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ScienceDirect
Neuromuscular Disorders 21 (2011) 41–46
www.elsevier.com/locate/nmd

Prevalence, mutation spectrum and phenotypic variability in Norwegian patients with Limb Girdle Muscular Dystrophy 2I

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Received 9 June 2010; received in revised form 19 August 2010; accepted 31 August 2010

Abstract

Mutations in the *FKRP* (Fukutin Related Protein) gene produce a range of phenotypes including Limb Girdle Muscular Dystrophy Type 2I (LGMD2I). In order to investigate the prevalence, the mutation spectrum and possible genotype–phenotype correlation, we studied a cohort of Norwegian patients with LGMD2I, ascertained in a 4-year period.

In this retrospective study of genetically tested patients, we identified 88 patients from 69 families, who were either homozygous or compound heterozygous for *FKRP* mutations. This gives a minimum prevalence of 1/54,000 and a corresponding carrier frequency of 1/116 in the Norwegian population. Seven different *FKRP* mutations, including three novel changes, were detected. Seventy-six patients were homozygous for the common c.826C>A mutation. These patients had later disease onset than patients who were compound heterozygous – 14.0 vs. 6.1 years. We detected substantial variability in disease severity among homozygous patients.

Keywords: Limb Girdle Muscular Dystrophy; FKRP

1. Introduction

Autosomal recessive Limb Girdle Muscular Dystrophy Type 2I (LGMD2I) was first described in a large consanguineous Tunisian family. The proximal limb muscle weakness and wasting predominantly affected the pelvic girdle, with variable age at onset and clinical course among siblings. All affected individuals had normal motor milestones. No distal arm- or facial muscle involvement, or cognitive impairment, was observed [1].

LGMD2I is caused by mutations in the Fukutin Related Protein (*FKRP*) gene, encoding a putative golgi resident glycosyltransferase [2,3].

The phenotypic spectrum caused by *FKRP* mutations is heterogeneous and includes severe disease with structural brain abnormalities (Walker–Warburg syndrome; WWS, or muscle-eye brain disease; MEB [4]), inability to achieve

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doi:10.1016/j.nmd.2010.08.008

walking function (MDC1C), early-onset, Duchenne-like phenotype (severe LGMD2I) or a milder disease with slow progression (mild LGMD2I) [5,2]. A continuum is seen between the mild and severe forms of LGMD2I [6]. Asymptomatic carriers of *FKRP* mutations on both alleles have also been described [6,7]. Cardiac and respiratory involvement is frequent [7–9].

The function of FKRP is unknown, but it is thought to be involved in the O-glycosylation of α-dystroglycan (α-DG), based on findings showing a reduction in molecular weight and band intensity seen on Western blots of muscular extracts from MDC1C patients, using glycan dependent anti α-DG antibodies [5]. Likewise, immunohistochemistry demonstrated reduced levels or absence of glycosylated alpha-dystroglycan in muscle sections from MDC1C and LGMD2I patients [5,2]. The O-glycan moiety of α-DG anchors the laminin-α2 chain, and plays an important role in stabilizing the muscle surface membrane and creating a link to the extracellular matrix. Phenotypic severity has been correlated to depletion of glycosylated α-DG and secondary reduction in laminin-α2 [10,3,11]. However, exceptions have been reported as severe depletion of glycosylated α-DG was found in patients with mild limb-girdle phenotypes [12].

More than 70 different *FKRP* mutations have been identified, of which approximately 40 cause LGMD2I [13]. The c.826C>A mutation is the most frequent among LGMD2I patients from Northern Europe, and patients homozygous for this mutation generally have a milder phenotype than those who are compound heterozygous [8]. Higher frequencies of other *FKRP* mutations have been found in Southern Europe and in other parts of the world [6,7,14]. Mildly affected patients with mutations other than the c.826C>A have been described [6,14], and similar to those who carry the c.826C>A mutation, substantial variability in clinical expression is seen among subjects with the same genotype [14].

