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Whole Exome Sequencing in Intellectual Disability Patients Identifies *de novo* Mutations in *KCNB1*, *SHANK2*, and *SYNGAP1* Genes and a Novel Mutation in *PPP1R3F*

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Abstract

Intellectual disability etiology still poses a challenge to clinicians and families. Here we aimed to dissect the genes causing intellectual disability in local families from Jordan. We recruited nine trio families with unexplained intellectual disability, and utilized whole exome sequencing to identify causative genes/mutations. Out of nine families, we identified the candidate causative genes in four (44% detection rate). Novel and known mutations were identified in *KCNB1*, *PPP1R3F*, *SYNGAP1*, and *SHANK2*. Mutations in *KCNB1*, *SYNGAP1*, and *SHANK2*were *de novo*, while *PPP1R3F* mutation was X-linked inherited from the mother. With a highly inbred population, it was unexpected to find the majority of our mutations to be *de novo* representing autosomal dominant inheritance as the major pattern for our sample of unexplained intellectual disability. Our data confirm previous data that *de novo* mutations in autosomal dominantly expressed genes represent the major cause of unexplained intellectual disability, even in highly inbred populations that usually shows enrichment of mutations in genes with autosomal recessive mode of inheritance.

Key words: Exome, Intellectual disability, *de novo*, *KCNB1*, *PPP1R3F*, *SHANK2*, *SYNGAP1*

1. Introduction

Intellectual disability (ID) is a complex neurodevelopmental disorder with a range of intellectual delay. It is characterized by intelligence quotient of less than 70 and deficits in adaptive behavior [Burack*et al.*, 2021]. Prevalence of ID is estimated to be (0.5-2%) with the majority of cases happening in children less than 15 years old (McBride *et al.*, 2021). According to recent estimates, more than 1,000 genes are implicated in causing ID in humans (Leblond*et al.*, 2021), with an expectation of more than 1000 yet to be discovered (Kaplanis*et al.*, 2020).

Studies in populations with common consanguineous marriages resulted in the discovery of mainly autosomal recessive ID genes (Rasheed *et al.*, 2021) whereas finding such genes is rare in outbred populations (Martin *et al.*, 2018). On the other hand, *de novo* variants were commonly found in studies from mixed and founder populations(Hamdan*et al.*, 2014; Jarvela*et al.*, 2021). Recent reports show that up to 48% of patients with ID and developmental disorders harbor pathogenic *de novo* mutations in protein-coding genes (Deciphering Developmental Disorders Study 2017; Kaplanis*et al.*, 2020).

Given the scarce studies done on the middle eastern and specifically the Jordanian population, we aimed to decipher the potential genetic etiologies in 9 families having at least one affected child with nonspecific ID through trio-whole-exome sequencing (trio-WES) and bioinformatic analysis. Even though the Jordanian community is largely inbred with a continuing prevalent consanguinity, most mutations found were *de novo* demonstrating the importance of such mutations even in highly inbred populations.

2. Methods

2.1. Participants

Patients with established perinatal diseases or chromosomal aneuploidies were excluded. We recruited 9 families with at least one child manifesting nonspecificID as the major phenotype. The study is approved by the ethics committee at Jordan University of Science and Technology (#24/123/2019) and conforms to the declaration of Helsinki. Goals of the study were explained to parents, and written informed consent was obtained before their inclusion in the study. A neuropediatrician examined all patients, and family histories and medical reports were obtained.

2.2. Trio-WES and bioinformatics analysis

Peripheral blood was collected from available family members, followed by DNA extraction using manufactures protocol (QIAamp DNA Blood mini kit, Qiagen, Hilden, Germany). Trio-WES was performed in Centogene laboratory (Rostock, Germany). One microgram of

