A novel GTPBP2 splicing mutation in two siblings affected with microcephaly, generalized muscular atrophy, and hypotrichosis

Isa Abdi Rad1,2 | Ali Vahabi2 | Elinaz Akbariazar2

1Cellular and Molecular Research Center, Urmia University of Medical Sciences, Urmia, Iran
2Department of Medical Genetics, Urmia University of Medical Sciences, Urmia, Iran

Correspondence
Elinaz Akbariazar, Department of Medical Genetics, Urmia University of Medical Sciences, Urmia, Iran.
Email: elinaz.akbari@yahoo.com

Abstract
A novel splice site mutation in the GTPBP2 gene was identified by whole-exome sequencing in two siblings with microcephaly and progressive generalized muscular atrophy associated with hypotrichosis.

KEYWORDS
GTPBP2 gene, hypotrichosis, microcephaly, muscular dystrophy

1 | INTRODUCTION

Microcephaly is a clinical diagnosis defined as a head circumference of less than two standard deviations from the mean for age and gender of the same population, although mostly head circumference of three standard deviations below the mean is considered as a clear-cut definition of microcephaly.1,2

Microcephaly is classified as nonsyndromic, where the underlying pathology affects exclusively the brain development, or as syndromic microcephaly in which other tissues or organs in the body affected in addition to the brain. However, determinations of syndromic or nonsyndromic forms do not imply distinct etiologies, and many environmental and genetic factors can play role in the pathogenesis of microcephaly.

GTPBP2 is a member of the GP-1 family (GTPase superfamily), which encodes a putative GTP-binding protein, with 44.2% amino acid sequence similarity to GTPBP1. Other members of this unique group are GTPBP1, AGP-1, and CGP-1. Human GTPBP2 gene is located on chromosome 6, locus 6p21.1 close to the vascular endothelial growth factor gene (VEGF) locus. GTPBP2 is highly conserved (99%) between human and mouse. Members of GP-1 family characterized by conserved GTP-binding motifs and involved in various conserved functions, including control of cell proliferation and differentiation, intracellular transportation, regulation of cytoskeleton, and protein synthesis.3

Immunohistochemical experiments showed that both GTPBP1 and GTPBP2 are expressed in the neurons of brain, smooth muscles, and in activated macrophages.3

It was shown that a homozygous splice donor site mutation in the intron 6 of Gtpbp2 (mice) resulted in mis-spliced mRNAs with premature stop codons and caused neurodegeneration, truncal ataxia, apoptosis of brain neurons, and degeneration of retina neurons.4 Also, GTPBP2 found as a binding partner of the ribosome recycling protein Pelota and loss of this protein in mice with a mutation in a CNS-specific tRNA gene causes ribosome stalling and widespread neurodegeneration.4

A splice site mutation in GTPBP2 was empirically shown to cause deletion of exon 9 of the gene with a truncated protein-lacking conserved C-terminus domains in a patient with neurodegenerative disorder and iron deposition in the brain.5

2 | CASE HISTORY/EXAMINATION

A family with two siblings affected with a microcephaly and generalized muscular atrophy was referred to Medical
Genetic Department, Urmia Medical University, for genetic analysis. Parents were consanguineous with F = 1/16 (first cousin) (Figure 1).

Similar clinical conditions inferred from the past medical history of siblings. There was no acquisition of psychomotor milestones, that is, no sitting, no speech, and no reaction to environment.

Physical examination carried out first on the proband (IV2) and then on his older affected sister (IV1), and both of them have similar clinical features including microcephaly (IV1: −8.9 SD and IV2: −7.5 SD), hypotrichosis of the scalp (present at birth and more prominent with time), prominent low-set ears, progressive generalized muscular atrophy, an extraflexion crease of thumb bilateral in IV1 and left unilateral in IV2, spastic limbs with pes cavus, and contractures of upper and lower limbs associated with spasticity, while DTRs were unobtainable (Figure 2).

After obtaining written informed consent, clinical exome sequencing was performed on the proband (IV:2). Exome sequencing data were mapped to the human reference (NCBI build 37.1, UCSC hg19) using Burrows-Wheeler aligner (BWA-MEM, version 0.7.10). All variants were annotated by ANNOVAR and Variant Effect Predictor (VEP). Variants with a read depth >20 and MAF <0.01 in the 1000 Genome Project, dbSNP, Exome Aggregation Consortium (ExAC), and ESP-6500 were extracted for further analysis. Finally, the candidate variants were checked in the Human Gene Mutation Database (HGMD), Varsome, and Clinvar.

After preliminary detection of mutation by clinical exome sequencing on the proband, Sanger sequencing carried out to validate the detected mutation and verify cosegregation in the studied family (III:1, III:2, IV:1).

In addition, identified mutation was searched in healthy controls database for the same ethnic group (Iranome; http://www.iranoome.ir/).

3 | RESULTS

A homozygous acceptor splice site mutation, that is, c.399-2A > G, was found in the GTPBP2 gene in the proband case. Direct sequencing of the region harboring the c.399-2A > G variant in all family members revealed complete segregation with the disease in this family (Figure 3). The detected variant is predicted to disrupt the highly conserved acceptor splice site of exon 4.

Although this variant considered a pathogenic variant in Varsome database, according to the American College of Medical Genetics (ACMG) guidelines this variant classified as likely pathogenic (class 2) as described below.

