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Array comparative genomic hybridisation and droplet digital PCR uncover recurrent CNV of the TTN segmental duplication region

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1 INTRO

Intragenic segmental duplication (SD) regions are potential hotspots for recurrent copy number variation and possible pathogenic aberrations. Using targeted array-CGH, we have detected recurrent CNVs of the SD region of titin (*TTN*). This region consists of a 9-exon-block thrice repeated, after which the two first exons of the block appear a fourth time (exons 172–180, 181–189, 190–198, and 203–204) and is located within the PEVK region of the gene. The structure of the *TTN* SD region is depicted in **Figure 1**.

Due to the limitations of the array-CGH methodology and the repetitiveness of the region, the exact copy numbers (CN) of the blocks could not be determined. Therefore, we developed complementary custom Droplet Digital PCR assays for the titin segmental duplication region to confirm true variation.

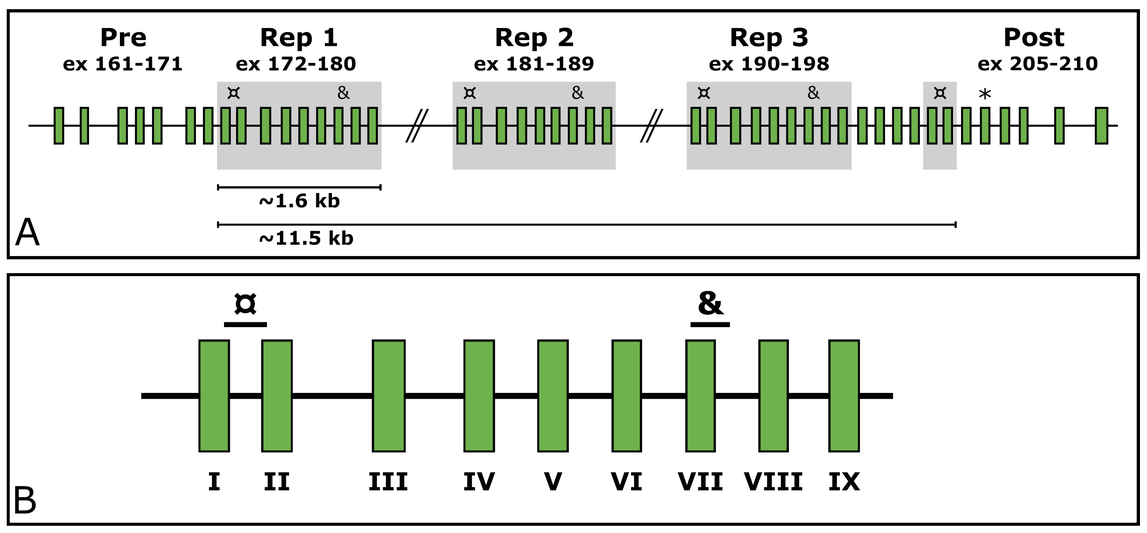

Figure 1. A schematic of the exons in the TTN SD region, with the targeted regions of the assays marked. Panel A shows the entire TTN SD region, and panel B is a zoom-in on the repeated segment. The TTN SD exon I assay is marked with a currency sign (¤), the TTN SD exon VII assay with an ampersand (&), and the TTN Post-SD assay with an asterisk (*). The black bars in panel B represent the length and location of the primer-probe pairs' amplicons.

2 MATERIALS & METHODS

Altogether, 62 samples from 42 neuromuscular disorder families were acquired for the study. Of these, 42 were index patient samples, and the remaining 20 samples were from unaffected family members. The patient phenotypes included nemaline myopathy (n = 18), distal nemaline myopathy (n = 2), asymmetric distal myopathy (n = 1), cap myopathy (n = 1), unspecified congenital myopathy with arthrogryposis (n = 1), and unspecified congenital myopathy (n = 12). Nine of the patients had previously received a final molecular genetic diagnosis. Causative CNVs of the *TTN* SD region were not expected in the cohort.

All samples were run on the NMD-CGH-array as previously describe [1,2], and subsequently run by four custom ddPCR assays [3], of which two targeted the SD region of *TTN*, one a region downstream of the SD, and one targeting the triplicate region of nebulin (*NEB* TRI) [2].

The performance of the assays was assessed by One-Way ANOVA, post-hoc Tukey's HSD, Bland-Altman analysis, Pearson correlation and regression analysis.

