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Targeted next-generation sequencing assay for detection of mutations in primary myopathies

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Abstract

Mutations in more than 100 different genes are known to cause hereditary primary myopathies. In patients with less distinct phenotypes several genes may have to be sequenced in order to make the correct diagnosis. The large number of possible candidate genes and overlapping phenotypes, as well as an enormous size of some of the genes such as DMD, TTN and NEB, constitute difficult challenges for molecular genetic diagnostics using conventional sequencing. Molecular characterization is nevertheless important for the final diagnosis and accurate management of the diseases. Targeted next-generation sequencing is a rapid and cost-effective method to sequence large numbers of genes simultaneously. We developed a targeted next-generation sequencing assay, MyoCap, for the coding exons and UTRs of 180 myopathy related genes including 42 novel genes that have not yet been associated with myopathies. DNA samples of four controls with known mutations and 61 patients negative for previous candidate gene approaches were sequenced. The genetic defect was totally or partly clarified in 21 patients with nine of them having potential disease-causing mutations in TTN. MyoCap provides higher read depth and coverage with lower price in the myopathy related genes compared to the whole exome sequencing and is thus very suitable for diagnostic use.

Keywords: Gene panel; Myopathy; Next-generation sequencing

1. Introduction

Myopathies are a clinically very heterogeneous group of disorders in which the primary symptom usually is muscle weakness. Most of them are genetic in origin and they can be further divided into several subgroups including muscular dystrophies, congenital myopathies, distal myopathies, myotonias, channelopathies, mitochondrial myopathies and metabolic myopathies, etc. Myopathies have varying ages of onset and severity of disease progression. The muscular dystrophies is the largest and most common group which lead to progressive weakness, muscle atrophy and incapacity frequently involving respiration or cardiac function [1]. Mutations in more than 400 different genes are known to cause hereditary neuromuscular disorders and of these genes about 100 are known to cause primary myopathies [2]. Mutations in several different genes can cause similar disease phenotypes and different mutations in a single gene may cause different phenotypes. The clinical and genetic heterogeneity is challenging for the clinical diagnostics and several genes may have to be sequenced in order to identify the causative mutation(s). Sanger sequencing has been the basic tool widely used for detecting mutations in myopathies. In many cases almost every exon of a gene had to be sequenced separately in order to cover the gene, and the final diagnosis frequently remained unsolved after candidate gene sequencing. Nevertheless, a correct final genetic diagnosis is critical for the correct management of the disease, genetic counselling and any aspect of therapeutic trials.

Muscle genes comprise some of the largest genes in humans e.g. *DMD*, *TTN* and *NEB* whose sequencing has been very laborious and expensive before the next-generation sequencing (NGS) era. Especially *TTN* has been a very challenging gene with its 363 exons. Due to its huge size *TTN* has not been routinely sequenced in diagnostic laboratories and only a limited amount of *TTN* mutations were identified before the general use of NGS methods. Novel *TTN* mutations detected by NGS methods have already been reported causing hereditary myopathy with early respiratory failure, centronuclear myopathy, core myopathy with heart disease and cardiomyopathy [3–8]. As

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Table 1
List of 180 genes in the MyoCap NGS assay.

