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Research Article

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Molecular Characterization of Galatosemia in Iran: Identification of Eleven Mutations

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Abstract

Introduction: A global prevalence of Autistic Spectrum Disorder (ASD) was estimated on review of epidemiological studies and has been found to be increasing. Advancements in genetic analysis have provided the ability to identify potential genetic changes that may contribute to ASD. TBC1D8 (TBC1 Domain Family Member 8) is a Protein Coding gene. Among its related pathways is cell cycle. The number of genes associated with autism is increasing. Whole exome sequencing (WES) identified the homozygous TBC1D8 variant.

Aim: To report for the first time a TBC1D8 missense variant (c. 1883G>A, p. (Arg628Gln) in 4 Libyan children (3 homozygous, 1 heterozygous)with severe neurodevelopmental phenotypes (ASD) and intellectual disability (ID). Based on the data of HGMD and ClinVar, variants in only a few autosomal recessive intellectual disability (ARID) genes seem to be reported frequently. None of the large ARID studies that were performed on over 100 families showed any particularly prevalent gene in ARID.

Method: Molecular genetic analysis of (WES) was carried out on blood samples from these children. The results were interpreted in the context of clinical finding, family history, and suspected mode of inheritance.

Results: The number of genes associated with autism is increasing. WES identified the TBC1D8 variant. According to the longest isoform (NM_001102426.1), the nomenclature of this variant is c.1883G>A, p. (Arg628Gln) in TBC1D8 which leads to an amino acid exchange. This variant has not previously reported or described in the literature (PubMed, HGMD).

Conclusion: We have provided evidence for a connection between TBC1D8 variant and ASD and ID; however, this evidence should be considered preliminary in the context of a single case report and such findings need to be replicated to gain insightin order to determine if ASD and ID are a characteristic of this variant.

Introduction

TBC1D8 (TBC1 Domain Family Member 8) is a Protein Coding gene. Cell cycle is one of the gene associated pathways. Annotations linked to gene ontology (GO) include calcium ion binding and activation of GTPase. An important paralog of this gene is TBC1D8B (HGNC: 17791) (GCID: GC02M101007).The chromosome location is 2q11.2. Only few of the novel ID genes seem to relate to molecular pathways and networks that have been previously implicated in dominant and X-linked forms of ID, such as pre- and post-synaptic signaling, transcription regulation and epigenetic mechanisms [1-3]. An exception is UBE2J2 encoding an ubiquitin-conjugating enzyme, which represents an emergent mechanism for regulating synapse function by post-translational modification through the ubiquitin pathway at the postsynaptic membrane [4]. Proteolysis by the ubiquitin proteasome pathway is recognized as a major molecular pathway leading to several neurodevelopmental and neurodegenerative diseases [5]. Previously identified gene networks that are commonly disrupted in ID and other neurodevelopmental disorders are composed of genes that are highly dosage-sensitive. Thus, these pathways might represent other neurobiological processes than those that are affected by recessive mutations [6]. In this article we report for the first time a homozygous TBC1D8 missense variant (c. 1883G>A, p. (Arg628Gln) in 3 Libyan children with severe neurodevelopmental phenotypes (ASD) and (ID).Surprisingly the fourth sibling carries the TBC1D8 variant in heterozygous state.

Intellectual disability is currently defined by the American Association on Intellectual and Developmental Disabilitiesas " a disability characterized by significant limitations both in intellectual functioning and in adaptive behavior as expressed in conceptual, social, and practical adaptive skills. This disability originates before age 18 " [7]. Additionally, newly published DSM-5 uses the term " intellectual developmental disorder " as the equivalent term for “intellectual disability” because upcoming ICD-11 will likely use the term “intellectual developmental disorder” to indicate involvement of impaired brain functioning early in life [8].Autistic spectrum disorder comprises a group of neurobiological disorders that are characterized by deficits in social interaction and communication and by abnormalities in behaviors, interests, and activities [8]. ASD has a complex genetic etiology, and monogenic disorders associated with the high penetrance of ASD are observed in less than 20% of ASD cases. Well-known single gene disorders associated with ASD are Fragile X syndrome, Rett syndrome,MECP2 duplication, Angelman syndrome, and Tuberous sclerosis [9].New USA government survey (2014) of parents suggests that 1 in 45 children aged 3 through 17 had been diagnosed with ASD. This is notably higher than the official government estimate of 1 in 68 American children with autism, as stated by National Center for Health Statistics [10].

