A ddPCR Method for Copy Number Variation Analysis in the Segmental Duplication Regions of Nebulin and Titin

Lydia Sagath¹, Vilma-Lotta Lehtokari¹, Carina Wallgren-Pettersson¹, Katarina Pelin¹,², Kísirí Kiiski¹
¹ The Folkhälsan Institute of Genetics, Finland
² Faculty of Biological and Environmental Sciences, University of Helsinki, Finland

INTRO
Nebulin and titin (encoded by the genes NEB and TTN) are sarcomeric giant proteins, and both harbour large segmental duplication (SD) regions known to be expressed in muscle. In NEB, this region contains eight exons repeated three times. In TTN, nine exons are repeated threefold, after which the two first exons of the block reappear alone a fourth time. Mutations in NEB are known to cause nemaline myopathy and copy number variations (CNV) of the NEB repetitive region are known to be pathogenic (Kiiski et al. 2016).

We are able to detect CNVs and determine copy number (CN) in the NEB SD using our custom Comparative Genomic Hybridisation arrays (aCGH; Sagath et al. 2018, Kiiski et al. 2013). The SD region of TTN, however, is shorter, making unique probe design more difficult.

Droplet digital PCR (ddPCR) allows us to quantify the CN of the SD regions of NEB and TTN by using sample partitioning, PCR and hydrolysis probes. By targeting unique sequences within the SDs we are able to determine the CN with greater precision than using other methods.

METHODS

We have designed custom assays to target different parts of the segmental duplication regions of NEB and TTN using Primer3Plus. The assays target the exons 4 and 8 of the repeated NEB region and exon-intron regions 1-2, 4-5 and 7-8 of TTN. The ddPCR was run with Bio-Rads QX200 ddPCR system and analysed with twodpcr.

RESULTS

We have optimised the ddPCR protocol for the custom assays. We are currently validating the NEB SD assays against data acquired with our custom CGH-arrays and gathering TTN SD data simultaneously.

As to date, we have run 50 samples with all assays. Of these, 35 are controls and 15 are patient samples with either normal NEB SD copy number, one-copy gains or losses.

We are expecting to be able to extrapolate the information gathered from the NEB ddPCR assays to the TTN SD ddPCR assays, as to be able to determine the copy number of the TTN SD with more precision than the custom CGH-array.

DISCUSSION

The proposed methods brings a novel segmental duplication region analysis method to the table with a short turnaround time, low costs and high preciseness. The ddPCR approach could be adapted to suit other similar SD regions in other genes, and thus paves the way for diagnostic use of ddPCR.

CONTACT
Lydia Sagath, MSc
Doctoral Candidate
lydia.sagath@helsinki.fi
+358-294125078
linkedin.com/in/lydiasagath
lydiasagath.com

FUNDING
This work is funded by Muscular Dystrophy UK, the Finska Läkarollskapet, the Medicinska understödföreningen Liv och Hälsa, l’Association Française contre les Myopathies, and the Folkhälsan Research Center.

REFERENCES


OBJECTIVES

- Develop a ddPCR assay for the CNV analysis of NEB and TTN
- Determine normal copy numbers for the TTN SD region
- Create estimates for the level of variation in TTN SD
- Find the putative pathogenic threshold for the TTN SD region

Figure 1. Copy number variation of the SD regions of NEB and TTN. In NEB, ~12% of samples (n=7) harbor variation in the SD region (in green). Of these, 5% are deletions and 7% are duplications. In TTN, variation in the SD region is seen in ~22% of the samples, and there is an equal distribution between duplications and deletions (in green).

Figure 2. A ddPCR schematic. Samples are partitioned into individual droplets, and the PCR reactions happens individually in each of them. In duplex reactions droplets can be categorized into four categories: target-positive/reference-positive, target-negative/reference-positive, target-negative/reference-negative, target-negative/reference-negative.

Figure 3. Nebulin SD exon 8 assay validation. ddPCR data validated against aCGH data of NEB SD exon 8 of samples with one-copy loss of the SD block (n=6), normal copy number of 6 (n=15), and gain of one SD block (n=7). Statistical significance between the groups is at least p<0.0005.

Figure 4. Nebulin SD exon 8 assay validation. ddPCR data validated against aCGH data of NEB SD exon 8 of samples with one-copy loss of the SD block (n=6), normal copy number of 6 (n=15), and gain of one SD block (n=7). Statistical significance between the groups is at least p<0.0005.

Figure 5. Nebulin SD exon 8 assay validation. ddPCR data validated against aCGH data of NEB SD exon 8 of samples with one-copy loss of the SD block (n=6), normal copy number of 6 (n=15), and gain of one SD block (n=7). Statistical significance between the groups is at least p<0.0005.