

Supplement 3: Preliminary CNV analysis on NGS panel sequencing data from 229 ataxia patients

We performed a preliminary CNV analysis on our sequencing data from n=179 patients sequenced by the HaloPlex gene panel kit and from n= 50 patients sequenced by a probe-based customized panel (Illumina Nextera Rapid Capture Custom Kit). All 179+50=229 patients were part of the original ataxia screening cohort described in the main text.

Preliminary CNV analysis on HaloPlex gene panel sequencing data. Sequencing data of n=179 ataxia patients were reanalyzed using our in-house software tool CnvHunter, which assigns copy number states in NGS data-sets comparing the mean coverage of every targeted exon with the normalized mean in 10 normal controls. This analysis did neither show homozygous exon deletions nor any heterozygous deletion or duplication exceeding 2 neighboring exons. As a positive controls we analyzed heterozygous deletions in the *ITPR1* gene in SCA15 patients (Synofzik *et al.*, 2011), demonstrating that this method is indeed robust enough to uncover multi-exon deletions. Since the sensitivity for detecting heterozygous copy-number alterations in HaloPlex data does not allow for single exon changes, this data at least excludes gross copy-number changes in *SYNE1* in this patient cohort.

CNV analysis on a probe-based customized panel (Illumina). Sequencing data on n=50 ataxia patients were reanalyzed based on a depth of coverage (DoC) comparison, comparing the patient group with a control group. The latter is composed of samples from n=17 index subjects of the same NGS runs as the patient group, but found to be positive for other disease gene point mutations. The analysis was calculated as follows: 1. For each sample, mean DoC was calculated for each target region and divided by the sum (Σ) of the mean DoC of all the target regions (=NMDoC, normalized mean DoC). 2. The NMDoCs of each patient sample were divided by the corresponding average NMDoC value from the control samples and multiplied by 100. This value is referred to as "relative mean DoC (RMDoC)" in the dot plot (see Figure

below). 3. For each target region in each sample, this calculation yielded a value for DoC which represents the percentage of the mean DoC of controls.

All 146 exons of *SYNE1* were analysed, except three (ex27, ex53, and ex60; NM_182961) which always exhibited a DoC too low for being analysed by NGS. These 3 exons represent $\leq 2\%$ of the coding sequence.

All the deletions identified in other genes with this calculation and confirmed by qPCR had a RMDoC around 0.5 (multi-exon deletion of ITPR1, single-exon deletion of ANO10, two-exon deletion of CACNA1A). In the current data set of n=50 ataxia patients, this analysis did neither show homozygous exon deletions nor any heterozygous deletion involving 2 neighboring exons. However, RMDoCs ≤ 0.7 occurred in 11 single-exons (which means 11 exons out of a total of 7.150 exons analysed; 143 x 50 samples), including only two exons in two different subjects with RMDoCs ≤ 0.6 . Only two patients showed more than one exon in the critical range of 0.5-0.7. Importantly, none of the patients carried another truncating *SYNE1* mutation on the other allele. Subsequent qPCR of exons with RMDoCs ≤ 0.7 showed normal copy number.

These results must be interpreted with caution, since no consensus currently exists on a method to perform CNV analysis on NGS data. However, these preliminary results do not provide evidence for any gross CNVs in the 179+50=229 ataxia patients studied here by NGS-panel based CNV analysis.

