EP.042

A DDPCR METHOD FOR THE DETECTION OF COPY NUMBER VARIATIONS IN THE NEBULIN TRIPLICATE REGION

Lydia Sagath[1,2], Vilma-Lotta Lehtokari[1,2], Carina Wallgren-Pettersson[1,2], Katarina Pelin[1,2,3], Kirsi Kiiski[1,2]

[1] Folkhälsan Research Center, Finland • [2] Department of Medical Genetics, Medicum, University of Helsinki, Finland • [3] Faculty of Biological and Environmental Sciences, University of Helsinki, Finland

Contact: lydia.sagath@helsinki.fi

1 INTRO

Copy number variations (CNVs) in the nebulin triplicate (*NEB* TRI) region (**Fig. 1**) are pathogenic when the copy number (CN) of the 8-exon duplication block deviates two or more copies from the standard number, three per allele.

We have established custom Droplet Digital PCR (ddPCR) assays to detect CNVs in the *NEB* TRI region. They allow sensitive, high-throughput, and cost-effective detection of CNVs within this region. They are suitable for implementation as a screening method for disease-causing CNVs of the *NEB* TRI region. We suggest that ddPCR may also be used to study other CNV-prone segmental duplication regions of the genome.

Table 1. Number and percentage of samples with different *NEB* TRI CNs.

	Copy number	No of samples	% of samples
Benign (n=83)	5	22	21.4
	6	49	47.6
	7	12	11.7
Pathogenic (n=20)	8	3	2.9
	9	5	4.9
	10	7	6.8
	11	2	1.9
	14	3	2.9
Total		103	100

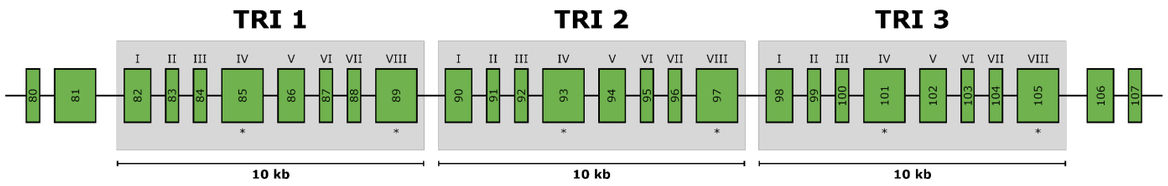

Fig. 1. A schematic figure of the exons in the NEB TRI region. The exons targeted by the ddPCR assays are marked with an asterisk (*).

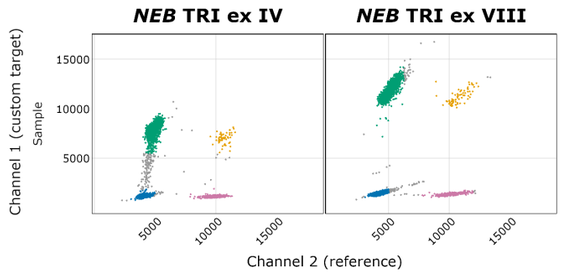

Fig. 2. Example 2D droplet intensity plots of *NEB* TRI ex IV and ex VIII assays on DNA and no-template controls as extracted from the twoddpcr output. The droplets excluded from further analysis ("rain") are displayed in grey.

2 M&M

We designed two ddPCR assays for the *NEB* TRI region targeting exons IV and VIII of the repeated block (**Fig. 1**). Altogether 130 samples were run on the Bio-Rad QX200 system. The raw data were filtered using the `twoddpcr` R-package (**Fig. 2**), after which thresholds of 10,000 accepted droplets and 100 droplets per cluster were applied. After filtering, 103 samples (**Table 1**) remained to be included in the downstream analyses.

Statistical analyses were performed in R. These included intra- and inter-assay analyses, linear regression modeling, and Cohen's Kappa for both detection of copy number and pathogenicity.

3 RESULTS & CONCLUSIONS

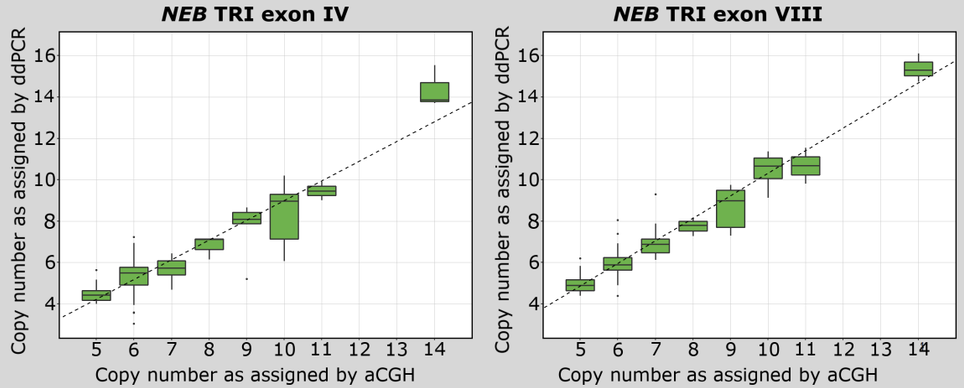

Fig. 3. Boxplots visualizing the CN of the *NEB* TRI exon IV and VIII assays in relation to the CN assigned by aCGH with the linear regression trend lines. The *NEB* TRI exon IV assay gives a relationship with a lower linear slope than the *NEB* TRI exon VIII assay, which seems to follow a 1:1 linear relationship adequately. The dashed lines represent the linear regression trend lines.

All statistical analyses were concordant with each other. Both assays detect CN differences; however, the *NEB* TRI exon VIII assay performed better in the given environment. The *NEB* TRI exon IV systematically evaluated the CN as lower than the aCGH, yielding an offset in CN in the analyses (**Fig. 3**).

Pearson correlation coefficients for exon IV and exon VIII assays against the aCGH-determined CN were 0.896 and 0.951, respectively. Linear regression models for the assays gave estimates of 0.953 and 1.090, respectively. Kappa values for the detection of copy number were 0.545 and 0.761, respectively. Kappa values for the detection of pathogenicity (**Table 2**) were 0.363 and 0.745, respectively. All values presented here were statistically significant at $p < 0.0001$.

Table 2. The Kappa tables for detection of pathogenicity of the assays *NEB* TRI exon IV (κ-value 0.363) and exon VIII (κ-value 0.745).

NEB TRI exon IV			
ddPCR \ aCGH	Benign	Pathogenic	
Benign	63	6	
Pathogenic	20	14	
Total	83	20	103

NEB TRI exon VIII			
ddPCR \ aCGH	Benign	Pathogenic	
Benign	76	2	
Pathogenic	7	18	
Total	83	20	103

Our results show that the ddPCR method is a viable option for the detection of CNVs of the *NEB* TRI region. Compared with custom aCGH, the method is rapid, high-throughput, and cost-effective, making it a practical method for the screening of a large number of samples simultaneously in a diagnostic setting. Furthermore, ddPCR could be implemented in the study of other repetitive regions of the genome.

Funded by Muscular Dystrophy UK, the Folkhälsan Research Foundation, the Magnus Ehrnrooth Foundation, the Finska Läkaresällskapet, and the Medicinska understödsföreningen Liv och Hälsa.