gene, one in Germany and the other in Brazil.[7,8] These reports quote the percentage positivity of the founder alleles to be 8.1% and 24%. These comparatively low figures are explained by the nature of these two investigations wherein they included all LGMDs seen in the region, of which calpainopathy formed only a part. Founder mutations have been reported from regions such as Russia,[16] Croatia,[17] Bulgaria,[18] northern Italy,[19] in the Amish community in northern Indiana in the USA,[20,21] and in the Gipuzkoa region of the Basque Country of Spain.[22,23]

The frequency of the founder mutation in the Agarwals suggests that the analysis of the two alleles could be the first step in the evaluation of LGMD in the Agarwals, bypassing the biopsy. Biopsies are invasive; calpain protein cannot be studied by immunostaining methods and facilities for western blotting of calpain protein on muscle tissue are available only in a few places in India. Moreover, issues in immunoblotting such as false positivity and false negativity make it a less favorable test.[1,4,5]

Allele-specific genetic testing is an important tool for the detection of preclinical stage of the disease, carrier detection, and offering genetic counseling.[2,5,6] In the Agarwals who practice intracommunal exogamy, carrier detection is important for premarital planning and prenatal planning. As demonstrated in Table 2, offsprings of a homozygous index case were obligate carriers and hence, premarital testing of their partners was advised to avoid transmission to the next generation. Similarly, three spouses of index cases undertook prenatal testing, the normal genotypes in the spouse making the probability of having diseased offspring negligible. Thus, the founder allele evaluation helped carrier detection and counseling in this study.

Conclusion

LGMD2A forms a majority of LGMDs in the Agarwals. The present investigation establishes the high sensitivity (89%) of two founder alleles in the calpain gene in the Agarwals having LGMD phenotype. This information is productive for diagnosis,

Table 1: Results of analysis of founder alleles in index cases

Genotype	Mutation 1	Mutation 2	Number of symptomatic subjects (29)
Homozygous	c.2338 G>C	c.2338 G>C	11
	c.2051-1G>T	c.2051-1G>T	3
Heterozygous	c.2338 G>C	c.2051-1G>T	10
Single founder mutation	c.2338 G>C		2
No founder mutation detected			3*

*Two of the three patients not showing the founder allele had homozygous mutations in the dysferlin gene (c.5713 C>T)

Table 2: Analysis of asymptomatic relatives of index cases

Mutation in index case	Asymptomatic relative	Mutation detected	Purpose
Affected mother Homozygous for exon 22 c.2338 G>C on 22 c.2338 G>C	Son	Single allele, exon 22 c.2338 G>C	Premarital
	Daughter	Single allele, exon 22 c.2338 G>C	Premarital
Wife Homozygous for exon 22 c.2338 G>C	Husband	No mutation	Prenatal
Wife Compound heterozygous intron 18/exon 19 c.2051-1G>T and exon 22 c.2338 G>C	Husband	No mutation	Prenatal
Husband Homozygous for exon 22 c.2338 G>C	Wife	No mutation	Prenatal

carrier detection, and counseling, offering community benefits at multiple levels. Testing the founder mutations can be used as the first diagnostic test, bypassing the muscle biopsy in the Agarwals having LGMDs.

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Conflicts of interest

There are no conflicts of interest.

