Journal of Affective Disorders 405 (2026) 121554

Contents lists available at ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com/locate/jad

Research paper

RELN biallelic variant as a candidate risk factor in a consanguineous Pakistani family with bipolar disorder and clinical heterogeneity

Aisha Nasir Hashmi [a,b,c], Ricardo S. Harripaul [b,d], Tahir Muhammad [b,d], Benjamin J. Lowther [b], Anna Mikhailov [b], Zehra Agha [a,e], Raheel Qamar [a,f], John B. Vincent [b,d,g,*], Maleeha Azam [a,**]

[a] *Translational Genomics Laboratory, COMSATS University Islamabad, Pakistan*
[b] *Molecular Neuropsychiatry & Development (MiND) Lab, Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, Toronto, Canada*
[c] *Bahria University College of Medicine, Bahria University of Health Sciences, Islamabad, Pakistan*
[d] *Institute of Medical Science, University of Toronto, Toronto, ON, Canada*
[e] *Department of Psychiatry, Jacobs School of Medicine and Biomedical Sciences, State University of New York at Buffalo, Buffalo, NY, 14203, USA*
[f] *Science and Technology Sector, ICESCO, Rabat, Morocco*
[g] *Department of Psychiatry, University of Toronto, Toronto, ON, Canada*

ARTICLE INFO

Keywords:
Autosomal recessive
Bipolar disorder
Consanguinity
Mutation
Pakistani population
Reelin

ABSTRACT

Background: Bipolar disorder (BD) is highly heritable, polygenic, multifactorial, and has complex genetic heterogeneity. This study aimed to identify rare variants contributing to the aetiology of BD in a consanguineous family from Pakistan.
Methods: Genome-wide SNP microarray and whole exome sequencing (WES) were used for variant identification in a large BD-affected consanguineous Pakistani family.
Results: In family BF04, we identified three main regions of homozygosity-by-descent (HBD) over 1 Mb in length, by far the largest being a 43.6 Mb segment on chromosome 7, and, through WES analysis, found one promising novel homozygous variant in *RELN* (NM_005045; c.2090G>A; p.(Gly697Asp)), within this HBD region segregating in all the BD-affected members.
Conclusion: Based on the clinical and genetic data, the present familial study revealed the plausible contribution of a novel variant of *RELN* in association with BD in the affected family. The present study's findings are valuable in understanding the genetic basis of the multifactorial phenotype BD and pave a better path for future functional studies.

1. Introduction

Bipolar disorder (BD) is a severe mental illness characterised by unpredictable mood swings that fluctuate between periods of mania and depression (Bipolar Disorder Type I, BD-I) or hypomania and depression (Bipolar Disorder Type II, BD-II) (Kerner, 2014). Mania is a phase of an abnormal and persistent state of elation and irritable mood with increased goal-oriented activity and energy, while in the hypomania phase, the threshold and severity of symptoms are lower compared to the manic phase, are not comorbid with the psychotic signs, and do not require hospitalization (American Psychiatric Association, 2013; Vieta et al., 2018). The depressive phase presents the same signs and clinical features as major depressive disorder (MDD), showing persistent feelings of sadness and low mood, fatigue, hopelessness, and social withdrawal that affect daily tasks, sleep, and appetite (American Psychiatric Association, 2013).

Psychiatric disorders such as BD have strong genetic inheritance, as demonstrated in numerous familial genetic studies, including twins and adoption studies. The estimated BD heritability is around 70%–90% (Smoller and Finn, 2003), with increased risk in first-degree relatives, and higher in a monozygotic (MZ) twin of a patient compared to a dizygotic (DZ) twin (MZ twins share 100% of DNA while DZ twins share 50%) (Smoller and Finn, 2003). However, segregation studies in BD-affected families have been unable to determine a clear 'Mendelian

* Correspondence to: J.B. Vincent, Molecular Neuropsychiatry and Development (MiND) Lab, Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, 250 College Street, Toronto, ON, M5T 1R8, Canada.
** Correspondence to: M. Azam, Translational Genomics Laboratory, Department of Biosciences, COMSATS University Islamabad, Tarlai Kalan, Park Road, Islamabad, 45550, Pakistan.
E-mail addresses: john.vincent@camh.ca (J.B. Vincent), malihazam@gmail.com (M. Azam).

https://doi.org/10.1016/j.jad.2026.121554
Received 26 January 2026; Received in revised form 25 February 2026; Accepted 28 February 2026
Available online 3 March 2026

pattern of transmission' for BD (Smoller and Finn, 2003), implying a more complex mode of transmission (Lan et al., 2007; Rice et al., 1978).

