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Targeted array comparative genomic hybridization – A new diagnostic tool for the detection of large copy number variations in nemaline myopathy-causing genes

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Abstract

Nemaline myopathy (NM) constitutes a heterogeneous group of congenital myopathies. Mutations in the nebulin gene (*NEB*) are the main cause of recessively inherited NM. *NEB* is one of the most largest genes in human. To date, 68 *NEB* mutations, mainly small deletions or point mutations have been published. The only large mutation characterized is the 2.5 kb deletion of exon 55 in the Ashkenazi Jewish population. To investigate any copy number variations in this enormous gene, we designed a novel custom comparative genomic hybridization microarray, NM-CGH, targeted towards the seven known genes causative for NM. During the validation of the NM-CGH array we identified two novel deletions in two different families. The first is the largest deletion characterized in *NEB* to date, (~53 kb) encompassing 24 exons. The second deletion (1 kb) covers two exons. In both families, the copy number change was the second mutation to be characterized and shown to have been inherited from one of the healthy carrier parents. In addition to these novel mutations, copy number variation was identified in four samples in three families in the triplicate region of *NEB*. We conclude that this method appears promising for the detection of copy number variations in *NEB*.

Keywords: Nebulin; NEB; Array comparative genomic hybridization (aCGH); NM-CGH; Copy number variation; Deletion; Nemaline myopathy

1. Introduction

Nemaline (rod) myopathy (NM) (MIM IDs: NEM1 #609284, NEM2 #256030, NEM3 #161800, NEM4 #609285, NEM5 #605355, NEM6 #609273, NEM7 #610687) is a heterogeneous group of rare muscle disorders characterized by muscle weakness and the presence of nemaline bodies (rods) in the muscle fibers. Nemaline bodies are aggregates of Z-disc and thin-filament proteins of the muscle sarcomere. NM has been subdivided into six clinical categories varying from very severe to milder forms of the disease [1].

Mutations in seven different genes coding for thin-filament proteins are known to cause NM (*NEB*, *ACTA1*, *TPM3*, *TPM2*, *TNNT1*, *CFL2*, *KBTBD13*). NM can be inherited as an autosomal recessive or dominant trait or it can be due to a new dominant mutation. The main cause of recessively inherited NM are mutations of the nebulin gene (*NEB*) [2–4]. In addition to NM, *NEB* mutations are also known to cause other myopathies; distal myopathy [5,6] and core-rod myopathy [7].

Nebulin is a gigantic structural protein (600–900 kDa) located mainly in the thin (actin) filaments of the

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sarcomeres in striated muscle. Nebulin has numerous different isoforms produced by alternative splicing of exons 63–66, 82–105, 143–144 and 166–177. In the central region of the gene there is an 8.2 kb triplicate region where eight exons are repeated three times (exons 82–89, 90–97 and 98–105) [8]. The alternative splicing patterns and precise structure of the region in each individual are difficult to study due to the high homology of the repetitive segment.

NEB is located in chromosome cytoband 2q23.3(152,341,850–152,591,001) (GRCh37/hg19). It consists of 183 exons spanning 249 kb of genomic sequence, making it one of the most extensive genes in the human genome. Mutation analysis of *NEB* is demanding due to the large size and complexity of the gene. Furthermore, there are no clear mutational hotspots in *NEB* and more than 140 different disease-causing *NEB* mutations have been found to date in 125 families. Most of the published *NEB* mutations are intragenic point mutations (55%) or small deletions (31%) of 2–19 bp [3]. Moreover, most of the NM patients are compound heterozygous, which contributes to making mutation analysis laborious.

Denaturing high-performance liquid chromatography (dHPLC) [3] followed by sequencing has been efficient in identifying small, heterozygous mutations, but additional methods are needed since both or the second mutations of many patients remain unidentified after analysis by dHPLC and sequencing alone. This may indicate that the dHPLC method does not detect all point mutations, but it is also possible for the second mutation to be a large deletion/duplication, an intronic mutation affecting splicing or a mutation in the promoter of *NEB* which could not be identified using dHPLC. Moreover the triplicate region in the middle of the gene might harbor mutations which have remained unidentified due to the difficulties that the repetitive segment creates.

The multiplex ligation-dependent probe amplification (MLPA) method has successfully been used in mutation detection and diagnostics of several genes [9–12]. Even though the MLPA technique optimally allows the copy number detection of tens of different genomic regions, there is no commercial kit available for the 183 exons of the *NEB* gene. Self-designed synthetic MLPA probes have previously been designed in our group for about one third of the *NEB* exons. (In this study, MLPA was used as a verification method for the new NM-CGH microarray.)

Only one large mutation, the 2.5 kb deletion of exon 55, has been described in *NEB*, but the frequency of such large copy number changes in *NEB* is unknown [13,14].

The array comparative genomic hybridization (aCGH, chromosomal microarray) technique has revolutionized the possibility to detect copy number variations in the genome. With aCGH it is possible to detect copy number variations from small submicroscopic duplications and deletions to entire chromosome copy number changes [15].

The entire human genome can be studied in only one single experiment. The resolution of the array depends on the number of the probes and the targeting of the microarray. Many different applications have arisen from the whole-genome microarray approach. Arrays can be targeted for example towards known syndromes. Furthermore it has become possible to design targeted custom arrays to cover densely only the genes of interest. One example of a high-density custom array is the DMD-CGH array targeted for mutation detection in the gigantic dystrophin gene where mutations cause dystrophinopathies [16]. In recent years different microarray methods have shown great success as novel mutation detection tools.

Here we describe the design and validation of a targeted custom NM-CGH microarray for mutation identification in NM. We hypothesized that there may be big *NEB* mutations that have not been identified. In order to study copy number variations, both pathogenic and benign, we designed a highly targeted custom NM-CGH microarray including all of the known causative genes for NM. The design includes the exons and introns as well as exon–intron boundaries, and ~25 kb upstream and downstream of each gene.

2. Materials and methods

2.1. Samples

This study included altogether 52 DNA samples from 43 different families. The samples were received either as isolated DNA or as blood, cell lines, muscle or skin biopsies from which DNA extraction was done using appropriate methods.

The primary reference sample was made by pooling 10 different samples from blood donors of the Finnish Red Cross. The anonymous reference samples have been given to the Folkhälsan Institute of Genetics and the Department of Medical Genetics, University of Helsinki as control samples for research use. The secondary reference used in this study was a commercial reference sample particularly meant for SNP microarray studies (Promega Corporation, Madison, WI, USA).

The validation samples of the NM-CGH microarray (Table 1) included five normal control samples from five different families with no indication of NM. Four samples from two families with known *NEB* mutations were used as positive controls. The first family was known to carry the *NEB* exon 55 deletion and samples from the second family was used to test the sensitivity and specificity of the mutation-specific probes for the previously described 10 bp deletion in *NEB* exon 122. The validation included testing of the probe quality with commercial control DNA (Oxford Gene Technology IP Limited, Oxford, UK), testing of the reference sample pool against a commercial reference (Human Genomic DNA, Male, Promega) as well as dye flip samples and replicate samples (data not shown).

The DNA samples of NM patients included altogether 43 samples from 36 families. These samples were divided into two groups. The first group consisted of 27 samples from 22 families where only the first of the two compound