In Libya, one prospective hospital-based study carried out on all children referred to a neurodevelopment clinic at Al-Khadra Teaching Hospital, Tripoli between 2005 and 2009 for ASD assessment showeda prevalence of one in 300 [11].Again in the 2011 study 200 children whose ages "ranged from younger than 3 years to over 12 years" were referred for ASD assessment because of their behavioral difficulties and

Keywords: TBC1D8; Gene; Homozygous variant

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speech and language disorders. ASD were diagnosed in 83% of cases (166 children) which gives a prevalence of 10: 1000 [12,13].The prevalence of the problem in Libya is probably higher or similar to that seen in the USA and UK.

Patients and Methods

These four Libyan children were born to non-consanguineous parents at term after uneventful pregnancy. Parents provided informed signed consent for genetic studies on blood samples from their children. The results were interpreted in the context of clinical finding, family history, and suspected mode of inheritance.

Children

Child 1 (girl) was the first sib. She is now 12 years old. Delayed developmental milestones including speech and language were noted since early infancy. At the age of 1 year: she was noted to have poor eye to eye contact; and the diagnosis of (ASD) and (ID) were made at 2 years of age. Child 2 (girl) is the second sib who is now 10 years old. Developmental delays were noted since early infancy. At the age of 20 months, her eye-to-eye contact was still absent. No seizures were reported. Speech was absent; and the diagnosis of (ASD) and (ID) were made at 4 years of age. Child 3 (boy) is the third sib who is now 9 years old. Developmental delays were also noted since early infancy. At the age of 18 months, his eye contact was absent. No seizures were reported. Speech was absent; and the diagnosis of (ASD) and (ID) were made at 5 years of age. Child 4 (girl) is the fourth sib who is now 7 years old. Developmental delays were noted since early infancy. At the age of 24 months, she was noted to have inconsistent imitation skills. Eye contact was absent. No seizures were reported. Speech was absent; and the diagnosis of (ASD) and (ID) were made at 5 years of age.

Genetic studies

Molecular genetic analysis of whole exome sequencing (WES) was carried out at Bioscientia Institute for Medical Diagnostics Gmbh, Center for Human Genetics, Ingelheim, Germany. More than 20000 genes of the patients DNA were enriched and sequenced .Filtering of the exome data targeted recessive, X-linked and dominantly inherited disease. Genomic DNA was fragmented and the exons of the known gene in the human genome, as well as corresponding exon-intron boundaries were enriched using Roche NimbleGen capture technology (SeqCapMedExome Library)amplified and sequenced simultaneously by illumina technology (next-generation sequencing NGS) using an illumina system. The target regions were sequenced with average coverage of 164 fold. For about 97% of the regions of interest a 15-fold coverage, for about 95% a 20-fold coverage was obtained. NGS data were aligned to the hg19 genome assembly. Variant calling and annotation was performed by an in-house developed bioinformatics pipeline identified single-nucleotide variants (SNVs) and indels were filtered against external and internal database focusing on rare variants with minor allele frequency (MAF) in gnomAD of 1% or less and removing known artifact and variants in regions with highly homologous regions. Classification of variants was conducted based on ACMG guidelines considering database entries (incl. HGMD), bioinformatics prediction tools and literature status [14].A change of pathogenicity classifications over time cannot be excluded. Variants annotated as common polymorphisms in databases or literature or that were classified as (likely) benign were neglected. Putatively pathogenic differences between the wild type sequence (human reference genome according to (UCSC Genome Browser: hg19,GRCh37) and the patients sequence mentioned and interpreted in this report were assessed using an in-house established quality score. Variants not passing the quality threshold were verified using polymerase chain reaction (PCR) amplification followed by conventional Sanger sequencing. Sample identity was ensured by internal quality management procedures. The laboratory Quality management is also recognized and accredited by Central Office of the Federal States for Health Protection for Drugs and Medical Devices (ZLG), German accreditation body (DAkkS) and College of American Pathologists (CAP).The recorded data in paper and/or in electronic form are stored in accordance with the legal requirements and their use and/or publication should be in pseudonymised form for scientific purposes. Confidentiality is maintained. Any sample material remaining at the end of the analysis is transferred, in accordance with §950 BGBI (Germany' s official gazette of federal law).