Many genetic studies have identified numerous genetic factors as candidate genes and highlighted the role and contributions of their genetic variations (polymorphisms or mutations), which range from smaller-effect-size (common variants or polymorphisms) to larger-effect-size (rare mutations), with a significant role in the aetiology of psychiatric disorders (Wray and Visscher, 2010; Yang et al., 2011). Next-generation sequencing (NGS) methods have further advanced our understanding of the contribution of genetics by identifying *de novo* mutations, single-nucleotide polymorphisms (SNPs), copy number variations (CNVs) (Kataoka et al., 2016; Toma et al., 2018), and rare mutations for psychiatric conditions (Goes et al., 2016; Monson et al., 2017; Rao et al., 2017).

Unfortunately, there is still limited data available on the genetics of BD in non-European populations, and more specifically in populations, such as Pakistan, that may have different genetic disease architecture due to the high proportion of consanguineous marriages (Aadil et al., 2017; Van Snellenberg and De Candia, 2009). However, survey-based studies conducted in different rural and urban areas of Pakistan reported approximately 60% of marriages were consanguineous, of which 80% were first cousins (Hussain and Bittles, 1998). In a study of three-generation BD type I families from Iran, of which 29% were consanguineous, the most common pattern of inheritance was autosomal recessive (Salehi et al., 2017). To date, no autosomal recessive genes or variants have been reported to be associated with BD. However, for several syndromic autosomal recessive disorders, *e.g.* Wilson disease (MIM 277900), symptoms of bipolar disorder have been reported frequently (Carta et al., 2025). The present study aims to identify candidate pathogenic variant(s) in Pakistani BD-affected families, using genome-wide SNP microarray and high-throughput next-generation sequencing, specifically using whole-exome sequencing (WES).

2. Materials and methodology

The current study was approved by the Ethics Review Board of the Department of Biosciences, COMSATS University Islamabad, Pakistan (CIIT/Bio/ERB/19/84, 25-03-2019) and by the institutional research ethics board of Centre for Addiction and Mental Health (CAMH), Toronto, Canada (#047/2020). All the subjects were informed about the study objectives, and written consent was obtained from the subjects. The privacy rights of all the participants were kept under consideration, and their identity was anonymized.

2.1. Family recruitment, blood sample and data collection

2.1.1. Family BF04

Family BF04 was a bipolar-affected family, recruited from the Wah Cantonment area of Pakistan. The family's pedigree is represented in Fig. 1, and the recorded clinical features and demographics of the BF04 family are given in Table 1. The selection criteria for the BF04 family were based on having multiple BD-affected members in the family. The subjects were diagnosed according to the standard diagnostic guidelines explained in the International Classification of Diseases, Tenth Revision (ICD-10), by qualified registered psychiatrists of the Pakistan Institute of Medical Sciences (PIMS) hospital, in Islamabad, Pakistan.

In the family (Fig. 1), the proband (III:6) was diagnosed with BD at the age of 20 years. The mother (III:1) has a history of mild to moderate depression but was completely asymptomatic for psychosis. It should be noted that, while the frequency of BD is~0.5% in Pakistan (Xue et al., 2026), frequency for depression is 29–66% in Pakistani women (Bhamani et al., 2016). The father (III:2) was unaffected. Individuals (IV:1, IV:2, IV:5, IV:7, IV:8, and IV:9) were unaffected siblings of the proband, and individuals IV:3 and IV:4 were identical twins. Individual IV:3 was diagnosed with MDD and psychosis, and was on medication, which was self-discontinued, whereas individual IV:4 presented with signs of euphoria, had a history of depression, and was diagnosed as bipolar II (BD-II). Individual IV:4 was currently in a depressive phase and on medication (Table 1).