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fragmented genomic DNA was used to enrich 60 MB of human exons using SureSelect Human All Exon V6 kit (Agilent, Santa Clara, California, USA) targeting >99% of regions in RefSeq, GENCODE, and the consensus coding sequence project (CCDS) databases. Exons were processed on NextSeq platform (Illumina, San Diego, CA, USA), and an average coverage of ~100X was obtained. More than 10X coverage was obtained for approximately 97% of targeted bases. All variants reported in this report had >45X depth of coverage. In-house bioinformatics pipeline was used to call base pairs, align reads to genome assembly GRCh37/hg19, filter out poor quality, and annotate variants. We considered all disease-causing variants in ClinVar (Landrum *et al.*, 2016), HMGD (Stenson *et al.*, 2020), and CentroMD (Trujillano*et al.*, 2016) databases and all variants in gnomAD(Karczewski*et al.*, 2020) (http://gnomad.broadinstitute.org/)database with an allele frequency of less than 0.01%. Identified variants in coding exons and their flanking intronic sequences were investigated taking into account multiple inheritance patterns (de novo autosomal dominant, autosomal recessive, and X-linked). Family history and clinical data were considered in evaluating identified variants. Candidate disease variants were confirmed by Sanger sequencing for probands.Deleterious variants were predicted by multiple commonly used algorithms, such as MutationTaster (Schwarz *et al.*, 2014), PolyPhen-2 (Adzhubei*et al.*, 2010), and SIFT (Sim *et al.*, 2012). The pathogenicity of variants was evaluated according to the American College of Medical Genetics guidelines (Richards*et al.*,2015).

3. Results

We recruited 9 families with ID and performed trio-WES for probands and their parents. All patients had normal brain MRI. The sequencing data were filtered by focusing on very rare variants (MAF<0.01%) and giving priority for those with potential effect on protein structure and function such as nonsense, splicing, and non-conservative missense mutations. Variants with low depth of coverage were excluded (Depth of coverage less than 20 readings). Only one of the nine families was consanguineous (family 799), in another family (family 751) the parents were distant relatives. Variants were analyzed in all modes of inheritance including sex-linked mode.

We identified 3*de novo*mutations in *KCNB1*, *SHANK2*, and *SYNGAP1*genes and a novel mutation in a boy inherited from the mother in X-linked *PPP1R3F* gene, all of which are already in ID etiology. All mutations had zero allele frequency in The Genome Aggregation Database (gnomAD) (Karczewski*et al.*, 2020) until the writing of the manuscript. Albeit, two of the mutations were reported before c.916C>T, p.Arg306Cys, in *KCNB1* (Saitsu*et al.*, 2015) and c.1735C>T, p.Arg579*, in *SYNGAP1* (Hamdan*et al.*, 2009). The other 2mutations are novelc.446C>G, p.Pro149Arg, in *PPP1R3F* and c.757C>T, p.Arg253*, in *SHANK2* (Table 1).

Table 1. List of identified mutations.

Family	Gene	Transcript	Variant	Previously published	Genotype	Inheritance	OMIM phenotype	OMIM#
750	*KCNB1*	NM_004975.2	c.916C>T, p.Arg306Cys	Saitsu*et al.*,, 2015	Heterozygous	AD/*de novo*	Developmental and epileptic encephalopathy 26	616056
751	*SYNGAP1*	NM_006772.2	c.1735C>T, p.Arg579*	Hamdan*et al.*,, 2009	Heterozygous	AD/*de novo*	Mental retardation, autosomal dominant 5	612621
766	*PPP1R3F*	NM_033215.4	c.446C>G, p.Pro149Arg	-	Hemizygous	X linked/ inherited from mother	-	-
804	*SHANK2*	NM_133266.4	c.757C>T, p.Arg253*	Berkel*et al.*,, 2010	Heterozygous	AD/ *de novo*	Autism susceptibility 17	613436

All mutations are *de novo* with zero allele frequency in gnomAD v2.1.1 database (https://gnomad.broadinstitute.org/) (Karczewski*et al.*,, 2020). AD, autosomal dominant inheritance.

Mutations in *KCNB1* gene cause Developmental and epileptic encephalopathy 26 (OMIM# 616056) (Torkamani*et al.*, 2014), and mutations in *SYNGAP1* cause Autosomal dominant mental retardation 5 (OMIM# 612621) (Hamdan*et al.*, 2009). *PPP1R3F*variant changes proline to arginine, which is predicted to be damaging/deleterious by MutTaster, Polyphen, and SIFT prediction tools (Table 2). *PPP1R3F* mutations are reported in patients with autism spectrum disorder (Piton *et al.*, 2011; DoostparastTorshizi*et al.*, 2018).*SHANK2* variant creates a termination codon at position 253 that is expected to produce a nonfunctional gene product. *SHANK2* mutation has been reported to cause autism spectrum disorder and mental retardation (Berkel*et al.*, 2010).

Table 2. Prediction software results and read depth for the identified mutations.

