


RESEARCH ARTICLE

WILEY

Molecular insight into *CREBBP* and *TANGO2* variants causing intellectual disability

Syeda Iqra Hussain¹ | Nazif Muhammad¹ | Niamatullah Khan¹ | Mobeen Khan¹ | Fardous Fardous² | Raheel Tahir¹ | Muhammad Yasin¹ | Sher Alam Khan¹ | Shamim Saleha¹ | Noor Muhammad¹ | Naveed Wasif^{3,4}  | Saadullah Khan¹

¹Department of Biotechnology and Genetic Engineering, Kohat University of Science and Technology (KUST), Kohat, Khyber Pakhtunkhwa, Pakistan

²Department of Medical Lab Technology, Kohat University of Science and Technology (KUST), Kohat, Khyber Pakhtunkhwa, Pakistan

³Institute of Human Genetics, Ulm University and Ulm University Medical Center, Ulm, Germany

⁴Institute of Human Genetics, University Hospital Schleswig-Holstein, Kiel, Germany

Correspondence

Saadullah Khan, Department of Biotechnology and Genetic Engineering, Kohat University of Science & Technology (KUST), Kohat 26000, Khyber Pakhtunkhwa, Pakistan.

Email: saad@kust.edu.pk; saadkhanwazir@gmail.com

Naveed Wasif, Institute of Human Genetics, University Hospital Schleswig-Holstein, Campus Kiel, 24105 Kiel, Germany.

Email: naveed.wasif@uksh.de

Funding information

Higher Education Commission, Pakistan

Abstract

Background: Intellectual disability (ID) can be associated with different syndromes such as Rubinstein-Taybi syndrome (RSTS) and can also be related to conditions such as metabolic encephalomyopathic crises, recurrent, with rhabdomyolysis, cardiac arrhythmias and neurodegeneration. Rare congenital RSTS1 (OMIM 180849) is characterized by mental and growth retardation, significant and duplicated distal phalanges of thumbs and halluces, facial dysmorphisms, and an elevated risk of malignancies. Microdeletions and point mutations in the CREB-binding protein (*CREBBP*) gene, located at 16p13.3, have been reported to cause RSTS. By contrast, *TANGO2*-related metabolic encephalopathy and arrhythmia (TRMEA) is a rare metabolic condition that causes repeated metabolic crises, hypoglycemia, lactic acidosis, rhabdomyolysis, arrhythmias and encephalopathy with cognitive decline. Clinicians need more clinical and genetic evidence to detect and comprehend the phenotypic spectrum of this disorder.

Methods: Exome sequencing was used to identify the disease-causing variants in two affected families A and B from District Kohat and District Karak, Khyber Pakhtunkhwa. Affected individuals from both families presented symptoms of ID, developmental delay and behavioral abnormalities. The validation and co-segregation analysis of the filtered variant was carried out using Sanger sequencing.

Results: In the present study, two families (A and B) exhibiting various forms of IDs were enrolled. In Family A, exome sequencing revealed a novel missense variant (NM 004380.3: c.4571A>G; NP_004371.2: p.Lys1524Arg) in the *CREBBP* gene, whereas, in Family B, a splice site variant (NM 152906.7: c.605 + 1G>A) in the *TANGO2* gene was identified. Sanger sequencing of both variants confirmed their segregation with ID in both families. The in silico tools verified the aberrant changes

Syeda Iqra Hussain and Nazif Muhammad contributed equally to this work.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2023 The Authors. *The Journal of Gene Medicine* published by John Wiley & Sons Ltd.

in the CREBBP protein structure. Wild-type and mutant CREBBP protein structures were superimposed and conformational changes were observed likely altering the protein function.

Conclusions: RSTS and TRMEA are exceedingly rare disorders for which specific clinical characteristics have been clearly established, but more investigations are underway and required. Multicenter studies are needed to increase our understanding of the clinical phenotypes, mainly showing the genotype–phenotype associations.

KEYWORDS

bioinformatics, molecular genetics, neurology

1 | INTRODUCTION

Worldwide, one in every 100 people is diagnosed with intellectual impairment, marked by significant difficulties in intellectual functioning and adaptive behavior, and typically manifests before the age of 18 years.¹ A definitive intellectual disability (ID) is diagnosed when an IQ test of a person reveals a score of 70 or below.² ID has a wide range of severities (mild, moderate, severe and profound) and can be isolated or in association with other congenital abnormalities or neurological malformations, such as epilepsy, sensory impairment and autism spectrum disorders.³

A congenital condition known as Rubinstein-Taybi syndrome (RSTS) (OMIM 180849) is marked by growth retardation, psychomotor developmental delay, large and duplicated thumb and hallux distal phalanges, and various characteristic dysmorphic traits. Broad nasal bridges, beaked noses, downturned palpebral fissures and micrognathia are examples of facial dysmorphology.⁴ Additionally, although poorly understood, patients with RSTS are at an elevated risk of developing tumors.^{5,6} The gene encoding the CREB-binding protein (CREBBP, often known as “CBP”), has been reported to cause RSTS.⁷ The transcriptional coactivator *CREBBP* carries out acetyltransferase activity on lysine residues of histone and non-histone proteins.⁸ *CREBBP* regulates the expression of several genes and participates in essential physiological functions such as growth, differentiation, DNA repair and apoptosis.⁹ *CREBBP* protein is a component of a molecular pathway that controls transcriptional activation, chromatin remodeling and gene expression. To better activate gene expression, it functions as a coactivator for other transcription factors such as CREB and p53.^{10,11} *CREBBP* also has histone acetyltransferase (HAT) activity, which it uses to acetylate histones and so loosen chromatin structure and increase gene accessibility. It plays a role in complexes that remodel chromatin, affects development, and aids in the control of genes through epigenetic mechanisms.^{12,13} The hereditary condition RSTS is linked to mutations in *CREBBP* and is characterized by ID and developmental delays.¹⁴ The mutations detected in RSTS patients range from point mutations to microdeletions eliminating the entire gene, leading to the haploinsufficiency of this dosage-sensitive gene.¹⁵ More specifically, studies of individuals with missense and

splice-site mutations in the *CREBBP* HAT domain revealed that loss of HAT activity is sufficient to cause the ID syndrome.¹⁶

