

Short Communication

First Report of *THOC6* Mutation in a Pakistani Family with Beaulieu-Boycott-Innes Syndrome

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ABSTRACT

The THOC6 protein is a component of the THO complex, which plays a role in transcription, mRNA processing, and export from the nucleus. Homozygous loss-of-function variants in the *THOC6* gene have been first identified in patients with Beaulieu-Boycott-Innes syndrome (BBIS). To date, 24 cases of THOC6-related BBIS from 18 families have been reported in 15 studies across various populations. In this study, we aimed to investigate the genetic basis of intellectual disability in a consanguineous family from Pakistan. The family had three siblings with intellectual disability, and whole exome sequencing was performed to identify pathogenic variants which were later validated through Sanger sequencing. This study was conducted at the Centre of Excellence in Molecular Biology, University of the Punjab, Lahore. We identified a novel homozygous missense mutation (c.508T>C; p.Tyr170His) in the *THOC6* gene in all three affected siblings of PKMR427. The mutation was found to segregate in the family along with the phenotype, and clinical assessment confirmed the typical features of BBIS. This is the first report of a *THOC6* gene mutation from Pakistan, and it substantiates the candidacy of *THOC6* as a gene involved in BBIS. It also expands the study spectrum of this syndrome.

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Authors' Contribution

MR enrolled the family, performed the experiments, analysed the data, and wrote the manuscript. MU did clinical evaluation of the patients and helped in the drafting of manuscript. AAK conceived the idea and designed the study. The final manuscript was reviewed and approved by all authors.

Key words

THOC6, Intellectual disability, BBIS, Exome sequencing, Pakistan

Intellectual disability (ID) is a heterogeneous disorder that is characterized by substantial limitations in cognitive abilities and adaptive behaviour (Association, 2013). Beaulieu-Boycott-Innes syndrome (BBIS; MIM: #613680) is a rare autosomal recessive neurodevelopmental disorder which is associated with the variations in *THOC6* gene (MIM: #615403). It is characterized mainly by moderate to severe intellectual disability, developmental delays, dysmorphic facial features, dental caries and dental malocclusion (Lemire *et al.*, 2020; Kiraz *et al.*, 2022). Other associated abnormalities include vertebral defects, anal deformations, cryptorchidism, renal and nervous system malformations, hearing loss and seizures. Facial features that have been reported in the literature include

short palpebral fissures, deep-set eyes, a tall forehead, a high anterior hairline, a hanging columella, thin or thick vermillion of the lips and a wide mouth (Amouzadeh *et al.*, 2020; Lemire *et al.*, 2020; Kiraz *et al.*, 2022; Ruaud *et al.*, 2022). The THOC6 protein is a subunit of the THO complex which coordinates the transcription process with its export from the nucleus. In situ hybridization revealed that *THOC6* gene is expressed in the midbrain and eyes. Cellular localization studies for this gene showed that the wild type protein becomes localized in the nucleus, whereas the mutant protein is found in the cytoplasm. The SiRNA knockdown of *THOC6* resulted in increased apoptosis leading to anatomical and neurological abnormalities. Consequently, the mutant gene protein cannot function properly (Beaulieu *et al.*, 2013).

We are hereby reporting the first Pakistani family with three siblings exhibiting the phenotype of BBI syndrome. In all three children, a novel bi-allelic missense mutation has been identified in *THOC6* gene (NM_024339.3; c.508T>C; (p.Tyr170His)) through whole exome sequencing. This finding will contribute to the existing data on BBI syndrome.

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Materials and methods

A consanguineous Punjabi family having one male and two female affected siblings in a single loop was recruited from Faisalabad, Pakistan (Fig. 1A). The diagnosis was made through neurological, morphological, behavioural, ophthalmological, auditory, skeletal and dermatological assessments by a physician and a neurologist. There were no healthy siblings in the nuclear family. Blood samples (10 mL) were collected from all affected individuals, and their normal mother and two uncles. Written informed consent was obtained from each participant.