Peripheral blood was collected from all the available affected and unaffected members of family BF04, and genomic DNA was extracted using the standard phenol-chloroform DNA extraction protocol (Sambrook and Russell, 2001). Brain Magnetic Resonance Imaging (MRI) was performed for two of the affected siblings (III:4 and III:6), at Murree Road IDC Imaging and Lab Services, Islamabad, using multi-planar, multisequential, non-contrast brain MRI using a Hitachi Aperto

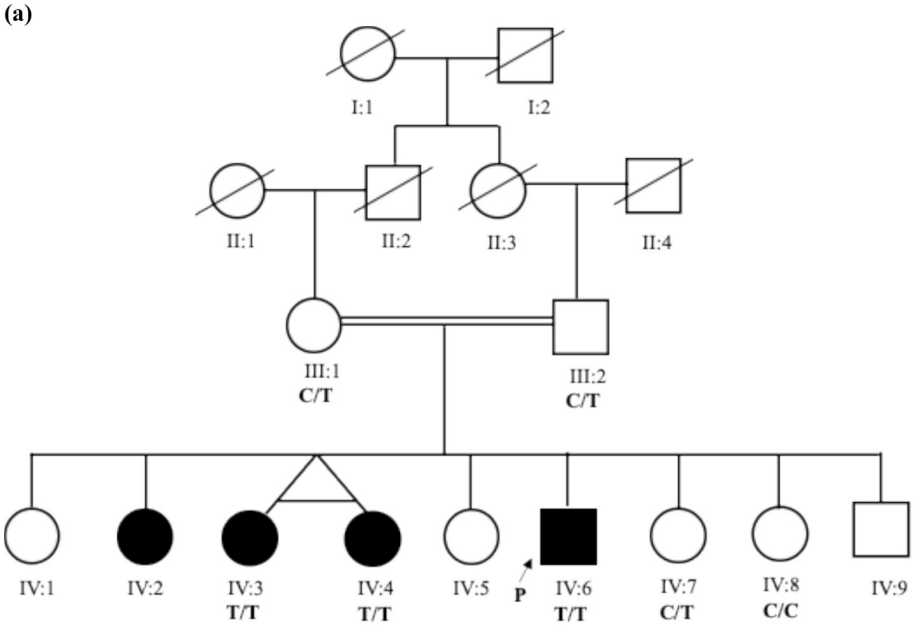

Fig. 1. Pedigree of family BF04. The genotype of variants [*RELN* G>A (represented as C>T genotype)] is mentioned below the individuals. The filled circles and squares are for affected females and males respectively. Generations in the pedigree are represented by Roman numerals and the individuals within each generation are symbolized by Arabic numerals. An arrow indicates the proband.

Table 1
Demographics and clinical features of the members of the family BF04.

Sample ID	Relation	Status at the time of Sampling	Diagnosed	Other ailments /notes/History	Gender	Age (yrs) At the time of sampling	Age at Onset (yrs)	Diagnosed Age (yrs)	Medication
BF04-III:1	Mother	Normal	Normal	History of mild-moderate depression, history of Head injuries, Age-related insomnia, and blood pressure fluctuation	Female	69	NA	NA	Never on medication
BF04-III:2	Father	Normal	Normal	History of respiratory tract operation	Male	70	NA	NA	NA
BF04-IV:2	Sister	Not sampled	MDD Psychosis	Mania-like symptoms	Female	40	NA	NA	On medication
BF04-IV:3	Sister	Depression	Psychosis (BD)	Pressured speech, aggression, mood swings,	Female	37	Not known	35	Self-discontinuation
BF04-IV:4	Sister	Euphoria/ Hypomania	Depression and psychosis (BD-Type 2)	Pressured speech, aggression, mood swings, auditory hallucinations	Female	37	Not known	35	Currently on medication
BF04-IV:6	Proband	BD-Mania	BD-Mania	Suicidal thoughts, fearful, talkative, aggressive, suspicious, grandiosity	Male	35	20	20	Currently on medication
BF04-IV:7	Sister	Normal	Normal	NA	Female	33	NA	NA	NA
BF04-IV:8	Sister	Normal	Normal	NA	Female	31	NA	NA	NA

0.4 Tesla instrument.

2.2. Microarray and homozygosity mapping

The SNP-microarray was performed using the Illumina Infinium CoreExome-24v1-4_A1 kit (~284,000 SNP markers), followed by Infinium HTS (high-throughput screening) assay (Illumina, San Diego, CA). Data was processed using Illumina's GenomeStudio 2.0, with the CNV partition plug-in for copy number variant analysis. The SNP-microarray genotype data were analysed to find HBD regions, using HomozygosityMapper (Seelow et al., 2009).

