A novel copy number variation detection array for the diagnosis of neuromuscular disorders

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Introduction

We have developed a novel 4x180k comparative genomic hybridization array, the NMD-CGH array for the diagnostics of neuromuscular disorders. It includes a selection of 187 genes related to nemaline myopathy and other neuromuscular diseases – amongst others, titin (*TTN*) and obscurin (*OBSCN*).

Our previous results have shown that copy number variations (CNVs) are a potential pathogenic cause in different neuromuscular disorders (**Table 1**). Our array allows for detection of CNVs in the segmental duplication (SD) regions of nebulin (*NEB*) and *TTN* (**Figure 1**). These regions are typically excluded from commercial CGH-array designs.

Even though NGS methods have improved recently, the array-CGH method is more sensitive and reliable in detecting CNVs to date.

Materials and Methods

The novel 4x180k NMD-CGH array is built upon the design of the previously published 8x60k nemaline myopathy CGH array [1]. It includes the known nemaline myopathy genes (*ACTA1*, *NEB*, *TPM3*, *TPM2*, *TNNT1*, *CFL2*, *KBTBD13*, *KLHL40*, *KLHL41*, *LMOD3* and *MYPN*) and 176 other known neuromuscular disease genes or candidates for such, including the genes of the 1st version of the MyoCap panel [2].

The genes are divided into three gene priority groups (**Table 2**):

- **Group 1**: the known nemaline myopathy genes
- **Group 2**: genes known or suspected to harbor pathogenic CNVs
- **Group 3**: other genes of interest in neuromuscular diseases

The backbone was increased threefold to allow for better alignment and detection of large CNVs throughout the genome.

Table 1. *Altogether 271 families have been analyzed on the NM-CGH and NMD-CGH arrays to date. The well-characterized Ashkenazi Jew founder mutation (deletion of exon 55 in nebulin) is not included in the aberration counts. NEB TRI = triplicate region of nebulin (exons 82-89, 90-97, 98-105).*

Families analyzed	271 families	100 %
Pathogenic aberrations in targeted genes	32 families	12 %
Pathogenic aberrations in *NEB* (excl.TRI)	11 different in 12 families	4.5 %
Pathogenic CNVs in *NEB* TRI	9 families	3 %
Benign CNVs in the *NEB* TRI	27 families	10 %

Table 2. *The genes are divided into three priority groups. F+R or F/R = probe targeting on either both the forward and the reverse strand or on one of the two, respectively. ± = the region covered upstream and downstream of the targeted genes. For the complete list of genes, please refer to appendix.*

	Group 1 (n = 12)	Group 2 (n = 29)	Group 3 (n = 146)
Exon	10 bp tiling, F+R	Adjacent probes, F+R	~4 probes/exon, F/R
Intron/promoter	20 bp tiling, F+R	Adjacent probes, F+R	~1-3 probes/intron, F/R
Flanking	± 25 kb	± 25 kb	± 25 kb

Results

We have validated the novel NMD-CGH array and it has shown sensitive and reliable detection of CNVs of different sizes in the targeted genes in all three targeting level groups (**Figure 1**). The array has also confirmed recurrent CNV in the *TTN* SD region.

Our NMD-CGH array allows for CNV detection in a large spectrum of genes related to neuromuscular diseases, and brings thus a new alternative to mutation detection in patients with neuromuscular disorders. The NMD-CGH array is available for diagnostic runs in our laboratory.

Figure 1. *A demonstration of the array results. NEB is targeted at group 1 level, TTN and MYH7 at group 2 level and MYOM and PYGM at group 3 level.*

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Appendix

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