Novel Mutation Found in Exon 12 of LAMA-2 Gene in Iranian Congenital Gravismeros in syndrome Patients

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Abstract
Muscular dystrophy (MD) is a group of multiple muscle diseases, which cause severe degeneration and dysfunctions in musculoskeletal system and hamper locomotion like walking or moving. Genetic is main cause of Muscular dystrophies and LAMA-2 is a marker gene for congenital myasthenia disease (CMD). Exon 12 and exon 56 of LAMA-2 gene sequenced in 20 Iranian patients of congenital Gravis myasthenia syndrome. Results presented novel mutation of (c.1798G>A homo p.G600R) in exon 12 in one patient. In exon 56, 10 patients carried benign mutation and none of them carried pathogenic mutation. 8 patients had no mutation in both exons. It seems that pathogen mutations of LAMA-2 gene in Iranian population of CMD are different from other populations.

Key words: LAMA-2; Gravis myasthenia syndrome

Introduction
Muscular dystrophy (MD) is a group of multiple muscle diseases, which affects the musculoskeletal system and hamper locomotion like walking or moving. Muscular dystrophies are characterized by progressive skeletal muscle weakness, defects in muscle proteins, and lead to degeneration and death of muscle cells and tissue in the final stage of disease [1,2]. Genetic is the main reason of almost all Muscular dystrophies but various inheritance patterns revealed in different muscular dystrophies. In addition several mutations in several genes are associated to muscular dystrophies types. Mutations in dystrophin gene are main cause of Duchenne and Becker types and mutations in EMD and LMNA genes are associated with Emery-Dreifuss muscular dystrophy [1]. Diagnosis of muscular dystrophy is based on muscle biopsy, increased creatine phosphokinase (CpK3), electromyography, electrocardiography and genetic tests [3]. Unfortunately still there is no specific treatment for muscular dystrophy but physiotherapy, aerobic exercise, yoga; low intensity catabolic steroids can help to maintain muscle tone. Also diet rich of nutrient supplements such as Vitamin D3, calcium, whey protein, creatine and glutamine could help to prevent contractures [4]. Laminin, is an extracellular protein, and a component of the basement membrane which is involved in attachment, migration, and organization of cells into tissues during embryonic development by interacting with other extracellular matrix components. Laminin has three subunits, alpha, beta, and gamma, which are bound to each other by disulfide bonds into a cross-shaped molecule. LAM-2 which is located in 6q22-q23 encodes the alpha 2 chain, which constitutes one of the subunits of laminin 2 (merosin) and laminin 4 (s-merosin). LAMA-2 length is over 260,000 base pairs which contains 64 exons. Mutations in this gene have been identified as the cause of congenital merosin-deficient muscular dystrophy [5]. Congenital myasthenia syndrome (CMD) is a severe form of congenital muscular disorder and characterized by significant dystrophic changes in skeletal muscles. Mechanisms of the muscle fiber degeneration in CMD are not completely clarified [6]. Several point mutations, deletions and duplications in several exons of LAMA-2 are associated with...
and a written consent was obtained. 20 Iranian patients of congenital Gravis myasthenia syndrome (10 male, 10 female) selected and blood samples collected from patients. MRI graph, pathological biopsy and clinical report of one of the patients presented in (Figures 1 & 2).

DNA extraction

DNA from lymphocytes of peripheral blood extracted using Roche Applied Science, DNA Isolation Kit for Mammalian Blood (Cat. No. 11 667 327 001). Integrity and quality of extracted DNA evaluated by using agarose gel electrophoresis.

PCR amplification

Primers for two exons of LAMA-2, exon 12 and exon 56 designed by Oligo 7 software and blasted on NCBI. Primers presented in Table 1. Full lengths of two mentioned exons were amplified by PCR using Roche Applied Science, PCR master mix (11636103001).

DNA sequencing

DNA cycle sequencing performed on automated sequencers ABI 3700 and results checked in NCBI, omim ‘polyphen’ ‘sift’ exom aniant data bases after using Finch TV software for compare with normal sequence.

Results

| Patients | Exon12 | Exon 56 |
|----------|--------|---------|
| p1       | c.7938 G>A hetero |          |
| p.Met 26491le |        |          |
| p2       | -      | c.1796G>A homo |
| p.G600R  |        |          |
| p3       | c.7938 G>A hetero |          |
| p.Met 26491le |        |          |
| p4       | -      | -        |
| p5       | c.7938 G>A hetero |          |
| p.Met 26491le |        |          |
| p6       | -      | -        |
| p7       | -      | -        |
| p8       | c.7938 G>A hetero |          |
| p.Met 26491le A |   |          |
| p9       | -      | -        |
| c.8076+6G>A |       |          |
| p10      | Hero   |          |
| p11      | c.7938 G>A hetero |          |
| p.Met 26491le |        |          |
| p12      | c.7938 G>A hetero |          |
| p.Met 26491le |        |          |
| p.R619 H |        |          |

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From 20 patients, results for exon 12 of LAMA-2 gene showed that just 3 patients (p2, p12, p16) had mutation in this exon and just one of them (p2) carried one damage mutation and two other patients had no mutations beyond normal single nucleotide polymorphisms (SNP). Other 17 patients had no mutation in exon 12. For exon 56 results showed 10 patients (p1,p3,p5,p8,p10,p11,p12,p13,p14,p15) had mutations entirely of heterogeneous nature and p10 also carried intronic variations. All of the mutations in exon 56 were being mutations and not related to the disease. 8 patients (p4,p6,p7,p9,p17,p18,p19,p20) had no mutation in neither exons. Summary of results presented in (Table 2).

**Discussion**

Muscular dystrophies are multi factorial and complex diseases with several ambiguities about mechanisms of muscle fiber degeneration, neuro degeneration and brain pathological symptoms. Prenatal diagnoses in these diseases are mandatory for prevention. Achievement to an accurate and chip prenatal diagnostic protocol for muscular dystrophies needs to find strong genetic markers, including population specific markers. Several previous reports indicated LAMA-2 as marker gene for Merosin-deficient congenital muscular dystrophy but studies showed that different population have different pathogenic mutations in this gene [9]. (c.1798G>A homo p.G600R) which found in p2 patient have been reported in 4 patients of 200 CMD patients in European population as germ line mutation [9] and another study in 2013 which assessed CMD patients of Arab tribe found it as probably damage mutation. Based on results which is first report about exon 12 of LAMA-2 in Iranian CMD patients, this mutation could be a new founded pathogen mutation in middle east population. (c.1856 G>A homo p.R619 H) mutation previously considered as single nucleotide polymorphism in Caucasians [10] and as benign mutation in European population [11]. Present study is in accordance with the previous investigations. Other pathogenous mutations, reported in exon 12 in previous reports have not been observed in Iranian population. In the Iranian population, the exon 56 mutations are of benign nature.

**Conclusion**

The Most significant findings in the present study is associated with the absence of mutations in both exons in 8 patient. This leads us to search other exons to find probable novel genetic markers for CMD patients in Iranian population. Further studies in bigger sample size by whole LAMA-2 exons sequencing along with deep sequencing of promoter, 3’ and 5’ UTRs and intronic regions and gene expression assessment may clarify the role of LAMA-2 mutations in different types of muscular dystrophies in different populations.

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