Gene	Variant	Chromosomal coordinates	MutationTaster	Polyphen-2	SIFT	Read depth
KCNB1	c.916C>T, p.Arg306Cys	chr20:47991181	Disease causing	Probably damaging	Deleterious	183
SYNGAP1	c.1735C>T, p.Arg579*	chr6:33408564	-	-	-	158
PPP1R3F	c.446C>G, p.Pro149Arg	chrX:49126778	Disease causing	Probably damaging	Deleterious	46
SHANK2	c.757C>T, p.Arg253*	Chr11:70336411	-	-	-	239

4. Discussion

In this study, we recruited 9 families affected with ID from Jordan. After doing trio whole exome sequencing for patients and their parents, we found 4 mutations in 4 genes associated with ID. Two of these mutations were reported before while the other two are novel. All 4 mutations are extremely rare, and none of them is listed in the gnomad database (Karczewski*et al.,* 2020).Molecular diagnostic yield attained in this study (44%) was similar to other recent studies utilizing trio WES to ascertain the genetic etiology of ID (Pode-Shakked*et al.,* 2021; Sheth*et al.,* 2021; Xiang *et al.,* 2021).

*PPP1R3F*is an X-linked gene, whose mutations have been associated with autism spectrum disorder. Mutations include c.733T>C (p.Phe245Leu) which was found in a child diagnosed with seizures and Asperger Syndrome. This mutation was inherited from the mother who suffered from learning disabilities and seizures herself (Piton *et al.,* 2011). Another mutation, c.2161A>G (p. Arg375Gly), was found in childhood-onset schizophrenia (Ambalavanan*et al.,* 2019). *PPP1R3F* is predominantly expressed in various brain regions such as cerebellum and frontal cortex and is a master regulator of 177 genes, 89 of which are highly expressed in various brain regions compared to other tissues (DoostparastTorshizi*et al.,* 2018). In this study, we found a novel mutation in a boy affected with ID. This mutation was classified as damaging/deleterious by several prediction tools. It changes a proline to Arginine c.446C>G, p.Pro149Arg. Proline is the only amino acid creating a ring with the polypeptide backbone, and it has very firm structure which curves the core chain of the protein in a distinctive way (Khan and Vihinen 2007). No other candidate disease-causing mutations were found in this patient.

Mutations in *SHANK2* are present in 0.17% of patients with ASD and mild ID (Leblond*et al.,* 2014). Many truncating mutations were reported in patients such as c.1384C>T (p.Arg462*) (Berkel*et al.,* 2010), c.1896dupA (p.Asp633Argfs) (Bowling *et al.,* 2017), and multiple-exon deletions (Leblond*et al.,* 2014). Additionally, many single point mutations were found in ASD, ID and Schizophrenia patients (reviewed in Eltokhi*et al.,* 2018). We found a *de novo* truncating mutation early in the gene that is expected to have a detrimental effect on gene product. It is novel and not reported before. No other candidate mutations were found in this patient. All this supports the candidacy of this mutation in causing the phenotype.

In conclusion, we have studied 9 families by trio-WES. The families came from a highly inbred population. We suspected having homozygous mutations prevalent in our sample. However, we found 4 mutations in 4 families (44% detection rate), and 3 of these mutations were *de novo* and all extremely rare. This supports previous reports (Al-Mubarak *et al.,* 2017; Järveläet *al.,* 2021; Wang *et al.,* 2019) pointing to the private and heterogeneous nature of the genetic architecture of ASD and ID even in highly inbred populations. Functional analysis is needed to confirm the role of the mutations found in causing disease. Whole genome sequencing and robust bioinformatic analysis might be needed to solve the genetic cause of disease in the other unresolved families.

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Details of the contribution of individual authors

AA designed the study, analyzed data, and wrote manuscript; MA recruited patients, made full clinical assessment; HH helped in collecting samples and helped carry out experimental work. All authors reviewed and corrected the manuscript.

Compliance with ethical standards

Informed consent

Informed consent is obtained from guardians of all patients. Institutional review board (IRB) approval is obtained from the Jordan University of Science and Technology. All procedures followed are in accordance with the IRB approval and with the Helsinki Declaration of 1975, as revised in 2000. Consent to publish is obtained, and the manuscript does not contain any individual person's data in any form.

Conflict of interest

Authors declare no conflict of interest.

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Availability of data

Detailed whole exome data in the manuscript are available upon request.

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