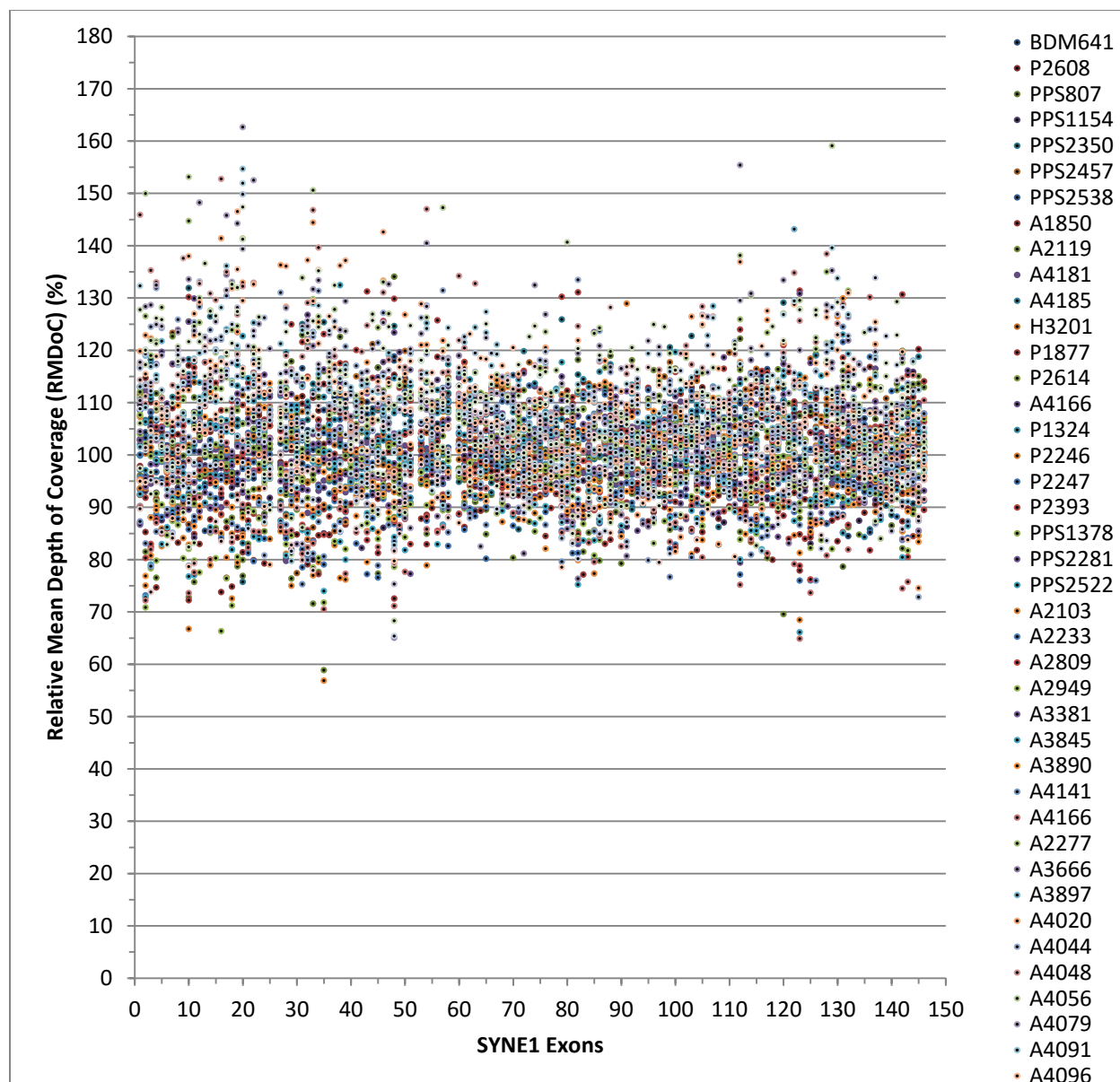


Figure: Relative mean of depth of coverage for 143 *SYNE1* exons in n=50 ataxia patients sequenced by a probe-based customized panel (Illumina). To screen for *SYNE1* exon deletions/duplications, panel-sequencing results were screened for lack of coverage, here indicated by a reduced relative mean depth of coverage. RMDoCs ≤ 0.7 occurred in only 11 instances, including only two subjects with RMDoCs ≤ 0.6 . Subsequent qPCR showed normal copy number .

References:

Synofzik M, Beetz C, Bauer C, Bonin M, Sanchez-Ferrero E, Schmitz-Hubsch T, et al.
Spinocerebellar ataxia type 15: diagnostic assessment, frequency, and phenotypic features. J
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