Homozygosity Mapper generated graphs showing red peaks for putative HBD regions on several different chromosomes. Each of these putative HBD regions was also checked at the genotype level to confirm haploidentity within suspected HBD regions and to exclude false-positive HBD peaks.

2.3. Whole exome sequencing

Whole exome sequencing (WES) for selected family members [BF04; III:1 and IV:6] was performed at The Centre of Applied Genomics (TCAG; www.tcag.ca) sequencing facility. The sequencing library for DNA samples was prepared using SureSelectXT2 Human All Exon V7, Agilent, 5191–4007, and SureSelectXT2 Reagent Kit, Agilent, G9621B kit (Agilent Technologies, Santa Clara, CA). Paired-end multiplex sequencing was performed on the Illumina NovaSeq 6000 SP sequencer. Pooled samples were loaded on one lane of the SP flow cell and subjected to paired-end sequencing, 150 cycles from each end for a total of 300 cycles as per the manufacturer's recommendations. Read alignment, QC steps, and variant calling were performed as previously described, using a custom in-house pipeline implemented on the Specialised Computing Cluster (SCC) at the Centre for Addiction and Mental Health (CAMH) (Harripaul et al., 2018).

2.4. WES data analysis

We used a filtering approach to identify rare mutations, keeping minor allele frequency [MAF] <1% (0.01) along with set parameters as described in Fig. 2. The filtered variants were further categorised mainly into homozygous and heterozygous variants, which were further annotated based on their functional role in pathogenicity, *i.e.* the type of mutation, such as missense variants, frameshift/non-frameshift, stop/gain, synonymous/nonsynonymous and splice site variants. We also focused on the HBD regions identified in each family (Supplementary File, Tables S1 &S2).

2.5. In silico analysis

In addition, HOPE analysis (Venselaar et al., 2010) was performed for the prediction of the effect of mutation/change of amino acid on conservation, protein chemistry and 3D structure prediction of protein caused by relevant mutation and domain changes. The domain annotation predicted from the HOPE analysis was also confirmed in the SMART (Simple Modular Architecture Research Tool) and Uniprot databases. The mutation was also checked in the protein-paint database (https://proteinpaint.stjude.org) to confirm novelty and that it has not previously been associated with BD or other psychiatric condition in any population.

2.6. Segregation validation

Finally, the selected variants from WES data were genotyped *via* standard PCR using specific primers (Supplementary File, Table S3), and the amplified products were Sanger-sequenced for validation. The segregation of these variants was checked in the available members of the families.

3. Results

3.1. Family BF04

We were able to recruit seven members (III:1, III:2, IV:3, IV:4, IV:6, IV:7, IV:8) of the family BF04, who were then sampled. Based on the clinical presentation of the phenotype, four of the family members (IV:3, IV:4, IV:6, IV:7) were analysed by Illumina Infinium CoreExome SNP Microarray. For homozygosity mapping, individuals (IV:3, IV:4, and IV:5) were taken as cases, and individual (IV:7) as a control. HomozygosityMapper indicated three candidate HBD regions, on chromosomes (Chr) 3, 7, and 20 (Fig. 3). The HBD regions/loci details are provided in (Supplementary Table S1).

