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Clinical and pathological heterogeneity of a congenital disorder of glycosylation manifesting as a myasthenic/myopathic syndrome

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Abstract

Congenital disorders of glycosylation are often associated with muscle weakness in apparent isolation or as part of a multi-systemic disorder. We report here the clinical and pathological features resulting from a homozygous mutation of *ALG2* in an extended family. Phenotypic heterogeneity is observed among the small cohort of patients reported to date and is highlighted by our study. Linkage analysis, homozygozity mapping and whole exome sequencing followed clinical and pathological characterization of patients who presented with a congenital limb girdle pattern of weakness with no ocular or bulbar involvement. Nerve stimulation studies were consistent with a congenital myasthenic syndrome. Severity and progression of disease was variable. Muscle biopsies showed myopathic features, ragged red fibers and a sub-sarcolemmal accumulation of structurally normal mitochondria. Whole exome sequencing revealed an indel mutation c.214_224delGGGGACTGGCTdelinsAGTCCCCG, p.72_75delGDWLinsSPR in exon 1 of *ALG2*. Mutation of *ALG2* manifested as a limb girdle pattern of muscle weakness with defects at both the neuromuscular junction and sarcomere. In addition the accumulation and distribution of mitochondria in the diseased muscle and the presence of ragged red fibers were supportive of a mitochondrial myopathy. *ALG2* mutation results in a heterogeneous phenotype and care should be taken in categorization and treatment of these patients.

Keywords: Congenital disorder of glycosylation; Myasthenia; Myopathy

1. Introduction

Congenital disorders of glycosylation (CDG) are a rapidly expanding subset of Mendelian diseases all having a recessive mode of inheritance with exception of the dominantly inherited EXT1/EXT2-CDG and the X-linked MGAT1-CDG and ALG13-CDG [1,2]. Glycosylation is centrally involved in many functions such as protein folding and function and intracellular transport [3]. Defects in glycosylation underlie mostly multi-organ disorders often involving the nervous system, hepatocytes, enterocytes, leucocytes and/or muscle, which are organs/cell types with high glycosylation demands [4]. Defects of N-glycosylation form the largest group of CDG described [2,5]. N-glycosylation is initiated by an amide linkage of N-acetylglycosamine (GlcNAc) to asparagine on target proteins and requires construction of a lipid-linked oligosaccharide (LLO) through the actions of a series of glycosyltransferases. Upon the addition of GlcNAc to asparagine, the glycan is further

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modified through sugar addition and deletion in a non-template driven manner under the influence of several enzymes competing for the same substrate [6]. Type I CDGs are characterized by deficient synthesis of LLO glycan precursors [7,8]. CDG-II alter the processing of protein bound glycans [4]. Wide phenotypic variation between CDG and within each type is common and may be related to the severity of mutations, genetic modifiers, and loss or substitution of specific glycans [9]. *ALG2* encodes alpha-1,3-mannosyltransferase, a member of the glycosyltransferase 1 family [10]. It is a transmembrane endoplasmic reticulum protein that catalyzes the transfer of mannosyl residues from GDP-Man to Man1GlcNAc2-PP-dolichol. Deficiency of ALG2 is associated with a severe multisystemic disorder that encompasses mental retardation, seizures, coloboma of the iris, hypomyelination, hepatomegaly and coagulopathy [11]. More recently two families have been reported with pathogenic mutations of *ALG2* but with relatively milder disease, essentially limited to dysfunction of the neuromuscular junction, and now diagnosed as a limb-girdle form of congenital myasthenic syndrome (CMS) [12]. The same study also reported a single family with mutations in *ALG14* resulting in a clinical diagnosis of probable CMS. A complex of *ALG13*, *ALG14* and *DPAGT1* (human *ALG7* orthologue) catalyzes the second step of LLO synthesis [13–15]. Not surprisingly mutations of *DPAGT1* have also been reported to cause a limb-girdle form of CMS [16,17]. In common with *ALG2*, mutations of *DPAGT1* may result in a severe multisystemic disorder [CDG1J (MIM 608093)] (delayed development, microcephaly, tremor, respiratory insufficiency, jaundice) or the milder CMS phenotype [16–19]. Here we report on a third ALG2-CDG family identified using linkage analysis and whole-exome sequencing, defining further and highlighting the broad clinical and pathological phenotype that is observed in common with other disorders of the N-linked glycosylation pathway.

2. Materials and methods

2.1. Subjects and nucleic acid extraction

Three patients of an extended consanguineous family from Saudi Arabia were examined at King Faisal Specialist Hospital and Research Center (KFSHRC) and diagnosed with limb girdle like congenital myopathy. All subjects were enrolled in an IRB-approved protocol (RAC# 2070005) with full informed consent. DNA was extracted from blood using standard procedures (Flexi Gene DNA Handbook, Qiagen). Blood was also collected in PAXgene tubes (Qiagen) for RNA extraction as recommended by the manufacturer. Samples were quantitated spectrophotometrically and stored at −20 °C.

2.2. Linkage and homozygosity mapping

All individuals (affected and unaffected) were genotyped using the Affymetrix Axiom array (Affymetrix, Santa Clara, CA, USA) following the manufacturer's protocol. Resulting genotypes were analyzed for shared runs of homozygosity (ROH) using autoSNPa (http://dna.leeds.ac.uk/autosnpa/). Linkage analysis was performed using the Allegro module of Easy Linkage assuming autosomal recessive inheritance and 100% penetrance.

2.3. DNA sequencing

Coding regions of candidate genes were amplified and sequenced using a BigDye Terminator kit (Applied Biosystems, Foster City, CA) and run on an ABI 3730xl automated sequencer (Applied Biosystems, Foster City, CA). SeqScape v.2.6 software (Applied Biosystems, Foster City, CA) was used to align sequence data.

2.4. FKTN expression

FKTN expression in patient peripheral blood was analyzed by RT-PCR. RNA was reverse transcribed using the SuperScript III First-Strand Synthesis System (Invitrogen, Carlsbad, CA, USA). Synthesized cDNA was amplified using primers specific for exons 8 and 9 of the FKTN transcript (forward-5′-ATGGTATCGACAATG CAACA-3′; reverse-5′-ATCATCTTTTCCCTGGAAGG-3′) generating a 175 bp amplicon. A 428-bp GAPDH fragment was used as an internal control.

2.5. Whole exome sequencing and analysis

Two micrograms of each DNA sample was treated to obtain the Ion Proton pre-capture library using the AB Library Builder System (Life Technologies, Carlsbad, CA, USA). Briefly, DNA was enzymatically sheared to produce fragments with a target range of 230–250 bp. Fragments were end repaired, ligated to adaptors and then nick-translated. The desired fragment lengths were obtained by running the samples through precast 2% agarose gel cassettes (Pippin Prep DNA Size Selection System; Sage Science, Beverly, MA, USA) for size selection. The purified adapter ligated fragments were subjected to library amplification using Platinum PCR Amplification Mix (Life Technologies, Carlsbad, CA, USA), P1 and P2 amplification primers and 10 amplification cycles to obtain the genomic pre-capture library. Libraries were quantified using an Agilent Bioanalyzer High-Sensitivity DNA chip (Agilent, Santa Clara, CA, USA). Target enrichment was performed with the TargetSeq human whole exome kit (Life Technologies, Carlsbad, CA, USA). The prepared exome library was further used for emulsion PCR on an Ion OneTouch System and templated Ion Sphere particles