References

1. Angelini C, Fanin M. Calpainopathy. 2005 May 10. In: Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, *et al*., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2015. Available from: http://www.ncbi.nlm.nih.gov/books/NBK1313/. [Last accessed on 2014 Nov 26].
2. Fanin M, Fulizio L, Nascimbeni AC, Spinazzi M, Piluso G, Ventriglia VM, *et al*. Molecular diagnosis in LGMD2A: Mutation analysis or protein testing? Hum Mutat 2004;24:52-62.
3. Bushby KM. Making sense of the limb-girdle muscular dystrophies. Brain 1999;122:1403-20.
4. Urtizberea JA, Leturcq F. Limb girdle muscular dystrophies: The clinicopathological viewpoint. Ann Indian Acad Neurol 2007;10:214-24.
5. Fanin M, Angelini C. Protein and genetic diagnosis of limb girdle muscular dystrophy type 2a: The yield and the pitfalls. Muscle Nerve 2015;52:163-73.
6. Fanin M, Nascimbeni AC, Tasca E, Angelini C. Hum How to tackle the diagnosis of limb-girdle muscular dystrophy 2A. Eur J Genet 2009;17:598-603.
7. de Paula F, Vainzof M, Passos-Bueno MR, de Cássia M Pavanello R, Matioli SR, V B Anderson L, *et al*. Clinical variability in calpainopathy: What makes the difference? Eur J Hum Genet 2002;10:825-32.
8. Hanisch F, Müller CR, Grimm D, Xue L, Traufeller K, Merkenschlager A, *et al*. Frequency of calpain-3 c.550delA mutation in limb girdle muscular dystrophy type 2 and isolated hyperCKemia in German patients. Clin Neuropathol 2007;26:157-63.
9. Ankala A, Kohn JN, Dastur R, Gaitonde P, Khadilkar SV, Hegde MR. Ancestral founder mutations in calpain-3 in the Indian Agarwal community: Historical, clinical, and molecular perspective. Muscle Nerve 2013;47:931-7.
10. Singhal BS, Gursahani RD, Udani VP, Biniwale AA. Megalencephalic leukodystrophy in an Asian Indian ethnic group. Pediatr Neurol 1996 14:291-6.
11. Sinha KK. Spinocerebellar ataxia 12 (SCA12). A tremor dominant disease, typically seen in India. In: Medicine Update 2005. Mumbai, India: Association of Physicians of India; 2005. p. 600-2.
12. Kendall FP, McCreary EK, Provance PG. Muscles: Testing and Function. Baltimore, MD: Williams & Wilkins; 1993. p. 185.
13. Aminoff MJ. Aminoffs Electrodiagnosis in Clinical Neurology. 6th ed. San Fransisco: Elsevier; 2012. p. 233-56.
14. Fardeau M, Hillaire D, Mignard C, Feingold N, Feingold J, Mignard D, *et al*. Juvenile limb girdle muscular dystrophy. Clinical, histopathological and genetic data from a small community living in the Reunion Island. Brain 1996;119:295-308.
15. Fanin M, Benedicenti F, Fritegotto C, Nascimbeni AC, Peterle E, Stanzial F, *et al*. An intronic mutation causes severe LGMD2A in a large inbred family belonging to a genetic isolate in the Alps. Clin Genet 2012;82:601-2.
16. Pogoda TV, Krakhmaleva IN, Lipatova NA, Shakhovskaya NI, Shishkin SS, Limborska SA. High incidence of 550delA mutation of CAPN3 in LGMD2A from Russia. Hum Mutat 2000;15:295.
17. Milic A, Canki-Klain N. Calpainopathy (LGMD2A) in Croatia: Molecular and haplotype analysis. Croat Med J 2005;46:657-63.
18. Todorova A, Georgieva B, Tournev I, Todorov T, Bogdanova N, Mitev V, *et al*. A large deletion and novel point mutations in the calpain-3 gene (CAPN3) in Bulgarian LGMD2A patients. Neurogenetics 2007;8:225-9.
19. Fanin M, Nascimbeni AC, Fulizio L, Angelini C. The frequency of limb girdle muscular dystrophy 2A in northeastern Italy. Neuromuscul Disord 2005;15:218-24.
20. Young K, Foroud T, Williams P, Jackson CE, Beckmann JS, Cohen D, *et al*. Confirmation of linkage of limb-girdle muscular dystrophy type 2 to chromosome 15. Genomics 1992;13:1370-1.
21. Richard I, Broux O, Allamand V, Fougerousse F, Chiannilkulchai N, Bourg N, *et al*. Mutations in the proteolytic enzyme calpain-3 cause limb girdle muscular dystrophy type 2A. Cell 1995;81:27-40.
22. Urtasun M, Saenz A, Roudaut C, Poza JJ, Urtizberea JA, Cobo AM, *et al*. Limb-girdle muscular dystrophy in Guipuzcoa (Basque Country, Spain). Brain 1998;121:1735-47.
23. Cobo AM, Sáenz A, Poza JJ, Urtasun M, Indakoetxea B, Urtizberea JA, *et al*. A common haplotype associated with the Basque 2362AG>TCATCT mutation in the muscular calpain-3 gene. Hum Biol 2004;76:731-41.