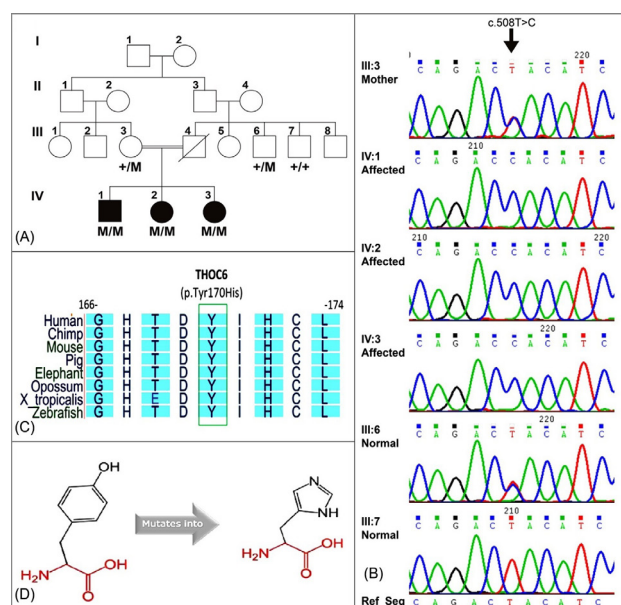


Fig. 1. (A) Pedigree of PKMR427 showing segregation of *THOC6* gene mutation, (B) sequencing chromatograms confirming the variant (c.508T>C), (C) highly conserved wild-type Tyrosine amino acid (p.Tyr170His) in various species, (D) graphical representation of amino acid change from tyrosine to histidine.

Standardized organic protocol was used to isolate genomic DNA from blood samples of six family members (Sambrook *et al.*, 1989). One affected individual (VI:1) was selected for whole exome sequencing on Illumina HiSeq2000 systems producing 100bp paired-end reads. These reads were mapped to human genome GRCh37/hg19 reference assembly. A filtration scheme (Riazuddin *et al.*, 2017) was followed to prioritize the variants on the basis of allele frequency, pathogenicity and brain expressions. The variants most likely to be of our interest were filtered out during exome data analysis. Orthologous protein sequences were checked for evolutionary conservation on UCSC genome browser. HOPE web-server (V 1.1.1) was employed for homology modelling to predict

mutation effect using Human Protein THOC6 sequence (NM_024339.3, UniProt ID: Q86W42) (Venselaar *et al.*, 2010).

The prioritized pathogenic variants were confirmed by Sanger sequencing, for their occurrence in patients and co-segregation with the disease phenotype in family. Following the PCR amplification (GeneAmp® PCR system 2700, Applied Biosystems), sequencing was carried out on ABI Prism 3100 Genetic analyzer (Applied Biosystems) present at CEMB, using BigDye (Applied Biosystems TM Big dye® Terminator v3.1 Cycle Sequencing Kit) and bidirectional primers (forward primer, 5'-GCCTATCCTGATTGACATTGAC-3'; reverse primer, 3'-CTCACCTCGTGCTTATAGACCTC-5'). Sequencing results were analyzed by SeqMan TM II (version 5.00.2221.0, DNASTAR Inc.) software. Primers were designed using Primer3 (Koressaar and Remm, 2007).

Results

Physical and clinical evaluations of Pakistani family PKMR427 revealed three affected siblings (one male and two females) with characteristic features of BBI syndrome (Fig. 2). All three patients suffered from moderate ID, diagnosed since early childhood, and exhibited facial dysmorphism and global developmental delays. Individual IV:1 was 17 years old at the time of evaluation. He was born through vaginal delivery after a full-term pregnancy of 38 weeks, and had a normal head size and shape. He had speech delay as well as delays in other developmental milestones such as sitting at 3 years and walking at 4 years. He could chew and eat soft food at the age of 1 year. He had dental caries, and dysmorphic facial features including upslanted palpebral fissures, hypertelorism, synophrys, a low hanging columella, a bulbous nose, a tall forehead and a thick vermillion of the upper lip. He also expressed slightly aggressive behaviour.

Individual IV:2 was 21 years old at the time of evaluation, and had normal head shape and size. She was born through vaginal delivery after full-term pregnancy of 37 weeks. She demonstrated delayed childhood milestones such as sitting at 2 years and walking at 3.5 years. She had not yet started speaking. She could eat soft foods at the age of 1 year. She started spoon-feeding and playing very late, around the age of 5 years. She had dental caries and dental malocclusion, and dysmorphic facial features including upslanted palpebral fissures, deep set eyes, hypertelorism, epicanthal fold, a bulbous nose, a thick vermillion of the lower lip and a low hanging columella. Additionally, she exhibited violent behaviour, including a hair-pulling disorder (Trichotillomania) in which she pulls her own hair from her head and even bites people who try to stop

her. She was diagnosed with tonic-clonic epilepsy at the age of 11 and continues to suffer from it. Her epilepsy was being treated by Sodium Valproate which also reduced her hair-pulling problem.



Fig. 2. Dysmorphic facial features of three patients of PKMR427 having BBIS phenotype.

Individual IV:3 was 19 years old at the time of evaluation. She was born through vaginal delivery after a full-term pregnancy of 38 weeks with a normal head size and shape. Despite reaching the age of four, she had only spoken a few words and had not yet developed proper speech. She began sitting at the age of 2.5 years and started walking at 3.5 years. Additionally, she displayed dysmorphic facial features such as upslanted palpebral fissures and a bulbous nose tip.