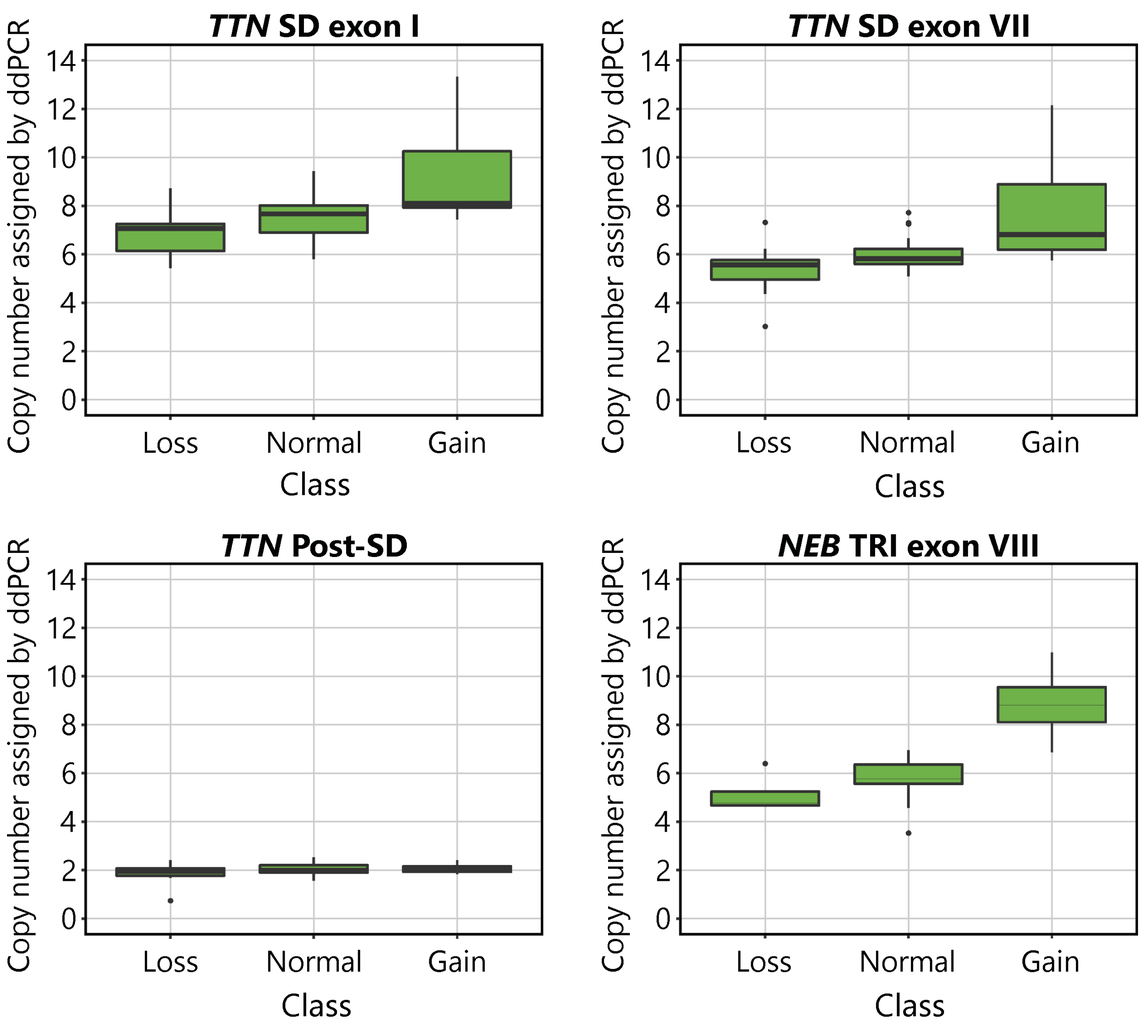

Figure 2. Interpreted ddPCR-derived CNs of the TTN SD exon I, SD exon VII, Post-SD, and NEB TRI exon VIII assays are plotted against the aCGH-determined CN class for the TTN SD region and CN class for the NEB TRI region. The expected normal CN were eight for the TTN SD exon I assay, six for the TTN SD exon VII, two for the TTN Post-SD assay, and six for the NEB TRI exon VIII assay.

3 RESULTS & CONCLUSIONS

Altogether 55 samples passed the initial quality filtering in all assays in at least two parallel wells. Of these, 36 were samples from neuromuscular disorder patients and 19 were samples from unaffected family members. Of all samples, 35 were classed as normal (CN = 6), 11 as losses (CN < 6), and 9 as gains (CN > 6) as per the CNV prediction of the NMD-CGH-array for the *TTN* SD region. Gains and losses in the *TTN* SD region were present in both patient and unaffected family member samples.

Result plots are presented in **Figure 2**. The CN of the *TTN* SD block ranged from 4.99 to 13.68 in the *TTN* SD exon I assay, 2.90 to 12.62 in the *TTN* SD exon VII assay, and 0.72 to 2.57 in the *TTN* Post-SD assay.

Statistical analyses found significant correlation and differences between groups in all assays, where expected.

While gains in the *NEB* TRI region have been defined a pathological threshold, no such threshold could be set for the *TTN* SD region CN in this cohort. The extensive splice isoforms of *TTN* may also hamper the effect of potentially pathogenic gains of the region, although patholocical effect by large gains cannot be excluded. The *NEB* TRI region seems to predispose the *NEB* gene to larger CNVs, and a similar effect could be seen in *TTN*.

Our results show, that the TTN SD region is subject for recurrent CNVs in patients with titin-unrelated neuromuscular disorders and family members thereof, and thus most probably also in the healthy population. Furthermore, we show ddPCR is a viable detection method of especially large gains in the TTN SD region.

REFERENCES

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