COMMENT

ADNP in reverse gear

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Breakthroughs in next-generation sequencing technologies, most notably whole-exome sequencing (WES), have increased genetic diagnostic yield. In individuals diagnosed with a neurodevelopmental disorder such as intellectual disability (ID) or autism, this has improved diagnosis to approximately 30% of cases [1]. However, this implies that more than two-thirds of patients remain without a molecular diagnosis. To face the challenge of increasing diagnostic yield, whole genome sequencing (WGS) is currently explored for use in routine diagnostics. In contrast to WES, where only the coding regions of the genome are investigated, in WGS, the sequence of the entire patient genome is compared to that of their parents. Despite this increase in resolution, currently, the reported diagnostic rate of WGS only marginally exceeds that of WES [2]. In their article in this issue, Georget et al. [3], performed WGS as the next step in the diagnostic odyssey in a young individual affected with developmental delay, hypotonia and dysmorphic features who was left without a diagnosis following routine clinical testing, including WES. They found an inversion in the genomic sequence of the *Activity-Dependent Neuroprotective Protein (ADNP)* gene that remained undetectable in the WES but was exposed following WGS. Mutations in the *ADNP* gene are associated with a diagnosis of Helsmoortel-Van der Aa syndrome (HVDAS; OMIM #615873) [4], in line with the clinical presentation of the patient in this study.

In order to determine whether the inversion inactivates the *ADNP* gene, the genomic rearrangement was studied in detail. The inversion reversed the order of exons 3–5 of the genomic sequence consisting of 6 exons of which the last three are protein-coding (Fig. 1). The intronic position of the inversion breakpoints explains why WES is not able to detect it. Counterintuitively, the RNA expression levels of *ADNP* in blood were not reduced when comparing the total number of transcripts between the patient and a large series of controls. However, such a finding is in line with earlier observations that transcript levels in patients with a

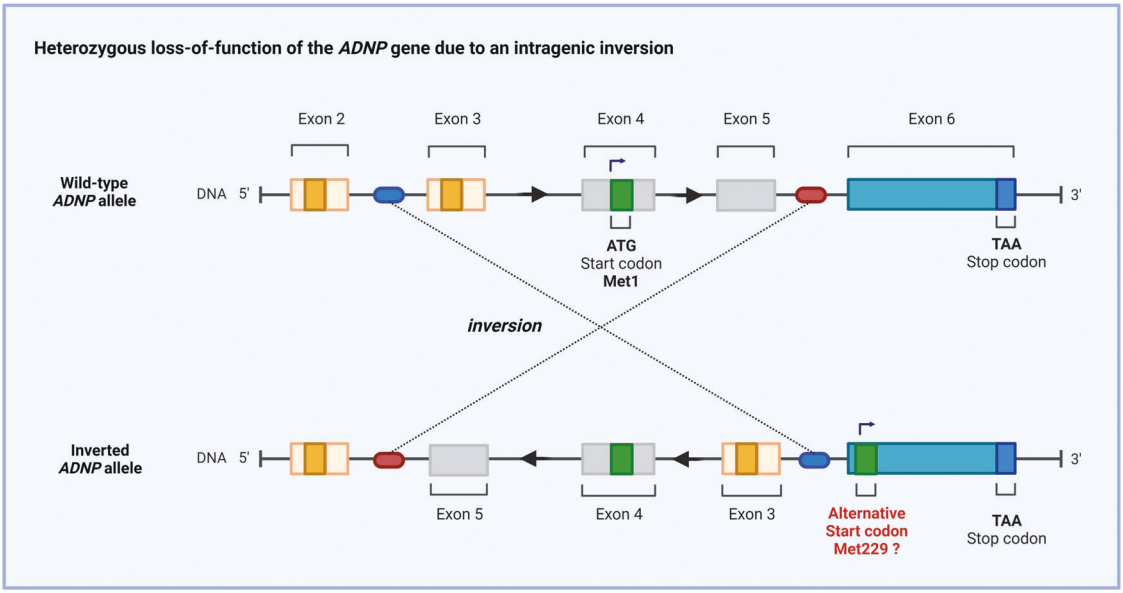

Fig. 1 Heterozygous loss of function of the *ADNP* gene due to an intragenic inversion. Schematic representation of the wild-type *ADNP* allele (above) together with the inverted allele (below). AluSq10 sequence (blue) a AluY (green) sequence are found at the breakpoints of the inversion of exons 3–5. Canonical *ADNP* gene transcription starts at the start codon (ATG) in exon 4 (Met1), which is skipped as a consequence of the intragenic inversion, causing potential transcription at the alternative start codon (ATG) in exon 6 (Met229).

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mutation in the last exon are not decreased when compared with controls [5]. As a next step, the authors looked at the translation potential of the rearranged *ADNP* gene. The rationale being that most of the coding sequence is located in the last and largest exon 6 and potentially undisturbed. When analyzing the RNA sequencing data of the patient, no transcripts including the exons in the reverse orientation were found. Though, some transcripts that skipped the inverted exons were detected. Such transcripts retained exon 6, but lacked the native start codon in exon 4. Exon 6 contained one candidate alternative start codon, a methionine at position 229 fulfilling the Kozak criteria. Theoretically, this alternative transcript from the mutant allele could thus translate an N-terminal truncated protein. However, such a protein has not been demonstrated and the presence of even a partially functional mutant ADNP protein is purely hypothetical. In each case, this thorough analysis demonstrates the absence of a functional ADNP protein transcribed from the mutant allele in the patient and confirms the diagnosis.

The intronic breakpoints are characterized by an AluSq10 sequence on one end and an AluY sequence on the other (Fig. 1). Both sequences are in inverse orientation and almost 80% identical, and the authors suggest non-allelic recombination as the underlying mechanism causative of the inversion. The *ADNP* gene is located in a large homozygous region. In this respect, it is of interest to mention that large stretches of homozygosity have been suggested to predisposed to somatic deletions as a consequence of an increased rate of allelic homologous recombination [6]. It can be speculated that this might underlie non-allelic homologous recombination (NAHR) in the observed region, in line with the observation of an increased mutational rate in large homozygous regions [7].

Thus, this case of a Helsmoortel-Van der Aa patient demonstrates a unique added diagnostic value of WGS over WES. It also shows the complexity of this type of analysis, as additional RNA sequencing and various translational predictions had to be performed to substantiate the causality of the inversion. It remains to be determined whether inversions in the *ADNP* gene or in fact in any disease gene are a common mutational mechanism or whether these are truly rare. Given that WGS is expected to replace WES in routine diagnostics in the near future, time will soon provide us with the answer!

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AUTHOR CONTRIBUTIONS

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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