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Molecular diagnosis of congenital muscular dystrophies with defective glycosylation of alpha-dystroglycan using next-generation sequencing technology

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Abstract

Targeted resequencing using next-generation sequencing technology is being rapidly applied to the molecular diagnosis of human genetic diseases. The group of muscular dystrophies may be an appropriate candidate for this approach because these diseases exhibit genotype–phenotype heterogeneity. To perform a proof-of-concept study, we selected four patients with congenital muscular dystrophies with defective glycosylation of alpha-dystroglycan. A custom-solution-based target enrichment kit was designed to capture whole-genic regions of the 26 muscular-dystrophy-related genes, including six genes implicated in alpha-dystroglycanopathies. Although approximately 95% of both coding and noncoding regions were covered with at least 15-read depth, parts of the coding exons of *FKRP* and *POMT2* were insufficiently covered. Homozygous and compound heterozygous *POMGnT1* mutations were found in two patients. Two novel noncoding variants of *FKTN* were identified in one patient who had a retrotransposon insertion mutation of *FKTN* in only one allele. The current targeted resequencing strategy yielded promising results for the extension of this method to other muscular dystrophies. As suboptimal coverage in a small subset of coding regions may affect the sensitivity of the method, complementary Sanger sequencing may be required.
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1. Introduction

The next-generation sequencing (NGS) technology has revolutionized the approach to the exploration of the genomes of various organisms. Moreover, this technology, combined with various methods of target enrichment, is rapidly being applied to the molecular diagnosis of rare Mendelian diseases. The preferred diseases or groups may have extreme genetic heterogeneity, which implies that multiple causative

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genes need to be sequenced. Recently published articles have addressed many diseases or groups of diseases: Charcot–Marie–Tooth disease [1], hereditary ataxia [2], congenital disorders of glycosylation [3], hypertrophic cardiomyopathies [4], and mitochondrial diseases [5]. The muscular dystrophies may be appropriate candidates for this approach because this group of disorders exhibits clinical and genetic heterogeneity. The congenital muscular dystrophies (CMDs) with defective glycosylation of alpha-dystroglycan are an example. Although this group shares common clinical and biochemical features, six causative genes have been identified to date: *FKTN*, *FKRP*, *POMT1*, *POMT2*, *POMGnT1*, and *LARGE*. Moreover, these genes, with the exception of *LARGE*, are also responsible for limb-girdle muscular dystrophies (LGMD) [6–10].

To develop comprehensive genetic diagnostic tools for the muscular dystrophies, related genes are selected for target enrichment and massively parallel sequencing. We demonstrated previously that the full mutation spectrum of the dystrophin gene could be successfully identified in 25 patients with Duchenne/Becker muscular dystrophy using this strategy [11]. To test the applicability of this approach to other muscular dystrophies with causative genes located in autosomal chromosomes, we selected four Korean patients with CMD with defective glycosylation of alpha-dystroglycan. The mutational spectrum of these four patients was not completely identified in the previous study [12].

In the present study, we describe the sequencing results of six genes that cause CMD with defective glycosylation of alpha-dystroglycan and discuss the advantages and limitations of a targeted resequencing strategy using next-generation sequencing technology in clinical molecular diagnostics.

2. Patients and methods

2.1. Patients

The study participants consisted of four of the 12 patients in our cohort with alpha-dystroglycanopathy, who were described in a previous article [12]. The diagnosis of CMD with defective glycosylation of alpha-dystroglycan was confirmed in all patients based on clinical features, radiological findings, and muscle immunohistochemistry. Extensive *FKTN* mutational analyses, including three-primer PCR for retrotransposon (RT) insertion mutations, direct sequencing of coding and junctional intronic regions, and RT–PCR-based sequencing, were performed. Patients 1–3 were negative for all of these tests, suggesting the presence of mutation in the other candidate genes. Patient 4 harbored an RT insertion mutation in only one allele. The clinical features of these four patients are summarized in Supplementary Table 1. The Institutional Review Board of the Seoul National University Hospital approved the study protocol (H-1206-076-414).

2.2. Target enrichment and massively parallel sequencing

Genomic DNA was extracted from whole blood of each of the patients using a QIAamp DNA Blood Midi Kit (Qiagen, California, USA) according to the manufacturer's instructions. We captured the target regions of 26 genes using a SureSelect Target Enrichment System Kit (Agilent Technology, California, USA) and sequenced them using an Illumina Genome Analyzer IIx instrument. The detailed methods regarding the custom kit design and massively parallel sequencing were described in our previous report [11]. The target regions included the total genic regions of 26 genes, such as *DMD*, *FKTN*, *FKRP*, *POMT1*, *POMT2*, *POMGnT1*, and *LARGE*, which are known causative genes of several muscular dystrophies. The genes and bait regions of the 26 genes are shown in Supplementary Table 2.

2.3. Single-nucleotide variant (SNV) and short insertion/deletion (indel) calls and screening for large rearrangements

All reads generated were aligned to the NCBI human genome reference build 37 using the GSNAP alignment tool [13]. In this study, we focused only on 24 genes located on autosomal chromosomes and used bases with $\geqslant$15 uniquely aligned reads and $\geqslant$20 mean quality scores for SNV and short indel detection. The variant calls were performed according to the algorithm described in our previous study [14].

Among the called SNVs and indels, we filtered variants detected in 25 control samples from patients with Duchenne/Becker muscular dystrophy, which were sequenced using the platform described in our previous study [11]. We selected additional novel variants among them via comparison with dbSNP build 135 and 137 healthy Asian exomes (51 Korean, 40 Mongolian, and 46 Asian HapMap samples, manuscript in preparation). The variants selected were annotated based on the RefSeq gene set. Variants including nonsynonymous SNVs, coding indels, and canonical splice-site variants were considered as pathogenic candidates, especially variants located in the six candidate genes (*FKTN*, *FKRP*, *POMT1*, *POMT2*, *POMGnT1*, and *LARGE*). For these six genes, we also checked bases with <15 uniquely aligned reads to find possible missed pathogenic variants that might be located in the suboptimally covered area. The SIFT and PolyPhen-2 programs were used to predict the pathogenicity of each nonsynonymous SNV [15,16].

All samples were screened for large rearrangements, such as large deletions and duplications, using the aberrant paired-end sequencing calls, as described in our previous study [11]. The pattern of coverage depth for each gene was also checked manually.

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