TANGO2-related metabolic encephalopathy and arrhythmia (TRMEA) (OMIM 616878) is an exceptionally uncommon neurological condition and is phenotypically diverse. A protein called TANGO2 (i.e. transport and Golgi organization two homologs), identified in 2016, has a role in reorganizing the Golgi membranes into the endoplasmic reticulum (ER). It is involved in a complex molecular pathway involving mitochondrial function, lipid metabolism, oxidative stress response and autophagy.¹⁷ Crucially, it aids in the breakdown of fatty acids for energy production, protects cells from oxidative damage, and keeps mitochondria healthy and efficient.¹⁸ Metabolic crises, muscle weakness, ID and cardiac abnormalities can result from mutations in the *TANGO2* gene that cause metabolic and neurodevelopmental diseases.¹⁹ Recently, exome sequencing has identified pathogenic *TANGO2* mutations in 15 patients presenting rhabdomyolysis, convulsions, increasing intellectual impairment, encephalopathy and cardiac arrhythmias when experiencing a metabolic crisis.^{20,21} More clinical and molecular knowledge is required for the clinicians and the families to comprehend the phenotypic spectrum of this rare condition.²²

The present study aimed to evaluate two Pakistani families showing various forms of ID. Exome sequencing was employed to identify the disease-causing variants and *in silico* tools were used to study the structural changes in the mutant protein.

2 | MATERIALS AND METHODS

2.1 | Collection of blood samples

The institutional ethical review board of Kohat University of Science and Technology, Kohat, Pakistan, approved the study (Ref. No. KUST/Ethical Committee/2191; Dated 06.10.2021), carefully following the recommendations of the Declaration of Helsinki. Two families (A and B) with ID were enrolled for the present study. Family A presenting autosomal dominant mode of inheritance was sampled from Billitang, Kohat. Four members, including three affected

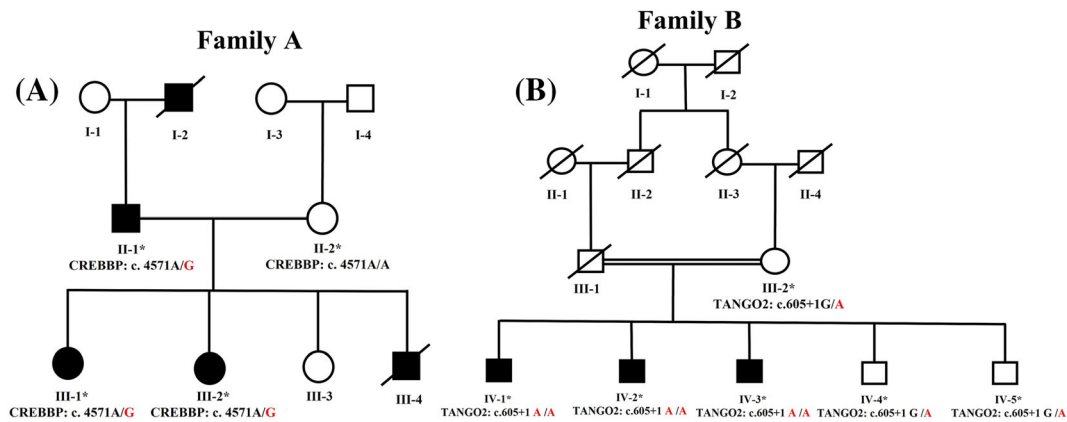


FIGURE 1 (A) Pedigree of family A showing the autosomal dominant pattern of ID and presenting the unaffected and affected individuals of Family A. (B) Pedigree of Family B showing the autosomal recessive pattern of ID and presenting the unaffected, heterozygous carrier and homozygous affected individuals of Family B.

individuals (II-1, III-1 and III-2) and an unaffected female (II-2) participated in the study. The affected females (III-1 and III-2) of the family presented relatively severe phenotypes compared to the affected male member (II-1). Similarly, Family B showing autosomal recessive mode of inheritance was recruited from district Karak of Khyber Pakhtunkhwa. Six members, including three unaffected and three affected individuals, participated in the study. Adults from both families were interviewed to compile the disease history, and pedigrees were constructed based on the provided information (Figure 1). The blood samples were collected into BD Vacutainer® (BD, Franklin Lakes, NJ, USA) blood collection tubes and preserved after gaining informed and written consent from both families. Standard phenol-chloroform extraction methods were used to isolate DNA from the blood samples, and the concentration of the extracted DNA was measured, using Qubit Fluorometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) to an accuracy of 40 ng/μl.

2.2 | Exome sequencing

For molecular analysis of both families with IDs, whole-exome sequencing was performed on the samples (Family A-III-1; Family B-IV-1). After performing whole-exome sequencing, the data were filtered to remove the variances.