Whole exome sequencing was performed for the proband (IV:6) and mother (III:1). The steps followed for WES variants filtration are indicated in Fig. 2. Two variants were obtained after filtration and annotation of exome sequencing data; (i) a homozygous non-synonymous SNV in *RELN* (Reelin), (7:103276895C>T (hg19); NM_005045.4: c.2090G>A; p.(Gly697Asp)), located on Chr 7, and (ii)) a homozygous

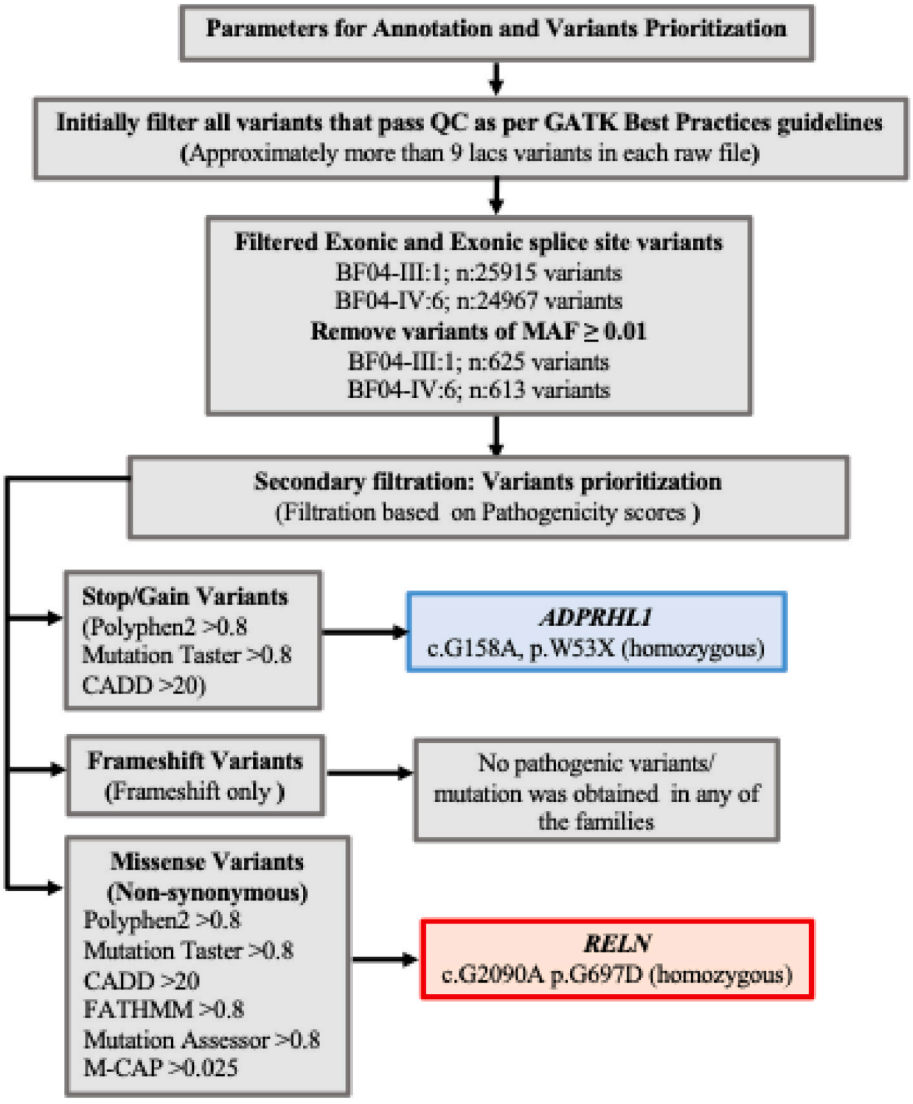

Fig. 2. Flow chart showing variant filtration steps from the whole exome sequencing data in Family BF04. The score mentioned represents the cut-off values for each type of variant which describes the pathogenicity/deleterious mutation. MAF: Minor allele frequency, n: number of variants, c. Chromosomal position, p. Amino acid position, *RELN*: Reelin, *ADPRHL1*: ADP-Ribosyl hydrolase Like 1, Polyphen2: Polymorphism phenotyping v2, CADD: Combine annotation dependent, FATHMM: Functional analysis through hidden Markov model (v2.3), M-CAP: Mendelian Clinically Applicable Pathogenicity.