Exome sequencing identified 79,567 variants which were processed through our filtration scheme. There were 11 variants each from a different gene, prioritized at the end. Ten were missense and one was splice site variant. Out of these, five variants were predicted as disease causing by one or more *in-silico* prediction tools and were further tested through Sanger sequencing for co-segregation with disease phenotype across the entire family. One homozygous missense variant Chr16:3076704T>C (c.508T>C, (p.Tyr170His)) in 8th exon of *THOC6* gene (NM_024339.3) was finally confirmed as co-segregating with ID phenotype in the family (Fig. 1A). Sanger sequencing was performed on six members of the family, and the resulting chromatograms (Fig. 1B) indicate that affected individuals carried a homozygous mutant variant, while the mother was a carrier of the mutation. Additionally, one uncle was identified as homozygous wild type and the

other as carrier of the variant. The variant was not detected in either dbSNP or the 1000G and ExAC databases. *In-silico* pathogenicity predicting tools predict it as having deleterious effect on protein structure and function. Mutation Taster predicted disease causing (Probability 0.9) as well as Polyphen2 probably damaging (score 0.9). PhyloP showed the thymine as highly conserved wild type nucleotide (score 4.085). The evolutionary conservation analysis of amino acids revealed that the tyrosine amino acid is highly conserved up to zebrafish (Fig. 1C). The physicochemical distance was determined to be 83, indicating moderate differences between tyrosine and histidine amino acids. The calculated CADD score was found to be 27.0. Furthermore, the expression profile of the *THOC6* gene in the brain tissue of *Drosophila*, as determined by NCBI Unigene EST profile, was 11 TPM.

Protein homology modelling by HOPE depicted that the mutant residue (Fig. 1D) is less hydrophobic and smaller in size than the wild type residue, therefore, this mutation may lead to loss of (hydrophobic) interaction either on the surface or in the core of protein. In view of more explanation, the wild type amino acid and other surrounding residues are highly conserved and present in WD4 region of protein which is very much repetitive. Variation of this wild type residue into another might affect this repeating stretch leading to overall effect on protein functions.

Discussion

ID is a heterogeneous group of disorders with complex phenotype, and is mostly caused by genetic variations (60%) (Mattioli *et al.*, 2019). *THOC6* is a causal gene for BBIS and the first-ever case with pathogenic missense mutation (p.Gly46Arg) in *THOC6* was reported by Beaulieu *et al.* (2013). To date, 24 BBIS patients from 18 families have been documented in 15 different studies from various populations (Hutterite, Saudi, Irish, French, Italian, Indian, Iranian, American, European and Turkish) (Amouzadeh *et al.*, 2020; Kiraz *et al.*, 2022; Lemire *et al.*, 2020; Ruaud *et al.*, 2022). In our study, the identified *THOC6* gene mutation (c.508T>C; (p.Tyr170His)) highlights the importance of genetic testing in the Pakistani population for intellectual disability cases.

Various clinical features were observed and reported in association with BBIS. Our patients displayed the characteristic phenotype of BBI syndrome but no cardiac or genitourinary anomalies were observed. However, the hair-pulling disorder was observed in our female patient (IV:2) who exhibited violent behaviour by pulling her own hair and biting other people who tried to stop her. She was also diagnosed with tonic-clonic epilepsy at the age of 11 years. Previously, epileptic seizures have been observed

in two research studies (Elmas et al., 2019; Mattioli et al., 2019) but type and severity of the seizures was not specified.

THOC6 is translated into a protein of 341 amino acid residues, which contains WD (Tryptophan-Aspartic Acid) repeats (Gupta et al., 2019). *In-silico* analyses predicted that the identified mutation (p.Tyr170His) is located in the WD4 repeat region of the protein which is crucial for the structural and functional stability of the encoded protein. Wild type nucleotide and amino acid of this variant was found highly conserved as well as this variant was not recognized in different genome databases.

Conclusion

In conclusion, this study reports the first-ever cases of BBIS in Pakistan and identifies a novel homozygous missense mutation (c.508T>C; p.Tyr170His) in *THOC6* as the genetic cause of the disorder in our family. The identified mutation highlights the critical role of the *THOC6* gene in normal intellectual development and suggests that genetic testing along with genetic counselling should be considered in families with intellectual disability in Pakistan.

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Ethical statement and IRB approval

This research study followed guidelines of the Helsinki Declaration and was approved by the IRB of Centre of Excellence in Molecular Biology (CEMB), University of The Punjab, Lahore, Pakistan.

Statement of conflict of interest

The authors have declared no conflict of interest.

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