First, the exonic regions of all 19,433 human genes were captured by the xGen Exome Research Panel v2 (Integrated DNA Technologies, Coralville, IA, USA). After capture, a Novaseq 600 system was used to sequence all captured regions (Illumina, San Diego, CA, USA). We acquired $\geq 20\times$ coverage in $> 98.9\%$ and $\geq 10\times$ coverage in $> 99.2\%$ of target sequences. Following the sequencing, the data were analyzed using open-source bioinformatics tools and proprietary software. bcl2fastq v2.20.0.422 (<https://emea.support.illumina.com/downloads/bcl2fastq-conversion-software-v2-20.html>) was used to convert and demultiplex base call sequence files to FASTQ files. Variant calling and annotation followed the alignment of the

sequencing data to the GRCh37/hg19 human reference genome was carried out using BWA-mem 0.7.17 (arXiv:1303.3997 [q-bio.GN]) to generate BAM files. BAM files were processed using GATK best practices (GATK, version 3.8; <https://www.broadinstitute.org>) for single nucleotide variants and small insertions/deletions (indel) variant calling to generate VCF files.^{23,24} For copy number variant calling based on depth-of-coverage (DOC) data, Conifer²⁵ and 3bCNV (<https://3billion.io/resources>) were used. The region of homozygosity (ROH) was mapped from the VCF file using AutoMap v1.2.²⁶

EVIDENCE tool (<https://3billion.io/resources>) was used to select variants according to the ACMG guidelines and each patient's phenotype. Variant filtration, categorization and similarity score for the patient's phenotype are three significant steps in this approach. For allele frequency estimation, a genome aggregation database (gnomAD; <http://gnomad.broadinstitute.org>) and the 3billion genome database (<https://3billion.io/main>) were utilized in the first step. According to ACMG guidelines, gene variations with more than 5% allele frequency were filtered out. Next, VarSome,²⁷ Human Gene Mutation Database (HGMD) (<https://www.hgmd.cf.ac.uk/ac/all.php>) Professional 2022.1, Pakistan Genetic Mutation Database (PGMD) (<https://www.pakmutation.kust.edu.pk>),²⁸ Database of Single Nucleotide Polymorphism (dbSNP) (<https://www.ncbi.nlm.nih.gov/snp/>) and ClinVar (<https://www.ncbi.nlm.nih.gov/clinvar>) were utilized for the evaluation of variants. Then, the assessment of each variant concerning disease phenotype was carried out using the ACMG guidelines.²⁹ Finally, in the third step, the patient's clinical phenotypes were converted to standardized human phenotype ontology terms (<https://hpo.jax.org>) and retrieved to determine the degree of similarity^{30,31} with each of 7000 rare genetic diseases (<https://omim.org/> and <https://www.orpha.net/consor/cgi-bin>). According to the ACMG guidelines, the similarity score between each patient's phenotype and symptoms related to that disease caused by priority variations varied from 0 to 10. Medical geneticists and physicians then manually evaluate probable alterations and related disorders. Bidirectional

Sanger sequencing was used to confirm single nucleotide variants and all indels.

2.3 | Segregation analysis

Sanger sequencing was used to validate and confirm the segregation of identified variants in the families. Primer3 (<https://primer3.ut.ee/>) was used to design the primers (flanking ~500 bp up- and downstream of the variants of interest). Two sets of primers were designed:

CREBBP-Forward 5'-CACACATGCATGGGACTC-3'
CREBBP-Reverse 5'-CACTGTGCAGAGAGTGCAG-3'
TANGO2- Forward 5'-CAGCTGTACCCCGTGTAG-3'
TANGO2-Reverse 5'-ATCAAAAGTCCCTGGTGTG-3'

2.4 | Structural analysis prediction and validation

The whole three-dimensional structure of the CREBBP cannot be found in the protein data bank (PDB), comprising a set of online resources (https://www.rcsb.org/?ref=nav_home). Alpha Fold, an online web server (<https://alphafold.ebi.ac.uk>), was used to predict the structure of the multiprotein template.³² The Ramachandran plot

and the ERRAT (<https://saves.mbi.ucla.edu/>) were made with Procheck (<https://saves.mbi.ucla.edu/>) to validate the 3D-modelled structure of the CREBBP, and to check the phi and psi angles.³³

2.5 | Protein mutagenesis

Biovia Discovery Studio (<https://www.3ds.com/products-services/biovia/products/molecular-modeling-simulation/biovia-discovery-studio/>) and Chimera (<https://www.rbvi.ucsf.edu/chimera>) were used to determine the 3D-structure of the mutant protein, which differs from the wild-type protein in crucial ways.³⁴ The wild-type protein served as a source for the mutant design. Biovia Discovery Studio was used to examine structural differences between wild-type and mutant proteins.

3 | RESULTS

3.1 | Clinical features of Family A

Four members of Family A, including three affected members (II-1, III-1 and III-2) (Figure 1A), were recruited in the study. The affected individuals displayed mild to severe ID and associated disease



FIGURE 2 (A-B) Physical appearance and facial features of the affected individual (III-1). (C-D) Physical appearance and facial features of affected individual (III-2) of Family A.

characteristics such as broad nasal bridges, beaked noses, short stature, facial features that are not typical, and unusually large thumbs and first toes (Figure 2). The additional clinical description of patient in Family A are summarized in the Supporting information (Table S1).

3.2 | Clinical features of Family B

From Family B, six members were recruited including three affected (IV-1, IV-2 and IV-3) individuals (Figure 1B). The affected individuals presented characteristic neurodevelopmental anomalies such as ID, seizures and difficulties in coordinating voluntary movements (ataxia) leading to awkwardness, an unsteady gait and speech difficulties (dysarthria) that were evident in the affected individuals. There is a continuum of ID from mild to moderate to severe. The additional clinical description of the patients in Family B is summarized in the Supporting information (Table S2).

3.3 | Molecular findings

Exome sequencing of Family A revealed a novel missense variant (NM_004380.3, c.4571A>G; NP_004371.2: p.Lys1524Arg) in exon-28 of the *CREBBP* gene. Sequencing analysis confirmed segregation of this variant in the family, consistent with the autosomal dominant inheritance pattern. Affected individuals were heterozygous (c.4571A/G), whereas the unaffected individuals were homozygous

for this variant (Figure 3A–C). Exome sequencing of Family B revealed a splice site mutation (NM_152906.7, c.605 + 1G>A) in the *TANGO2* gene. The segregation of this splice site variant was confirmed in the family by Sanger sequencing. Parents were heterozygous (c.605 + 1G/A) for this splice site mutation, whereas affected individuals were homozygous (c.605 + 1A/A), confirming the autosomal recessive mode of inheritance (Figure 3D–F).