stop/gain variant in *ADPRHL1* (ADP-Ribosyl-hydrolase Like 1), (13:114107595C>T; NM_138430.4:c.158G>A:p.Trp53*), located on Chr 13. The *RELN* variant was found within the 43.6 Mb HBD region (Supplementary Table S1) and segregated in the BD-severely affected family members, as confirmed through Sanger sequencing. An additional variant within the Chr 7 HBD region (*CALD1*; 7:134617932C>A; NM_033157.4; c.412C>A; p.(Gln138Lys)) was excluded, as most predictive algorithms suggest the variant is benign/tolerated (Supplementary Table S2). The details of the WES variants are provided in Table 2. Integrated Genomics Viewer (IGV; https://www.broadinstitute.org/scientific-community/software/integrative-genomics-viewer) images of variant reads are represented in (Supplementary File, Fig. S1a & b), and the chromatograms are represented in (Fig. 4 & Supplementary File, Fig. S2). The *RELN* c.2090G>A is only present in the gnomAD South Asian data, with the minor allele frequency (MAF) = 0.0006816 and no reported homozygotes (gnomAD v4.1.0; accessed 12 Jan 2026). Copy number variations (CNV) analysis did not identify any likely candidate genomic variants.

3.2. *In silico protein analysis of RELN Gly697Asp*

To predict the effect of the identified variant p.(Gly697Asp) that was located in the protein-coding region (exon 18) of the human *RELN* gene, including the effects on protein conformation or 3D structure, *in silico* protein analysis was performed *via* HOPE. This provides insight into the predicted effects of amino acid change on protein chemistry and mechanism. The normal and mutant 3D structures of the RELN protein are represented in (Fig. 5a, b& c). The change in an amino acid residue p.(Gly697Asp) is shown in Fig. 6. Each amino acid carries different properties depending upon its size, charge, and hydrophilic and hydrophobicity value. In our current findings in the *RELN* gene, the wild-type (normal) residue (glycine; G) has been replaced by aspartic acid (D), at position 697 in the Reelin protein. The mutant residue (aspartic acid) is larger than glycine (wild-type residue) and is negatively charged, while glycine is neutral and has greater hydrophobicity than aspartic acid.

Gly697Asp is located in one of seven EGF (epidermal growth factor) domains, which is 29 amino acids long (position 673–701). The domain annotation predicted from the HOPE analysis was also confirmed in the