2. Subjects and methods

Patients were ascertained either through the Department of Medical Genetics at the University Hospital of North Norway or Centre for Medical Genetics and Molecular Medicine at Haukeland University Hospital, which are the only institutions performing *FKRP* genetic testing in Norway. Most patients tested for *FKRP* mutations had a typical LGMD phenotype, while others had elevated CK or symptoms resembling myopathy (exertional muscle pain, weakness with other than LGMD-distribution, myoglobunuria etc.). Patients were included in this study only if they had disease causing *FKRP* mutations on both alleles. Subsequently, information concerning age at onset of symptoms, presenting symptom(s), current involvement of upper limbs, current ambulatory status and need for ventilatory support was collected via a questionnaire sent to 83 eligible patients (1 patient had died, and 4 patients were diagnosed in December 2008 after questionnaires were sent). Clinical onset of disease was defined as the first time the patient noticed one or more of the following symptoms: Reduced walking distance, difficulty climbing stairs or rising from a seated or crouched position, muscle pain after physical exertion, or muscle weakness in the arms. Ambulatory status was graded in 4 categories: Walking without aid, walking with an aid (e.g. stick, crutch or frame), the need for a wheelchair at walking distances >200 m, and wheelchair dependence.

DNA was extracted from peripheral blood cells according to standard procedures. *FKRP* coding exon 4 was PCR-amplified in two overlapping segments with primer combinations FKRP-1F: 5′GACAATCAGCTGCTGCCTTCCC 3′/FKRP-2R: 5′GTCTCCTT GTTGCAGCCGAACC 3′ and FKRP-1R: 5′ ACAGAGCTTCTCCACATCCAG 3′/FKRP-2F: 5′GGCACCAGCCTCTTTCTGCAG 3′, using REDTaq Ready Mix PCR Reaction Mix (Sigma). Betaine (0.4 M) was added to the PCR reaction mixtures. DNA sequencing was performed using sequencing primers and BigDye 3.1 kit reagents (Applied Biosystems). Sequencing reactions were run on an automated sequencer unit (3130xl, Applied Biosystems/Hitachi) and analyzed using the SeqScape v2.5 software (Applied Biosystems).

The study was approved by the regional board of research ethics, and each subject gave informed consent. The SPSS statistical software was used for statistical analyses. Differences between groups were tested by *t*-tests and chi-square tests. Two-sided values of $p < 0.05$ were considered statistically significant.

3. Results

In the 4 year study period, a total of 326 individuals were tested for *FKRP* mutations: 230 had no *FKRP* mutations, 8 had a mutation on one allele, and 88 from 69 families had *FKRP* mutations on both alleles. Among these 88 subjects, the common c.826C>A mutation accounted for 163 alleles, whereas c.962C>A (p.Ala321Glu) accounted for 6 alleles, c.899T>C (p.Val300Ala) for 4 alleles and c.913C>T (p.Pro305Ser), c.1323T>G (p.Phe441Leu) and c.170_189del20 (p.Glu57AlafsX68) for 1 allele each (Table 1).

The c.913C>T, c.1323T>G and c.170_189del20 are novel mutations. The c.170_189del20 mutation is a frame-shifting deletion predicted to cause a severely truncated FKRP polypeptide, if expressed. Mutations c.913C>T and c.1323T>G are missense mutations causing replacement of proline (Pro) with serine (Ser) at residue 305 and phenylalanine (Phe) with leucine (Leu) at residue 441, respectively. Both substitutions represent major alterations in side chain properties of the affected residues, and both Pro305 and Phe441 are evolutionary conserved from insects. Neither c.913C>T, nor c.1323T>G, could be detected in more than 200 Norwegian normal chromosomes.

Of the 88 LGMD2I patients identified, 76 patients from 59 families were homozygous for the common c.826C>A mutation, and 11 patients from 9 families were compound heterozygous (c.826C>A/other) (Table 1). Among the compound heterozygotes, the most frequent mutations on