3.4 | Secondary structure prediction

The PDBsum tool (<http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/>) was used to determine the prediction of the secondary structure for the wild-type *CREBBP*. It contains six sheets, one beta alpha beta unit, nine beta hairpins, seven beta bulges, 19 strands, 42 helices, 37 helix–helix interactions, 89 beta turns and 23 gamma turn.

3.5 | Structural analysis prediction and validation of wild-type *CREBBP*

Using the Ensemble genome browser (https://asia.ensembl.org/Homo_sapiens/Info/Index), we retrieved the *CREBBP* protein sequence. The three-dimensional structure was created using AlphaFold (<https://alphafold.ebi.ac.uk/>) (Figure 4). For structure validation, EERAT was utilized, which calculated a quality factor score of 73.6%.

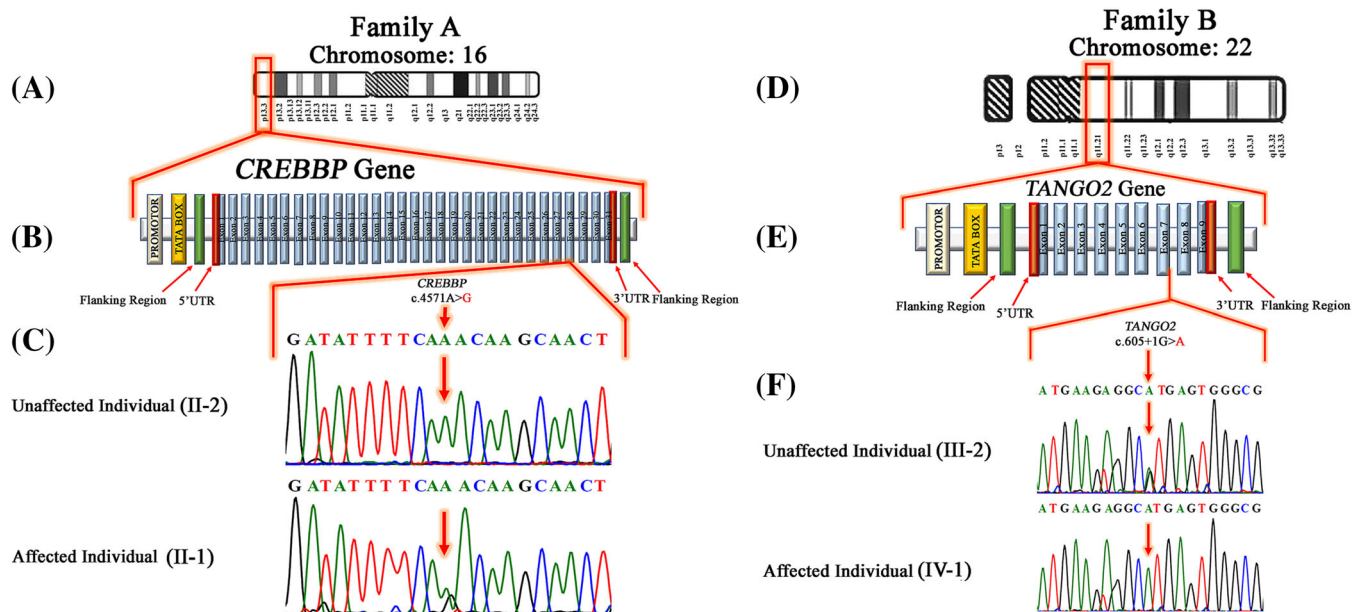


FIGURE 3 (A) Representing the location of *CREBBP* (p13.3) at chromosome 16. (B) Representing the typical structure of the *CREBBP* gene comprising 31 exons and also indicating the location of missense variant (c.4571A>G; p.Lys1524Arg) in exon 28 (C) chromatograms of a homozygous unaffected mother (II-2) and heterozygous affected father (II-1) of Family A. (D) Location of *TANGO2* (q11.21) at chromosome 22. (E) The typical structure of the *TANGO2* gene comprising nine exons, indicating the location of splice-site variant (c.605 + 1G>a) in the intronic region. (F) Chromatograms of the heterozygous unaffected mother (III-2) and homozygous affected son (IV-1) of Family B.

