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

Lydia Sagath[1], Vilma-Lotta Lehtokari[1], Carina Wallgren-Pettersson[1], Katarina Pelin[1,2], Kirsi Kiiski[1]

[1]The Folkhälsan Institute of Genetics and the Department of Medical and Clinical Genetics, Medicum, University of Helsinki, Helsinki, Finland
[2]Department of Biosciences, Division of Genetics, University of Helsinki, Helsinki, Finland.

Introduction

We have developed a novel 4x180k comparative genomic hybridization array for the diagnostics of neuromuscular disorders (the NMD-CGH array) based on our previously published targeted nemaline myopathy (NM) 8x60k CGH array (the NM-CGH array). It includes, in addition to the 11 published (+1 unpublished) NM causing genes (*ACTA1*, *NEB*, *TPM3*, *TPM2*, *TNNT1*, *CFL2*, *KBTBD13*, *KLHL40*, *KLHL41*, *LMOD3* and *MYPN*), a selection of 176 genes related to other myopathies. Amongst others, titin (*TTN*) and obscurin (*OBSCN*) are included.

Our previous results as well as other studies have shown that copy number variations (CNVs) are a potential pathogenic cause in different neuromuscular disorders. 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 NM-CGH-array. The neuromuscular disease genes were chosen based off the published neuromuscular disease next generation sequencing panel MyoCap (Evilä ***et al.*** 2016). We have divided the targeted genes into three groups based on targeting level (table 1). Group 1 contains the known NM genes, group 2 a set of genes known or suspected to harbor pathogenic CNVs and group 3 contains other genes of interest in terms of neuromuscular diseases. The backbone was increased from 1 probe/1Mb to 1 probe/300kb to allow for better alignment as well as detection of large CNVs throughout the genome.

Table 1. *The 187 muscle genes included on the 4x180k array design have been divided into three different groups according to the amount of targeted probes per bp. Group 1 consists of the known nemaline myopathy genes, group 2 of genes proven or suspected to harbor pathogenic CNVs and group 3 contains other genes of interest. For the complete list of genes, please refer to the material at http://www.lydiasagath.com/eshg2017.html, or scan QR code.*

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

Results

As to date, we have analyzed samples from 261 families with NM or related muscle disorders on our custom NM- and/or NMD-CGH-arrays, and identified 15 different disease-causing aberrations in the nebulin gene (***NEB***) in 31 of the families. The majority of these pathogenic variations were detected only in one to three families each. Copy number variants (CNVs) affecting the ***NEB*** triplicate region (TRI, nebulin exons 82-89, 90-97, 98-105) were detected in 16% of the NM families, and in 5% of the families the CNV was interpreted to be pathogenic. Pathogenic CNVs have been found in 12% of all families analyzed.

We have recently 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 has confirmed recurrent CNV in the ***TTN*** segmental duplication region.

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

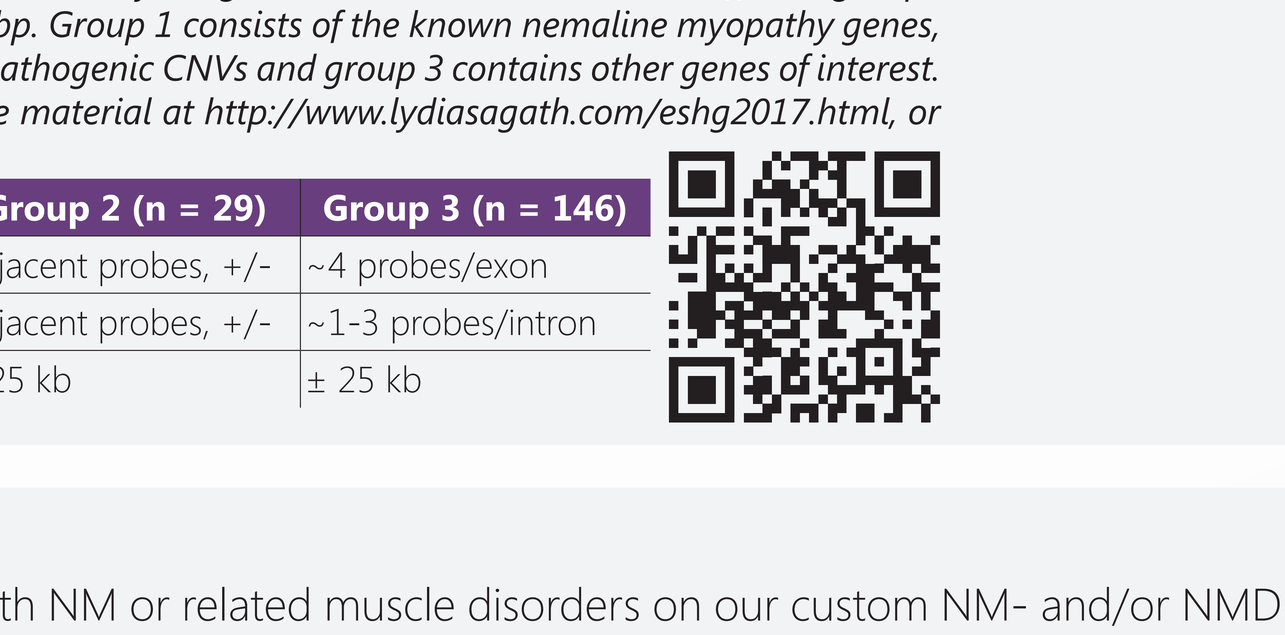
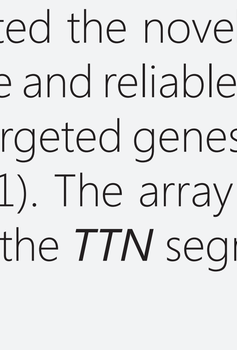


Figure 1. *A demonstration of the array results. NEB is targeted at group 1 level, TTN and MYH7 at group 2 level and MYOM and PYGM at group 3 level.*

Contact

Lydia Sagath, MSc
Doctoral candidate

lydia.sagath@helsinki.fi
+358-50-3496096
linkedin.com/in/lydiasagath



Funding

This work is funded by Muscular Dystrophy UK, the Sigrid Jusélius Foundation, Finska Läkaresällskapet, Medicinska understödsföreningen Liv och Hälsa, Association Française contre les Myopathies, and the Folkhälsan Research Center.

References

A. Evilä, M. Arumilli, B. Udd, P. Hackman. **Targeted next-generation sequencing assay for detection of mutations in primary myopathies**. Neuromuscul Disord, 26(1):7-17 (2016).

K. Kiiski, L. Laari, VL. Lehtokari, M. Lunkka-Hytönen, C. Angelini, R. Petty, P. Hackman, C. Wallgren-Pettersson, K. Pelin. **Targeted array comparative genomic hybridization - a new diagnostic tool for the detection of large copy number varaitions in nemaline myopathy-causing genes**. Neuromuscul Disord, 23(1):56-65 (2013).