In summary, GTPBP2: c.399-2A > G variant met the PVS1 and PM2 criteria:

PVS1 (very strong): null variant (nonsense, frameshift, canonical ± 1 or 2 splice sites, initiation codon, single-or multiexon deletion) in a gene where LOF is a known mechanism of disease.
PM2 (Moderate): absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium.

In summary, the ACMG guideline states that one very strong (PVS1) plus one moderate (PM1-PM6) item denotes to a likely pathogenic variant.

4 | DISCUSSION

We identified a homozygous acceptor splice site variant (c.399-2A > G) in GTPBP2 gene as possible cause of disease in patients with the clinical manifestations of the microcephaly, hypotrichosis, progressive generalized muscular atrophy, and spastic limbs by whole-exome sequencing (WES). The detected variant is predicted to disrupt the highly conserved
acceptor splice site of exon 4. The mis-spliced GTPBP2 mRNA is expected to cause deletion of 37 amino acids encoded by exon 4 at the N-terminus.

GTPBP2 gene was first identified in mice brain cDNA library and human T-cell line cDNA library. Mouse GTPBP2, encoding a protein consisting of 582 amino acids and carrying GTP-binding motifs. The amino acid sequence of mouse GTPBP2 revealed 44.2% similarity to mouse GTPBP1. GTPBP2 protein was found highly conserved between human and mouse (over 99% identical), thereby suggesting a fundamental role of this molecule across species. Human GTPBP2 locus codes for two forms of GTPBP2 protein: GTPBP2A and GTPBP2B, because of alternative splicing of GTPBP2 transcript, so they have the same amino acid sequence except GTPBP2B is missing 74 amino acids at the N-terminus. GTPBP2A and GTPBP2B have an N-terminal GTPase domain, followed by two conserved C-terminal domains that are conserved between GTPBP1 and GTPBP2 and they are related to elongation factors (eEF1A, eRF3).

In the Xenopus embryos, GTPBP2 is maternally expressed and localized to the egg pole. During cleavage and early gastrulation stages, GTPBP2 transcripts separated to the nascent ectodermal and mesodermal cells and expressed in the brain, eyes, somites, ventral blood island, and branchial arches. Also, GTPBP2 is a nuclear protein and binding partner of Smad1 (principal transcription factor downstream of BMP ligands). C-terminal effector region of GTPBP2 interacts with MH1 domain of Smad1 and colocalizes with Smad1 to nuclear foci and interacts with several transcription factors and affects BMP canonical signaling. Morpholino knockdown of GTPBP2 showed reduction in animal cap responses to BMP4. BMP/Smad1 signaling is required for neural differentiation program in the nascent ectoderm and acts together with, but upstream of Wnt signals for patterning of the ventral-posterior mesendoderm.

GTPBP2 is a positive regulator of canonical Wnt/β-catenin signaling. Wnt/β-catenin signaling is required for polarity of embryos and maintenance of normal tissue homeostasis in Xenopus embryos, and dysregulation of Wnt/β-catenin signaling causes diseases and birth defects. Reported documents showed that knockdown of GTPBP2 disrupts activation of Wnt signaling and results in axial patterning defects and reduces induction of organizer genes in Xenopus embryos.

GTPBP2 knockdown increases Axin protein levels, and it points an inhibitory role for Gtpbp2 on the activity of the β-catenin destruction complex. Axin is a rate-limiting component of Wnt signaling and required for efficient targeting of β-catenin by Gsk3β.

A homozygous splice site mutation, nmf205, in GTPBP2 gene caused neurodegeneration accompanied by truncal ataxia symptoms in mice. Also, a splice site variant, c.1237-1G > T, in the GTPBP2 gene is associated with almost similar phenotype in man. The homozygous variant c.1237-1G > T in the GTPBP2 gene was shown to segregate in a family with neurodegeneration accompanied by iron deposition in the brain. In the last variant, c.1237-1G > T affected family members presented with developmental delay and intellectual disability, scoliosis and kyphoscoliosis, pectus carinatum, variable dystonia, mild to moderate ataxia, autonomic dysfunction, chronic motor neuronopathy, cerebellar vermis atrophy, anomalies of the retina, and thin sparse brittle hair (PMID:26675814).

Considering published evidences, and cosegregation of the detected variant, c.399-2A > G, in GTPBP2 gene in the family members, and also autosomal recessive pattern of inheritance, we consider this variant as a pathogenic mutation. And to the best of our knowledge, this is the first report of GTPBP2 related hypotrichotic microcephaly with generalized muscular dystrophy.

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CONFLICT OF INTEREST
The authors declare that there is no conflict of interests.

AUTHOR CONTRIBUTIONS
IAR: contributed to clinical evaluation of the cases, analyzed data, and co-wrote the paper; AV: analyzed data and co-wrote the paper; EA: performed experiments, analyzed data, and wrote the paper.

ETHICAL APPROVAL
This research and all methods were performed in accordance with the ethical principles, the national norms, standards, relevant guidelines, and regulations for conducting Medical Research in Iran. Written informed consent was obtained from the family included in this study.

DATA AVAILABILITY STATEMENT
All data generated or analyzed during this study are included in this case report. Details are available from the corresponding author upon reasonable request.

ORCID
Isa Abdi Rad https://orcid.org/0000-0002-3931-669X
Ali Vahabi https://orcid.org/0000-0003-2647-1743
Elnaz Akbariazar https://orcid.org/0000-0001-8047-7156

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