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Reanalysis of trio whole exome sequencing (WES) data with a novel variant prioritization workflow reveals a de-novo missense variant in *EBF3* gene associated with hypotonia and developmental delay

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Clinical whole exome sequencing (WES) is considered as a powerful approach for identifying disease-causing variants, despite it yields a diagnosis in 25-30% of patients. While some of these undiagnosed patients have a pathogenic variant in already sequenced exome, it may not be identified in the initial analysis. The main challenge in this field is the data analysis and interpretation, in terms of detecting the causative mutation from thousands of variants of unknown significance. A recent study reported that 10% of the undiagnosed patients could get a precise diagnosis through the reanalysis of the same WES data, using different workflows and with the help of growing knowledge in the literature.

We developed a variant prioritization workflow taking into account distinct symptoms of patients and the variants in the genes that can give rise to those symptoms. In this study, we present a reanalysis of trio WES, which was left as unsolved through several attempts. To identify disease-causing variants, WES was performed on genomic DNA extracted from samples submitted from the proband, biological mother, and biological father. We have applied our internal workflow to WES raw data. After the variant annotation step, homozygous and compound heterozygous variants with minor allele frequency (MAF) <0.1% in the databases, i.e. gnomAD and 1KGP were filtered. Heterozygous variants, which were found in at least one of these databases, were excluded. The intronic variants that were further away from ±10 bases of exon-intron boundaries were eliminated. Then, the variants were prioritized based on the symptoms of the proband via using inhouse variant prioritization workflow and collecting evidence from various resources and literature. Via integrating several computational analyses, a de-novo heterozygous mutation, c.C487T (p. Arg163Trp), in *EBF3* gene (OMIM *607407) was prioritized as the most prominent variant. *EBF3* gene encodes a member of the early B-cell factor (EBF) family transcription factors that has crucial roles in neurogenesis and development. Heterozygous mutations in *EBF3* are associated with Hypotonia, Ataxia, and Delayed Development Syndrome; HADDS (OMIM #617330), which is a neurodevelopmental syndrome characterized by congenital hypotonia, delayed psychomotor development, variable intellectual disability with speech delay, ataxia and variable dysmorphic facial features. Although the pathogenic mechanisms of *EBF3* mutations remain unclear, a number of missense, nonsense, and intronic variants, and copy number variations are described so far. Interestingly, all of the reported missense variants are located in the DNA binding domain, which is highly conserved in EBF3 protein. Five of these missense variants affect the same amino acid residue (Arg163), which is in the Zn2+ finger Collier/Olf/Ebf (COE) motif. In this respect, a recent study conducted molecular dynamics simulations and demonstrated that p.(Arg163Trp) can cause decreased DNA binding affinity and differential transcriptional activation. The overlapping phenotypic features of our proband with all previously reported cases include generalized hypotonia with global developmental delay, mild facial dysmorphisms such as frontal bossing and low-set ears, speech delay, decreased pain response, hyperactive deep tendon reflexes, strabismus and bilateral esotropia. As it is the case for some of the reported cases, our proband had normal evaluations for brain magnetic resonance imaging (MRI), electroencephalography (EEG), chromosomal microarray, and comprehensive biochemical metabolic testing.

In conclusion, by employing our internal WES data analysis and variant prioritization pipeline on a previously unsolved exome, we identified a pathogenic de-novo missense variant in EBF3 gene. Targeted Sanger sequencing was used to confirm the variant in proband and to show the absence of the alteration in parental samples. Hence, the proband is diagnosed with HADDS. To sum up, here we highlight the potential of reanalysis of WES data for undiagnosed individuals.

Keywords: *EBF3*, Hypotonic Ataxic Developmental Delay Syndrome (HADDS), Reanalysis, Unsolved Exome, Whole Exome Sequencing (WES)