Diagnosis and Management

ASD and ID were determined as follows: First, according to history, parent interview and neurodevelopmental symptoms, including cognition, motor performance, hearing, speech and vision; second, through examination of IQ (intelligence quotient), defined by a total human intelligence score derived from standardized tests (below 70); third, according to limitations in environment adaptation, including self-care and skills of communication [15,16]. Neurodevelopmental assessment with the help of The Vineland Adaptive Behavior Scale (VABS),DSM5,Modified check list of autism in children(M-CHAT)and Conner's Autism Rating Scale(CARS) revealed the children were suffering from autism along with severe ID with IQ score was about 25 to 30 [17-19].

Auditory Brainstem Response (ABR) showed normal hearing. EEG revealed no abnormal epileptic activities; and MRI brain showed normal brain structure with no evidence of encephalopathy, degenerative changes or atrophy. Their complete blood pictures, urea and electrolytes, liver and thyroid function tests and creatinine kinase were normal. The metabolic screen including serum bicarbonate, arterial blood gases, vitamin D and B12 were normal. Molecular genetic analysis of whole exome sequencing (WES) identified the homozygous missense variant in TBC1D8 gene in all the children (Table 1).

Gene (Isoform)	**OMIM-P (Mode of inheritance)**	**Variant**	**Zygosity**	**MAF gnomAD [%]**	**Literature [PIMD]**
TBC1D8	--	c.1883G>A, p.(Arg628Gin) chr2:101648738	Hom.	0,0014	--

(NM_001102426.1)					

Table 1: Molecular genetic analysis of whole exome sequencing (WES).

Results

The number of genes associated with autism is increasing [20]. WES identified the homozygous TBC1D8 variant. The following is NCBI Reference Sequence:

LOCUSNM_001102426 (4226bp - mRNA - linear - PRI 07-MAY-2020)

DEFINITION:Homo sapiens TBC1 domain family member 8 (TBC1D8), transcript variant 2, mRNA.

ACCESSION:NM_001102426

VERSION:NM_001102426.2

KEYWORDS:RefSeq.

Source

Homo sapiens (human)

The nomenclature of this variant is c.1883G>A, p. (Arg628Gln) in TBC1D8 which leads to an amino acid exchange. 8 out 10 bioinformatic in silico programs predict a pathogenic effect for this variant. In silico analysis of the variant showed that 17 out of 30 tools predicted a possible pathogenicity [Prediction (17/30)=Functional (11/22)+Conservation (6/8)].

Although many deleteriousness prediction methods have been developed, their prediction results are sometimes inconsistent with each other. The predictive performance of 18 current deleteriousness-scoring methods were comprehensively evaluated, including 11 function prediction scores (PolyPhen-2, SIFT, MutationTaster, Mutation Assessor, FATHMM, LRT, PANTHER, PhD-SNP, SNAP, SNPs&GO and MutPred), 3 conservation scores (GERP++, SiPhy and PhyloP) and 4 ensemble scores (CADD, PON-P, KGGSeq and CONDEL). It has been found that FATHMM and KGGSeq had the highest discriminative power among independent scores and ensemble scores, respectively [21].

