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# **Truncating biallelic variant in *DNAJ1* is associated with intellectual disability and seizures**

## **Running head: *DNAJ1* in intellectual disability and seizures**

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**Abstract:**

Intellectual disability poses a huge burden on the health care system, and it is one of the most common referral reasons to the genetic and child neurology clinic. ID is genetically heterogeneous, and it is associated with several other neurological conditions. Exome-sequencing is a robust genetic tool and has revolutionized the process of molecular diagnosis and novel gene discovery. Besides its diagnostic clinical value, novel gene discovery is prime in reverse genetics, when human mutations help to understand the function of a gene and may aid in better understanding of the human brain and nervous system. Using WES, we identified a biallelic truncating variant in *DNAJ1* gene in a multiplex Saudi consanguineous family. The main phenotype shared between the siblings was intellectual disability and seizure disorder.

## **Introduction:**

Intellectual disability (ID) is a neurodevelopmental disorder that is defined as an impairment in intellectual and adaptive functioning <sup>1</sup>. ID is a relatively common medical condition affecting at least 1% of the general population, and it can result from myriad of causes <sup>2</sup>. ID can be generally classified based on other associated systemic findings as syndromic and nonsyndromic. ID is genetically heterogeneous and the level of genetic involvement in ID has not thus far been elucidated; however, genetics is the most likely culprit in the majority of cases even when the child lacks syndromic or other systemic features <sup>2</sup>. Comparative genomic hybridization (CGH) is considered now the first-tier diagnostic test to order, nevertheless with the introduction of next-generation sequencing (NGS), many now are advocating exome sequencing (WES) as the first-tier test <sup>3</sup>. WES has shown to be cost-effective and led to a shorter diagnostic interval <sup>4</sup>. The diagnostic yield of NGS in CGH negative syndromic and nonsyndromic ID has been reported as 68 and 50%, respectively <sup>2</sup>. Several studies have demonstrated a high diagnostic yield in sequencing inbred populations that reached up to 50% <sup>2,5-7</sup>. More importantly, NGS has accelerated the process of novel gene discovery or candidate gene proposal, and it led to an unprecedented expansion in the number of genes linked to ID with an estimated more than 2,500 genes thus far <sup>8</sup>. Gene hunting in inbred populations is easier due to autozygosity mapping in multiplex families, which narrows the field of search and ending a journey of diagnostic odyssey. Reaching for an accurate diagnosis is particularly important for inbred populations where autosomal recessive founder mutations make a relatively large fraction of the molecular pool, and perhaps, a preventable cause of ID and other neurodevelopmental disorders <sup>9,10</sup>.

*DNAJ1* gene encodes a member of the DNAJ family of proteins, also known as heat shock protein 40 (Hsp40 or Hsc40), which acts as co-chaperone for Hsp70 and Hsp90. DNAJ proteins are responsible for several essential cellular processes including folding polypeptide chains, modulate protein assembly, disassembly, translocation through membranes into cellular compartments, assist in conformational maturation and maintenance and finally targeting of proteins for degradation through proteolytic processes of client proteins <sup>11</sup>. Herein, we show that a biallelic truncating variant in *DNAJ1* gene may cause ID and seizures in humans.

## **Human subjects and method**

### **Human Subjects:**

Three siblings from a Saudi consanguineous family presented to our practice. Their parents are a first-degree consanguineous couple with three affected and five healthy children. (Fig 1) All the affected siblings underwent a clinical evaluation by neurologists and clinical geneticists. Our institution's standardized clinical-exome consent was obtained from their parents, and the study received ethical approval from King Abdullah International Medical Research Center (KAIMRC). Additional informed consent was obtained from all individual participants for whom identifying information is included in this article.

### **Molecular analysis:**

#### **Autozygome analysis:**

Blood samples were collected from all affected individuals, the parents and two healthy siblings in EDTA tubes, and DNA was extracted from whole blood. We started with whole-genome array-based comparative genomic hybridization (aCGH) and genome-wide single nucleotide polymorphism (SNP) for the affected siblings and their parents as previously

described<sup>12</sup>. We did not detect any significant copy number changes; however, it showed large regions of homozygosity between the affected siblings and their parents. ( Table S1)

### **Exome sequencing:**

We then proceeded with WES in all affected patients and their parents as previously described<sup>13</sup>. The generated library is sequenced on an Illumina platform to obtain an average coverage depth of ~100x. Typically, ~97% of the targeted bases are covered >10x. An end to end in-house bioinformatics pipeline including base calling, alignment of reads to GRCh37/hg19 genome assembly, primary filtering out of low-quality reads and probable artifacts, and subsequent annotation of variants, were applied. Evaluation is focused on coding exons along with flanking +/-20 intronic bases. All inheritance patterns were considered. In addition, provided family history and clinical information were used to evaluate eventually identified variants.

### **Results:**

The three affected siblings share a strikingly similar phenotype and features, and they look completely different from their unaffected siblings. All of them are overweight, intellectually disabled with a seizure disorder, have moon facies, protruding ears, and short neck. Epilepsy gene panel was negative. (Table 1) (Fig 4) (See Table S2 for a detailed clinical synopsis)

Initial filtering for intellectual disability and seizure disorders variants revealed no mutations. All the affected siblings were homozygous for a truncating variant in *DNAJAI* gene (NM\_001539.3) c.511C>T p.(Gln171\*). We confirmed the variant by Sanger sequencing in all patients, and the variant segregated well in unaffected family members. (Fig 2). This variant has not been previously reported in a large-scale exome sequencing databases such as Exome

Aggregation Consortium (ExAC), dbSNP/1000 genome, or Exome Sequencing Project (ESP) and in the Genome Aggregation Database (gnomAD) databases. Also, this variant was absent from 2000 ethnically matched controls in local database and our institute's database Majeen (1200 exomes).