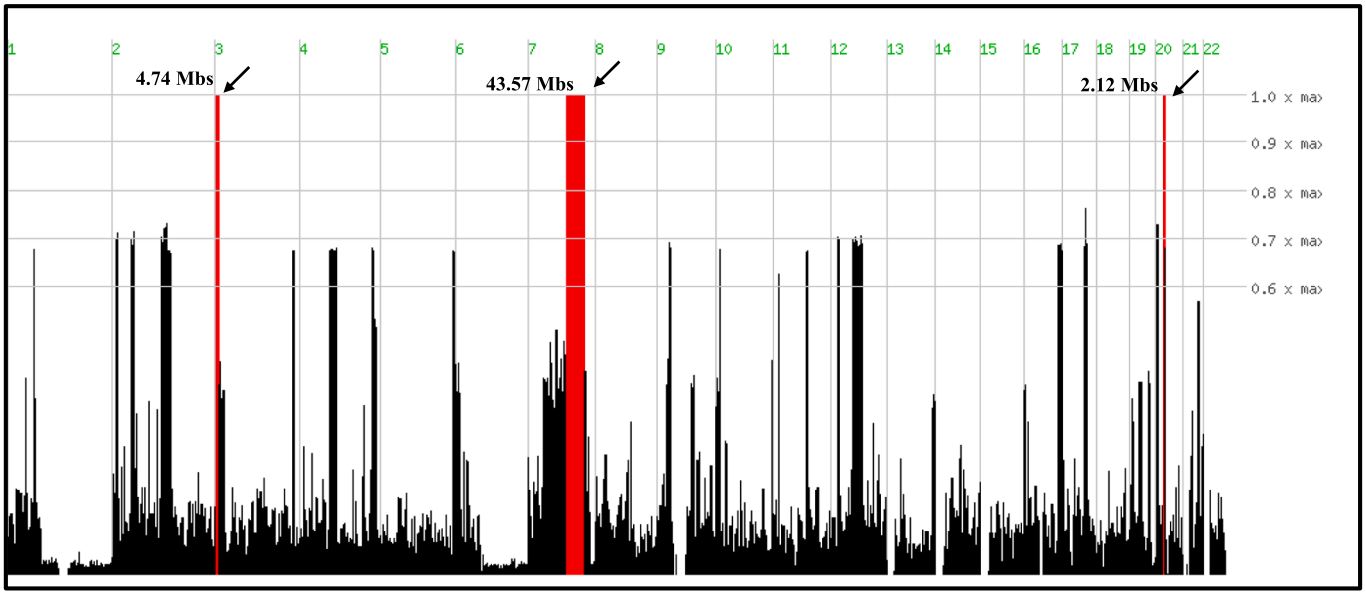

Fig. 3. Graphical representation of homozygosity mapping results using HomozygosityMapper. Plot showing shared homozygous regions as red bars on chromosomes 3, 7, and 20. The top x-axis of the graph represents the chromosome number. The y-axis represents a threshold homozygosity score of 0.8 [scores below 0.8 are non-significant (black lines), while those above 0.8 are suggestive homozygous regions (red lines)].

Uniprot and SMART databases (Supplementary File, Fig. S3). Moreover, the wild-type residue (Gly) is conserved across vertebrate lineages (UCSC genome browser, Supplementary Fig. S4). Based on HOPE conservation scores as well as a wide range of predictive algorithms including AlphaMissense, CADD, Polyphen2, ClinPred, and M-CAP (see Supplementary Table S2), p.(Gly697Asp) is likely to be damaging to the Reelin protein function and/or stability.

EGF repeats are commonly found in either secreted proteins or the extracellular domain of membrane proteins. The domain's three-dimensional structure is encoded by six cysteine residues that form three disulfide bonds. Many EGF domains carry posttranslational modifications, specifically *O*-glycans, which are often critical for the correct function of the protein (Haltom and Jafar-Nejad, 2015). The Gly697 residue lies within a motif for *O*-glycan *N*-acetylglucosamine (GlcNAc) addition (consensus motif: CXXGX(S/T)**G**XXC; RELN: CDPGFS**G**PAC; G697 residue in bold type) (Haltom and Jafar-Nejad, 2015). This consensus motif forms a β-hairpin that has a structural role in the protein (Copley et al., 2001; Ranaivoson et al., 2016). Thus, the substitution of an aspartic acid residue at Gly697 in the consensus motif would likely disrupt this motif, prevent GlcNAc addition, and disrupt the function of this EGF domain.

Additionally, the p.(Gly697Asp) mutation is not listed in the protein-paint database (Fig. 6), and it has not previously been associated with BD or any other psychiatric condition in any population, according to the protein-paint database (www.proteinpaint.stjude.org), which supports the novelty of this variant.

4. Discussion

Family BF04 was a BD-affected consanguineous family, in which an autosomal recessive mode of inheritance was consistent with the observed segregation of BD. In the studied family (BF04), two variants were obtained after filtration and annotation of exome sequencing data, which include a missense variant in *RELN* (NM_005045; c.2090G>A; p.(Gly697Asp)) and a stop/gain variant in *ADPRHL1* (c.158G>A:p.Trp53*). *ADPRHL1* is not a known gene for psychiatric disorders such as MDD, BD, and SCZ and has low expression in the brain; the variant also did not segregate with the disease in the family. On the other hand, *RELN* (c.2090G>A), as expected, given its location with a large HBD region, was found to segregate with BD in the affected members. In the family, the parents were heterozygous (G/A), while all the affected members were homozygous (A/A), and the available healthy sibling carried a homozygous wild-type allele (G/G) for the identified variant (NM_005045; c.2090G>A) in *RELN*.