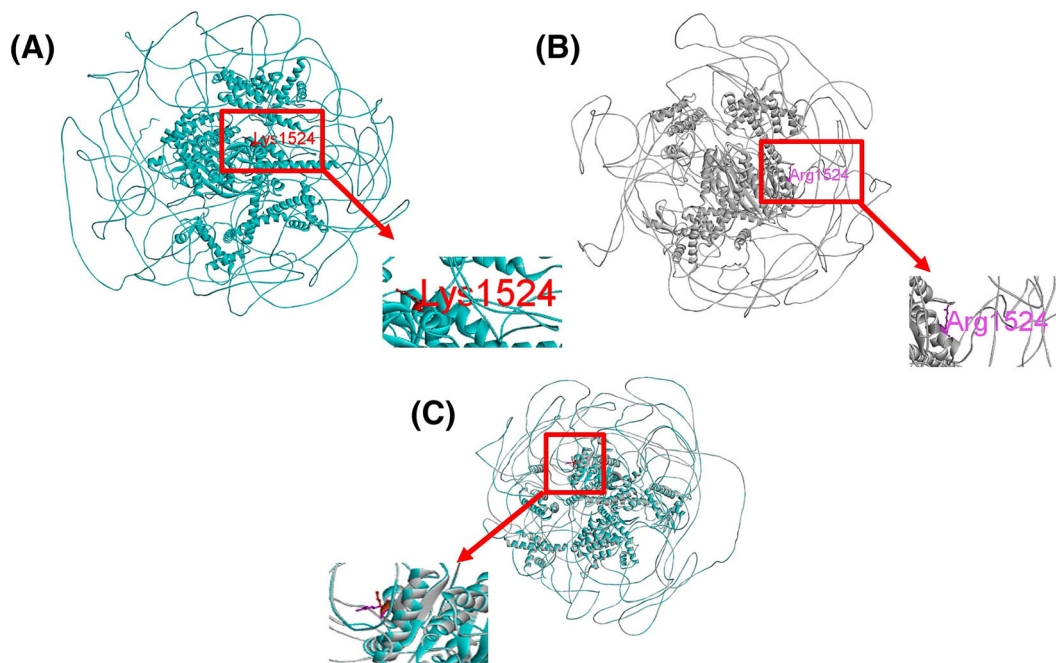


FIGURE 4 (A) Structure of wild-type CREBBP protein shown in blue, and Lys 1524 protein residue highlighted in red. (B) The structure of the mutant CREBBP protein is shown in gray, and Arg 1524 protein residue is highlighted in violet. (C) Superimposition of wild-type CREBBP protein presented by the blue colored band and Lys residue (red) and mutant CREBBP protein presented by the gray colored band and arginine residue (violet).

3.6 | Mutagenesis of CREBBP and comparison with wild-type CREBBP

The structure of the CREBBP mutant was aligned to the wild-type structure. Both wild-type and mutant structures were superimposed using Biovia Discovery Studio for checking the conformational changes. Conformational changes were observed between the wild-type CREBBP and mutant CREBBP protein altering the protein structure and function (Figure 4C).

4 | DISCUSSION

RSTS is a congenital illness with a unique morphology that its symptoms can identify. In RSTS, the cardinal symptoms are typical facial characteristics, large thumbs and halluces, microcephaly, mental impairment, talon cusps, and slow growth. In addition, several other RSTS associated abnormalities have been noted, including tumors, keloid tissues, congenital heart disease, genitourinary anomalies and joint abnormalities.³⁵ The patients enrolled in the present study showed characteristic facial dysmorphism, broad thumbs, ID and cryptorchidism, which are well known characteristics in male patients.³⁶ Only a few studies have described the associated central nervous system malformations,³⁷ including corpus callosal agenesis in a Brazilian boy with RSTS.³⁸ Thorough clinical surveys of RSTS patients may identify more central nervous system deformities.

CREBBP is involved in gene transcription, comprising a transcriptional coactivator with acetyltransferase activity on both histone and non-histone protein lysine residues. It activates itself after attaching to phosphorylated CREB,⁷ subsequently activating gene transcription and recruitment of other transcription factors.³⁹ Additionally, it binds to GLI3, a transcription factor protein, in the Sonic Hedgehog-Patched-Gli (SHH) pathway.⁴⁰ SHH is expressed throughout the human embryo, from the notochord and floorplate of the neural tube through the brain and the zone of polarizing activity in the developing limbs and the stomach.⁴⁰ On the other hand, CREBBP plays an essential role in cell expansion, transformation and development, along with the intrinsic HAT activity.¹⁵ *CREBBP*^{-/-} knockout studies have explained the critical role of *CREBBP* in brain development and neuronal morphogenesis.⁴¹

Acute metabolic crisis (hypoglycemia, increased lactate and mild hyperammonemia) or developmental delay, regression and/or seizures are all possible manifestations of *TANGO2*-related metabolic encephalopathy and arrhythmias.⁴² The initial symptoms might range from profound lethargy to severe muscular weakness, ataxia and/or disorientation.²¹ Rhabdomyolysis can occur at any stage of life, manifesting with sudden and severe epileptic attacks.⁴² Myoglobinuria has been reported in patients as young as 5 months old. Myoglobinuria can cause acute kidney injury and renal failure by damaging kidney filtration tubules.^{21,42} Acute illness is associated with short-term abnormalities in electrocardiogram findings, the most prevalent of which is QT prolongation.⁴² Recurrent ventricular tachycardia, often known as torsade de pointes, is most common during severe illness.⁴²

Some individuals with metabolic disorders do not have acute symptoms such as a metabolic crisis, but may still have walking or talking difficulties.⁴² Almost everyone has some degree of intellectual impairment and more than 75% of individuals with epilepsy report experiencing seizures outside of acute situations. Moreover, one-third of the general population has hypothyroidism.⁴² According to the findings of our research, the affected members of Family B had symptoms of intellectual impairment. The affected individuals had neurodevelopmental abnormalities such as intellectual incapacity, seizures and difficulty in coordinating voluntary movements (ataxia), which resulted in discomfort, an unsteady gait and speech issues (dysarthria).

Two Pakistani families with ID were recruited for the clinical and genetic investigations. A novel missense variant (NM_004380.3, c.4571A>G; NP_004371.2: p.Lys1524Arg) in exon-28 of the *CREBBP* gene was identified in Family A. According to ACMG guidelines,⁴³ this missense variant is likely pathogenic (PM1, PM2, PM3, PP1 and PP3). It was observed that the patients with the *CREBBP* variant were experiencing growth retardation in height and weight, seizures, and a lower-than-average IQ. Molecular analysis of Family B revealed a splice site mutation (NM_152906.7: c.605 + 1G>A) previously reported by Lalani et al.²¹ in the *TANGO2* gene. They hypothesized that disrupting the Golgi and ER and increased ER stress because of *TANGO2* mutations could contribute to the disease mechanism. The splice site variant is categorized as pathogenic (PVS1, PM2, PP1 and PP3) after following ACMG guidelines.⁴³ The patients showed typical features of metabolic encephalopathy and arrhythmias associated with *TANGO2* in an autosomal recessive pattern. Previous studies have identified biallelic pathogenic mutations in this gene causing loss of protein function.²¹