### **Structural and functional analysis**

DNAJA1 is composed of a N-terminal J-domain followed by a G/F-rich region and two zinc finger motifs, which connect to the peptide binding fragment located at the C-terminus. The 3D structure of this protein was modeled using I-TASSER, which selected as the best template the crystal structure of the type I Hsp40 Ydj1 from yeast with a sequence identity of 45% (Fig. 3) (I-TASSER template Z-score 5.58, model C-score -2.78)<sup>14</sup>. The premature stop codon at Gln171 omits 227 of the 397 protein residues. This truncation results in the loss of two of the CXXCXGXG zinc-binding motifs, most of the peptide-binding fragment, and the putative C-terminal dimerization domain (Fig 3). Only the J-domain and the G/F-rich regions are preserved, and hence the Gln171\* mutant would only preserve functions associated with these regions.

### **Discussion:**

DNAJ family is the largest HSP family in humans and is characterized by its highly conserved J-domain<sup>15</sup>. Hsp40 co-chaperones (also called DNAJ proteins), which are broadly expressed throughout the nervous system and within neuronal subcompartments, work in synchrony with Hsp70 and Hsp90 to help in ATP hydrolysis for protein folding<sup>16,17</sup>. Several chaperones and co-chaperones were linked to many human diseases, for instance, the dysfunction of the Hsp70 chaperone machinery has been shown to be associated with



Parkinson's disease<sup>18</sup>. Hsp70 is a housekeeping protein (proteostasis) that assists in the folding and function of the human proteome, and it is considered one of the most important chaperones as they play important role in synaptic plasticity through its interactions with Hsp40<sup>19,20</sup>. The nervous system in particular seems to be susceptible to chaperonopathy due to the sustained nature of neurotransmission. Thus, the synaptic proteome is perilously liable to misfolding and aggregation<sup>21</sup>. This occurs because of the relatively great distance between synapses and the neuronal cell body, where the majority of proteins are synthesized and folded<sup>22</sup>. Nevertheless, there are small subsets of proteins that are locally translated at postsynaptic spines and the presynaptic termini<sup>23,24</sup>. Synaptic function requires a large complement of specialized proteins, such as neurotransmitter receptors, synaptic vesicle, exocytosis and endocytosis and all of which are essential for neurotransmission<sup>21</sup>. Through dedicated synapse-specific proteostasis machinery localized to pre- and post-synaptic compartments, of which Hsp60, Hsp70 and Hsp90 are an integral component, neurons maintain their specialized structure and function<sup>21,25</sup>. The synaptic chaperone complement also includes other Hsp40/DNAJ client proteins such as DNAJC5 (CSPa), DNAJC6 (auxilin) and Receptor Mediated Endocytosis 8 (RME-8) which all participate in either synaptic exocytic and endocytic machinery and neuroprotection through regulating distinct steps of the synaptic vesicle cycle<sup>21,26</sup>. Recent studies link ID and seizures to some genetic defects that encode proteins involved in the aforementioned steps that may ultimately affect their roles in synaptic proteostasis and plasticity<sup>27,28</sup>.

The proposal for *DNAJ1* as the most likely culprit in this family comes from several lines of evidence. It falls within the area of homozygosity that is shared between the affected siblings, and it segregated well in the unaffected family members. The three affected siblings share a strikingly similar phenotype, and their phenotype differs completely from their unaffected siblings. We did not identify any variant in known ID and epilepsy genes, and the truncating

nature of this variant, which only preserves the J-domain and the G/F-rich regions, further supports this variant candidacy. The J-domain is able to interact with Hsp70 and stimulate ATP hydrolysis, possibly with the intervention of the G/F domain as observed in *E. coli* DnaJ<sup>29</sup>. Hence, the mutated DNAJA1 might conserve the ability to promote ATPase activity in Hsp70. However, it would no longer be able to interact with the hydrophobic side chains of nonnative polypeptides through the peptide-binding fragment and prevent their aggregation. Consequently, the Gln171\* mutant loses its capability of delivering misfolded polypeptides to Hsp70 to continue the folding process.

In summary, we identified *DNAJA1* as a strong candidate gene for intellectual disability and seizure disorder in a Saudi family. However, given the lack of functional studies or a second hit in another family, further research in this area is needed as the current literature is relatively deficient in defining the role of chaperones in human disease overall and in the CNS particularly.

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### **Contributorship Statement**

SA and FA created the presented idea and wrote the manuscript. SA, DB and FA contributed to the patient care and diagnosis. SA, AA, DB, and FA prepared the tables. SA, AA, STA, FJG and FA prepared the figures. SA, AA, STA, FJG, DB and FA edited the manuscript.

### **Conflict of interest**

The authors declare no conflict of interest.

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## FIGURE LEGENDS

Figure 1: Family pedigree of the current cohort.

Figure 2: Electropherograms of the cohort, their parents and two healthy family members.

Figure 3: Computational analysis of the 3D structure of DNAJA1. The structure has been inferred based on PDB id 1nlt. The region lost in the Gln171\* mutant is colored in grey and includes both zinc finger motifs (zinc is shown as grey sphere), most of the peptide-binding fragment and the putative C-terminal dimerization domain. The G/F rich region is colored in blue.

Figure 4: Clinical pictures of the three siblings showing moon facies, protruding ears, short neck, and concave nail beds.

## TABLES

Table 1: Current cohort phenotype.

Table S1: Regions of homozygosity in the current cohort.

Table S2: Detailed clinical synopsis