Known disease-causing genes							Other genes	
ABHD5	*CNTN1*	*ETFDH*	*LDB3*	*MYPN*	*QDPR*	*TNNT1*	*CELF1*	*MYL12B*
ACADS	*COL6A1*	*FHL1*	*LDHA*	*NEB*	*RYR1*	*TNNT3*	*CMYA5*	*MYLIP*
ACADVL	*COL6A2*	*FKRP*	*LDHB*	*NEBL*	*SCN4A*	*TPM1*	*FBXO32*	*MYLK*
ACTA1	*COL6A3*	*FKTN*	*LMNA*	*NTRK1*	*SEPN1*	*TPM2*	*FHL2*	*MYLK3*
ACTN3	*CPT2*	*FLNC*	*MATR3*	*OBSCN*	*SGCA*	*TPM3*	*KY*	*MYLK4*
ACVR1	*CRYAB*	*GAA*	*MBNL1*	*PABPN1*	*SGCB*	*TRIM32*	*LAMP1*	*MYLPF*
AGL	*CSRP3*	*GBE1*	*MEGF10*	*PDLIM3*	*SGCD*	*TRIM55*	*LIFR*	*MYOM2*
ANO5	*DAG1*	*GNE*	*MSTN*	*PFKM*	*SGCG*	*TRIM63*	*MBNL2*	*MYOM3*
ATP2A1	*DES*	*GYS1*	*MTM1*	*PGAM2*	*SLC22A5*	*TTN*	*MBNL3*	*MYOZ1*
B3GNT1	*DMD*	*ISCU*	*MTMR14*	*PGK1*	*SLC25A20*	*TTR*	*MYBPC2*	*MYOZ3*
BAG3	*DMPK*	*ISPD*	*MYBPC3*	*PGM1*	*SOX10*	*VCP*	*MYH1*	*NBR1*
BIN1	*DNAJB6*	*ITGA7*	*MYH2*	*PLEC*	*SQSTM1*	*VMA21*	*MYH4*	*OBSL1*
CACNA1A	*DNM2*	*KBTBD13*	*MYH3*	*PLN*	*SYNE1*		*MYL1*	*PDLIM5*
CACNA1S	*DPM2*	*KCNE1*	*MYH7*	*PNPLA2*	*SYNE2*		*MYL4*	*PDLIM7*
CAPN3	*DPM3*	*KCNE3*	*MYH8*	*POMGNT1*	*TCAP*		*MYL5*	*PLEKHG4*
CAV3	*DUX4*	*KCNJ2*	*MYL2*	*POMGNT2*	*TIA1*		*MYL6*	*SRF*
CFL2	*DYSF*	*KCNQ1*	*MYL3*	*POMT1*	*TMEM43*		*MYL6B*	*SYNE3*
CHKB	*EMD*	*KLHL9*	*MYLK2*	*POMT2*	*TMEM5*		*MYL7*	*SYNPO2*
CKM	*ENO3*	*LAMA2*	*MYOM1*	*PRKAG2*	*TNNC1*		*MYL9*	*TMOD3*
CLCN1	*ETFA*	*LAMP2*	*MYOT*	*PTRF*	*TNNI2*		*MYL10*	*TNNC2*
CNBP	*ETFB*	*LARGE*	*MYOZ2*	*PYGM*	*TNNI3*		*MYL12A*	*TNNI1*

titin is a very central protein in muscle it is likely that many more myopathy phenotypes caused by *TTN* mutations will be identified.

NGS methods have enabled a rapid and cost-effective screening of the whole genome, the whole exome or selected targeted genes or genomic areas. Targeted NGS gene panels for neuromuscular disorders have been reported during recent years [9–14]. Whole exome sequencing (WES) has also been used but it is more expensive and the coverage is usually not as good as with the targeted methods using smaller gene sets [15]. Whole genome sequencing (WGS) is the most extensive of the NGS methods. It covers both coding and noncoding regions and does not require PCR or hybridization steps to enrich target regions. Compared to WES and WGS the targeted NGS methods are less expensive, have higher average read depth, require less storage space and do not bring the problem of incidental findings outside the studied disease [15].

We describe here a targeted NGS panel, MyoCap, which was developed for sequencing of 180 myopathy related genes simultaneously, and the results obtained in 61 patients with undetermined progressive muscle disease that escaped our previous vigorous diagnostic attempts using extensive genetic and protein studies.

2. Patients and methods

2.1. Patients

DNA samples of 65 myopathy patients were obtained from clinicians in 15 different countries. Four of the patients served as mutation controls with previously reported mutations and 61 patients were unclarified cases from previous diagnostic approaches, e.g. linkage studies, Sanger sequencing of candidate genes and muscle protein examinations after full clinical evaluations including muscle imaging. Of the undetermined patients 14 had a dominant disease, 11 a recessive disease and 36 were sporadic.

The study was approved by the local ethics committees and samples were obtained according to the Helsinki declaration.

2.2. Gene selection and probe design

A total of 180 genes with previously reported mutations causing myopathy or genes related to these were selected for the MyoCap panel in 2013 (Table 1). 42 of the genes selected have not been reported to cause muscle diseases, but were included because of direct interactions of the proteins with known mutational regions of established myopathy genes. Target region contained coding exons and 3′ and 5′ UTRs of transcripts present in databases RefSeq, Ensembl, CCDS, Gencode and VEGA. The total size of the target region was 1.3 Mb containing 3999 exons. For DNA capture a custom SeqCap EZ Choice Library (Roche NimbleGen) was designed using NimbleDesign (Roche NimebleGen) and 50–105-mer probes were allowed to have up to 5 close matches in the genome.

2.3. DNA capture and next-generation sequencing

DNA capture, enrichment and next-generation sequencing were performed at the Institute for Molecular Medicine Finland (FIMM). DNA library preparation was performed using NEBNext DNA Sample Prep Master Mix Set 1 Kit (New England BioLabs) and 4-plex pooled barcoded DNA libraries were captured using the designed SeqCap EZ Choice Library (Roche NimbleGen). Paired-end sequencing (100 bp) was performed using Illumina HiSeq 1500 with a sequencing depth of 100×.

2.4. Next-generation sequence data analysis

Sequencing data were processed using our in-house developed next-generation sequencing pipeline. FASTX tool kit