We have investigated the effects of the mutation (p.Lys1524Arg) on the stability and function of the *CREBBP* protein. To identify its potential role in causing ID in Family A, we have performed in silico analyses. The function of a protein is dependent on its conformational and dynamic interactions, ligand binding ability, protein–protein interactions and protein flexibility.^{44,45} The changes in the protein's flexibility alter its activity and ability with respect to binding to the substrates and affect its interactions with other molecules.⁴⁶ In the present study, the identified sequence variant was assumed to alter the structure and function of the *CREBBP* protein, changing its molecular flexibility, and leading to the gain or loss of protein function. Superimposition of wild-type and mutant *CREBBP* protein revealed a significant difference in the protein's conformation and likely abortion of its normal function causing ID in Family A. By interfering with typical cellular functions or signaling pathways, the gain and loss of function of proteins in ID may accelerate the disease progression.⁴⁷ In the present study, we looked for a likely association between genetic variations and the severity or presence of particular clinical symptoms in the probands by carefully reviewing the clinical descriptions of our patients compared to previous studies.^{48–50} This clinical comparison revealed only the standard features, including the characteristic dysmorphic traits of RSTS, postnatal growth retardation, moderate to severe ID and various physical abnormalities. We could not establish a

significant genotype–phenotype correlation. Future studies may confirm our in silico findings and expand our understanding of the molecular mechanisms underlying IDs.

More than 300 million people worldwide suffer from rare genetic disorders, affecting about one in 2000 persons.⁵¹ The correct diagnosis can have far-reaching consequences for the patient and their loved ones, providing insight into disease's cause, progression and prognosis and making it simpler to find support groups and access genetic counseling.⁵² Data sharing and global collaboration among clinicians and scientists may lead to future gene therapy development.⁵³ Recent advances in next-generation sequencing technology, such as whole-exome sequencing and whole-genome sequencing, have led to the discovery of pathogenic mutations in many previously unresolved rare genetic disorders.⁵³ Some additional screening testing may further improve the diagnostic standards such as Newborn Screening Program (NBS) and non-invasive prenatal testing (NIPT). NBS has gained global recognition as an exceptionally effective public health initiative for promoting health and preventing diseases. Presently, NIPT for aneuploidy is widely employed globally as an initial screening modality for the prevailing fetal screening.^{54,55}

5 | CONCLUSIONS

The present molecular genetic study found a novel variant in the *CREBBP* gene and a mutation in the *TANGO2* gene, thus further extending the mutational spectrum and allelic heterogeneity of *CREBBP* and *TANGO2* and its association with ID. Next-generation sequencing techniques have not only improved the understanding of the genetic heterogeneity of the syndrome, but also widened the phenotypic spectrum of RSTS by encompassing the broader field of chromatinopathies rendering phenotype/genotype correlations more complex. Affected individuals with TRMEA frequently experience life-threatening complications preceded by common neurological symptoms. Additional work on disease pathophysiology and potential therapeutics is needed.

ACKNOWLEDGEMENTS

The current research is partially supported by HEC, Pakistan through Indigenous 5000 PhD Fellowship Project Phase-II, Batch-VI (520[Ph-II]/2BS6–162/HEC/IS/2020). We are indebted to all the research participants for their volunteer contribution in the present genetic study. We are thankful to 3Billion for providing us whole-exome sequencing facility for the current study (<https://3billion.io/index>). We are thankful to Professor Reiner Siebert for his moral support. Open Access funding enabled and organized by Projekt DEAL.

CONFLICTS OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

SK and NM were responsible for study conception and planning. SIH, NM, NK, MY, SAK and RT were responsible for samples and data

collection, as well as analysis and interpretation of data. SIH, NM, SK and NW were responsible for the preparation and writing of the manuscript. NM and SS were responsible for effective participation in the research guidance. NM and SIH were responsible for critical review of the literature and bioinformatic analysis. SK, NM and NW were responsible for critical review and final approval of the manuscript submitted for publication.

DATA AVAILABILITY STATEMENT

The raw data (sequence, photographs, and pedigrees) are stored in a password protected computer at Kohat University of Science and Technology, Kohat, and are available upon request. The identified variants in the present study have been deposited in ClinVar (<https://submit.ncbi.nlm.nih.gov/clinvar>) with accession numbers SCV003935868 and SCV003935867.

ETHICAL STATEMENT

The present molecular study was approved by the institutional ethical review board of Kohat University of Science and Technology (Ref. No. KUST/Ethical Committee/2191; Dated 06.10.2021), Kohat, Pakistan. Signed informed consent was obtained from legal guardians of the patients.