This variant has not previously reported or described in the literature (PubMed, HGMD) so far. The variant is found in 0.0014% of the overall population (4 heterozygous, 0 homozygous; X hemizygous; GnomAD). (Riazuddin and his colleagues reported a c.1501C>T, p. (Leu501Phe) variant of TBC1D8 in a Punjabi consanguineous family (PKMR66) among the other 30 novel candidate genes for autosomal recessive intellectual disability with neither epilepsy nor autistic spectrum disorder (Table 2) [22].

Family	Ethnicity	Gene	Position	Transcript	cDNA mutation	Protein change	variation	Protein function
PKMR66	Punjabi	TBC1D8	Chr2: 101652537G>A	NM_001102426.1	c.1501C>T	p.(Leu501Phe)	Missense	G-protein modulator 31.0 99.37

Table 2: Homozygous DNA variants in a single gene in a Punjabi ID consanguineous family

Discussion

A global prevalence of (ASD) was estimated on review of epidemiological studies and has been found to be increasing [23]. Advancements in genetic analysis have provided the ability to identify potential genetic changes that may contribute to ASD. Interestingly, based on the data of HGMD and ClinVar, variants in only a few autosomal recessive intellectual disability (ARID) genes seem to be reported frequently. None of the large ARID studies that were performed on over 100 families showed any particularly prevalent gene in ARID. It is also important to note that a diagnosis from WES should not end with laboratory classification of a variant. For instance, the classification of a variant of unknown significance (VUS) from a laboratory should be further assessed in the clinical setting. With increased knowledge over time of phenotypes related to different genetic disorders, exome reanalysis may change the clinical interpretation of a VUS [24]. Homozygous mouse models (Tbc1d8 em1 (IMPC) J/Tbc1d8 em1 (IMPC) J) showed abnormal behavior, cataract and male infertility (MGI Reference; 1927225 ID=J: 188991) [25,26].

Numerous existing techniques for pathway examination rely upon prevailing databases. The information utilized, isn't in every case totally clarified. Many genes interactions in databases are somewhat theoretical as they depend on scientific facts and are pulled from a particular cell type or illness. Additionally most authoritative pathways are manufactured utilizing the information got from an inadequate number of analyses with limited cell models [27].

This series of four Libyan siblings with ASD and ID sum-ups novel presentations of a variant in protein coding genes TBC1D8 (c. 1883G>A, p. (Arg628Gln). The four siblings had what seemed to be similar presentation of ASD at initial evaluation and have proceeded through the similar clinical course, progressed in the same way but unfortunately they have been making slow and disappointing progress despite very good educational input. Generally speaking, these four cases provide novel presentations of ASD and ID and highlight the importance of WES investigations.

To our knowledge, the variant has not been described in the literatures so far. The variant is found in 0.0014% of the overall population (4 heterozygous, 0 homozygous; X hemizygous, gnomAD).Taken together, it is clear that the detected homozygous TBC1D8 variant may contribute to the phenotype of these siblings.

Conclusion

In conclusion, in this article, we have provided evidence for a connection between TBC1D8 variant and ASD and ID; however, this evidence should be considered preliminary in the context of a single case report and such findings need to be replicated in order to determine if ASD and ID are a characteristic of this variant; Indeed, we report these findings to encourage other clinicians and professionals to look for such variants when dealing with children who suffers from ASD and ID. The knowledge about the causes of human genetic disorders constantly improves due to the continuous identification of novel disease genes.

Compliance with Ethical Standards

Funding source

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Financial disclosure

The authors have no financial relationships relevant to this article to disclose.

Conflict of Interest

We have no conflicts of interest to disclose.

All procedures performed were in accordance with the ethical standards of the hospital.

Informed consent was obtained from all individual participants included in this case report.

Author contributions statement

AMZ made a substantial contributed to the conception and design of the work.

AMZ drafted the work or revised the article critically for important intellectual content.

AMZ provided approval for publication of the content.

SA supervised the collection and labeling of blood samples.

SA word processed the daft and the main article.

AMZ and SA Agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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