ORCID

Naveed Wasif  <https://orcid.org/0000-0002-3455-8833>

REFERENCES

- Muhammad N, Hussain SI, Rehman ZU, et al. Autosomal recessive variants c. 953A> C and c. 97-1G> C in NSUN2 causing intellectual disability: a molecular dynamics simulation study of loss-of-function mechanisms. *Front Neurol*. 2023;14:1168307. doi:10.3389/fneur.2023.1168307
- Boat TF, Wu J. Committee to evaluate the supplemental security income disability program for children with mental disorders. Board on the Health of Select Populations 2015.
- Vissers LE, Gilissen C, Veltman JA. Genetic studies in intellectual disability and related disorders. *Nat Rev Genet*. 2016;17(1):9-18. doi:10.1038/nrg3999
- Rubinstein JH, Taybi H. Broad thumbs and toes and facial abnormalities: a possible mental retardation syndrome. *Am J Dis Child*. 1963;105(6):588-608. doi:10.1001/archpedi.1963.02080040590010
- Miller RW, Rubinstein JH. Tumors in Rubinstein-Taybi syndrome. *Am J Med Genet*. 1995;56(1):112-115. doi:10.1002/ajmg.1320560125
- Siraganian PA, Rubinstein JH, Miller RW. Keloids and neoplasms in the Rubinstein-Taybi syndrome. *Med Pediatr Oncol*. 1989;17(5-6):485-491. doi:10.1002/mpo.2950170526
- Petrif F, Giles RH, Dauwerse HG, et al. Rubinstein-Taybi syndrome caused by mutations in the transcriptional co-activator CBP. *Nature*. 1995;376(6538):348-351. doi:10.1038/376348a0
- Bannister AJ, Kouzarides T. The CBP co-activator is a histone acetyltransferase. *Nature*. 1996;384(6610):641-643. doi:10.1038/384641a0
- Goodman RH, Smolik S. CBP/p300 in cell growth, transformation, and development. *Genes Dev*. 2000;14(13):1553-1577. doi:10.1101/gad.14.13.1553
- Korzus E, Rosenfeld MG, Mayford M. CBP histone acetyltransferase activity is a critical component of memory consolidation. *Neuron*. 2004;42(6):961-972. doi:10.1016/j.neuron.2004.06.002
- Yu S, Reddy JK. Transcription coactivators for peroxisome proliferator-activated receptors. *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*. 2007;1771(8):936-951. doi:10.1016/j.bbalip.2007.01.008
- Zhang N, Shi L, Wang Y. CREB-associated glycosylation and function in human disease. *Adv Clin Exp Med*. 2022;31(11):1289-1297. doi:10.17219/acem/151026
- Zong W, Gong Y, Sun W, Li T, Wang Z-Q. PARP1: liaison of chromatin remodeling and transcription. *Cancer*. 2022;14(17):4162. doi:10.3390/cancers14174162
- Wang Q, Wang C, Wei WB, Rong WN, Shi XY. A novel CREBBP mutation and its phenotype in a case of Rubinstein-Taybi syndrome. *BMC Med Genomics*. 2022;15(1):182. doi:10.1186/s12920-022-01335-4
- Bentivegna A, Milani D, Gervasini C, et al. Rubinstein-Taybi syndrome: spectrum of CREBBP mutations in Italian patients. *BMC Med Genet*. 2006;7(1): 77. doi:10.1186/1471-2350-7-77
- Murata T, Kurokawa R, Krones A, et al. Defect of histone acetyltransferase activity of the nuclear transcriptional coactivator CBP in Rubinstein-Taybi syndrome. *Hum Mol Genet*. 2001;10(10):1071-1076. doi:10.1093/hmg/10.10.1071
- Katti P, Love-Rutledge S, Murray SA, Hinton A Jr. The role of mitochondrial endoplasmic reticulum contact sites in human health and disease. *Front Mol Biosci*. 2023;10:1223354. doi:10.3389/fmolb.2023.1223354
- Sutton VR, El-Hattab AW. Inborn errors of metabolism. In: *An issue of pediatric clinics of North America*. Elsevier Health Sciences; 2018.
- Hoebcke C, Cano A, De Lonlay P, Chabrol B. Clinical phenotype associated with TANGO2 gene mutation. *Archives de Pediatrie*. 2021; 28(1):80-86. doi:10.1016/j.arcped.2020.11.004
- Kremer LS, Distelmaier F, Alhaddad B, et al. Bi-allelic truncating mutations in TANGO2 cause infancy-onset recurrent metabolic crises with encephalocardiomyopathy. *Am J Hum Gen*. 2016;98(2):358-362. doi:10.1016/j.ajhg.2015.12.009
- Lalani SR, Liu P, Rosenfeld JA, et al. Recurrent muscle weakness with rhabdomyolysis, metabolic crises, and cardiac arrhythmia due to bi-allelic TANGO2 mutations. *Am J Hum Gen*. 2016;98(2):347-357. doi:10.1016/j.ajhg.2015.12.008
- Dines JN, Golden-Grant K, LaCroix A, et al. TANGO2: expanding the clinical phenotype and spectrum of pathogenic variants. *Genet Med*. 2019;21(3):601-607. doi:10.1038/s41436-018-0137-y
- McKenna A, Hanna M, Banks E, et al. The genome analysis toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. *Genome Res*. 2010;20(9):1297-1303. doi:10.1101/gr.107524.110
- DePristo MA, Banks E, Poplin R, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. *Nat Genet*. 2011;43(5):491-498. doi:10.1038/ng.806
- Krumm N, Sudmant PH, Ko A, et al. Copy number variation detection and genotyping from exome sequence data. *Genome Res*. 2012;22(8):1525-1532. doi:10.1101/gr.138115.112
- Quinodoz M, Peter VG, Bedoni N, et al. AutoMap is a high performance homozygosity mapping tool using next-generation sequencing data. *Nat Commun*. 2021;12(1):518. doi:10.1038/s41467-020-20584-4
- Kopanos C, Tsiolkas V, Kouris A, et al. VarSome: the human genomic variant search engine. *Bioinformatics*. 2019;35(11):1978-1980. doi:10.1093/bioinformatics/bty897
- Qasim I, Ahmad B, Khan MA, et al. Pakistan genetic mutation database (PGMD); a centralized Pakistani mutome data source. *Eur J Med Genet*. 2018;61(4):204-208. doi:10.1016/j.ejmg.2017.11.015
- Kleinberger J, Maloney KA, Pollin TI, Jeng LJB. An openly available online tool for implementing the ACMG/AMP standards and guidelines for the interpretation of sequence variants. *Genet Med*. 2016; 18(11):1165. doi:10.1038/gim.2016.13

30. Köhler S, Schulz MH, Krawitz P, et al. Clinical diagnostics in human genetics with semantic similarity searches in ontologies. *Am J Hum Gen.* 2009;85(4):457-464. doi:10.1016/j.ajhg.2009.09.003
31. Greene D, BioResource N, Richardson S, Turro E. Phenotype similarity regression for identifying the genetic determinants of rare diseases. *Am J Hum Gen.* 2016;98(3):490-499. doi:10.1016/j.ajhg.2016.01.008
32. Wei G-W. Protein structure prediction beyond AlphaFold. *Nat Mach Intell.* 2019;1(8):336-337. doi:10.1038/s42256-019-0086-4
33. Kleywegt GJ, Jones TA. Phi/psi-chology: Ramachandran revisited. *Structure.* 1996;4(12):1395-1400. doi:10.1016/S0969-2126(96)00147-5
34. Pettersen EF, Goddard TD, Huang CC, et al. UCSF chimera—a visualization system for exploratory research and analysis. *J Comput Chem.* 2004;25(13):1605-1612. doi:10.1002/jcc.20084
35. Roelfsema JH, Peters DJ. Rubinstein-Taybi syndrome: clinical and molecular overview. *Expert Rev Mol Med.* 2007;9(23):1-16. doi:10.1017/S1462399407000415
36. Cantani A, Gagliesi D. Rubinstein-Taybi syndrome. Review of 732 cases and analysis of the typical traits. *Eur Rev Med Pharmacol Sci.* 1998;2(2):81-87.
37. Sener RN. Rubinstein-Taybi syndrome: cranial MR imaging findings. *Comput Med Imaging Graph.* 1995;19(5):417-418. doi:10.1016/0895-6111(95)00023-2
38. Guion-Almeida ML, Richieri-Costa A. Callosal agenesis, iris coloboma, and megacolon in a Brazilian boy with Rubinstein-Taybi syndrome. *Am J Med Genet.* 1992;43(6):929-931. doi:10.1002/ajmg.1320430604
39. Miller RS, Wolfe A, He L, Radovick S, Wondisford FE. CREB binding protein (CBP) activation is required for luteinizing hormone beta expression and normal fertility in mice. *Mol Cell Biol.* 2012;32(13):2349-2358. doi:10.1128/MCB.00394-12
40. Villavicencio EH, Walterhouse DO, Iannaccone PM. The sonic hedgehog-patched-gli pathway in human development and disease. *Am J Hum Gen.* 2000;67(5):1047-1054. doi:10.1016/S0002-9297(07)62934-6
41. Schoof M, Launspach M, Holdhof D, et al. The transcriptional coactivator and histone acetyltransferase CBP regulates neural precursor cell development and migration. *Acta Neuropathol Commun.* 2019;7(1):199. doi:10.1186/s40478-019-0849-5
42. Lalani SR, Graham B, Burrage L et al. TANGO2-related metabolic encephalopathy and arrhythmias. 2018.
43. Richards S, Aziz N, Bale S, Bick D, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17(5):405-423. doi:10.1038/gim.2015.30
44. Henzler-Wildman K, Kern D. Dynamic personalities of proteins. *Nature.* 2007;450(7172):964-972. doi:10.1038/nature06522
45. Salo-Ahen OM, Alanko I, Bhadane R, et al. Molecular dynamics simulations in drug discovery and pharmaceutical development. *Processes.* 2020;9(1):71. doi:10.3390/pr9010071
46. Dixit A, Verkhivker GM. The energy landscape analysis of cancer mutations in protein kinases. *PLoS ONE.* 2011;6(10):e26071. doi:10.1371/journal.pone.0026071
47. Kleefstra T, Kramer JM, Neveling K, et al. Disruption of an EHMT1-associated chromatin-modification module causes intellectual disability. *Am J Hum Gen.* 2012;91(1):73-82. doi:10.1016/j.ajhg.2012.05.003
48. Pérez-Grijalba V, García-Oguiza A, López M, et al. New insights into genetic variant spectrum and genotype-phenotype correlations of Rubinstein-Taybi syndrome in 39 CREBBP-positive patients. *Mol Genet Genom Med.* 2019;7(11):e972. doi:10.1002/mggg.3.972
49. Schorry EK, Keddache M, Lanphear N, et al. Genotype-phenotype correlations in Rubinstein-Taybi syndrome. *Am J Med Genet a.* 2008;146(19):2512-2519. doi:10.1002/ajmg.a.32424
50. Hennekam R. Rubinstein-Taybi syndrome. *Eur J Hum Genet.* 2006;14(9):981-985. doi:10.1038/sj.ejhg.5201594
51. Nguengang Wakap S, Lambert DM, Oly A, et al. Estimating cumulative point prevalence of rare diseases: analysis of the Orphanet database. *Eur J Hum Genet.* 2020;28(2):165-173. doi:10.1038/s41431-019-0508-0
52. Umair M. Rare genetic disorders: beyond whole-exome sequencing. *J Gene Med.* 2023;e3503. doi:10.1002/jgm.3503
53. Umair M, Waqas A. Undiagnosed rare genetic disorders: importance of functional characterization of variants. *MDPI. Genes (Basel).* 2023;14(7):1469. doi:10.3390/genes14071469
54. Scotchman E, Chandler NJ, Mellis R, Chitty LS. Noninvasive prenatal diagnosis of single-gene diseases: the next frontier. *Clin Chem.* 2020;66(1):53-60. doi:10.1373/clinchem.2019.304238
55. Alyafee Y, Al Tuwaijri A, Umair M, et al. Non-invasive prenatal testing for autosomal recessive disorders: a new promising approach. *Front Genet.* 2022;13:1047474. doi:10.3389/fgene.2022.1047474

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Hussain SI, Muhammad N, Khan N, et al. Molecular insight into CREBBP and TANGO2 variants causing intellectual disability. *J Gene Med.* 2024;26(1):e3591. doi:10.1002/jgm.3591