RELN lies within the identified homozygous region (43.57 Mb) on chromosome 7, indicated by the run of homozygosity in the family. Through joint meta-analysis, this 7q22 region has previously been reported in association with BD and SCZ (Badner and Gershon, 2002), and this has been further corroborated through other studies reporting *RELN* association for SCZ (Luo et al., 2019; Sobue et al., 2018; Yin et al., 2020), and BD (Goes et al., 2010; Ovadia and Shifman, 2011). In addition, *RELN* has also been observed in association with other neurological disorders, including autism spectrum disorder (ASD), (Lammert and Howell, 2016), autosomal dominant epilepsy (Fang et al., 2022; Michelucci et al., 2017), and autosomal recessive neuronal migration disorders (Guerrini and Parrini, 2010). ClinVar (www.ncbi.nlm.nih.gov/clinvar/; accessed 19 Jan 2026) lists 62 *RELN* variants as either pathogenic or likely pathogenic for autosomal recessive lissencephaly or Norman-Roberts syndrome, all of which are loss-of-function (LoF) or likely LoF; 31 variants either pathogenic or likely pathogenic for familial temporal lobe epilepsy 7 (autosomal dominant), predominantly LoF/likely LoF, but with three missense variants included; two missense variants for neurodevelopmental disorder, either as a variant of uncertain significance or conflicting classification of pathogenicity; also one variant as likely pathogenic for schizophrenia. *RELN* is considered a "high confidence" gene for ASD, with an SFARI score of 1 and an Eagle score of 7.2, with 228 variants reported (gene.sfari.org/database/human-gene/RELN; accessed 19 Jan 2026).

However, in the current study, the variant (c.2090G>A; p.(Gly697Asp)), found in exon 18 of *RELN,* has not been reported previously with any of the above-mentioned disorders and is present at a very low frequency in gnomAD v4.1.0 control populations (MAF = 6.816E-04 in South Asians, MAF = 3.908E-05 overall, and a single homozygote in over 800,000 individuals). Moreover, there are currently no reports of this variant in ClinVar (accessed 4 Oct 2023), though other missense variants close by, and even within the same EGF domain, have been listed in ClinVar as variants of uncertain significance, in association with Lissencephaly 2 (autosomal recessive; also known as Norman-Roberts syndrome) and epilepsy. Brain MRI for two of the affected siblings (II:4 and II:6) show no signs of lissencephaly or pachygyria

Table 2
Whole exome sequencing identified and filtered variants in the family BF04.

Exome Identified Variants	Chr. No.	Variant Type	Exon	Cytoband/Location (hg19)/ Change	Amino Acid Change	MAF (Global)	MAF (SAS)	Mutation Taster Score	MCAP Score	FATHM Score	Segregated Yes/No	Known/Novel Gene for		Phenotypes
												Psychiatric condition	Neurological disorder	
RELN	7	Non-synonymous (missense) Homozygous	18	7q22.1 103276895C>T; NM_005045.4 c.2090G>A	p. (G697D)	3.908E-05	6.816E-04	1	0.043	2.26	Segregated in BD affected	Yes	Yes	SCZ, Autism, Epilepsy, autosomal dominant (ETL7: MIM 616436), Neuronal migration disorders, autosomal recessive (Lissencephaly 2: MIM 257320)
ADPRHL1	13	Stop/gain Homozygous	1	13q34 114107595C>T; NM_138430.4 c.158G>A	p.W53*	1.859E-06	0	1	–	–	No	No	No	NA

RELN: Reelin; *ADPRHL1*: ADP-Ribosyl hydrolase Like 1; c. chromosomal position; p. amino acid change position; G: glycine; D: aspartic acid; W: tryptophan; X: stop codon; MAF: Minor allele frequency (from gnomAD v4.1.0 (accessed 12 Jan 2026)); SAS: South Asians; NA: Not applicable.

Fig. 4. Electropherograms using Sanger sequencing traces of *RELN* (c.2090G > A). The arrow indicates the position of the nucleotide change.

(Supplementary Fig. S5), however, quantitative analysis was not possible, and thus, more subtle signs cannot be excluded for these family members. Other clinical features, such as profound intellectual disability and epilepsy, are clearly not present in BF04 members.

RELN encodes the important secreted glycoprotein reelin, which is approximately 440 kDa in size. Reelin is composed of multiple domains, including a Reeler domain, F-spondin domain (N-terminal domain), followed by an H region and 8 Reelin-specific repeats (RR), and then a highly basic region (Ranaivoson et al., 2016). Each RR is divided into sub-repeats termed A and B, which are homologous and separated by an EGF domain (Ichihara et al., 2001; Ranaivoson et al., 2016). The variant found in the current study, p.(Gly697Asp), is in one of the seven EGF

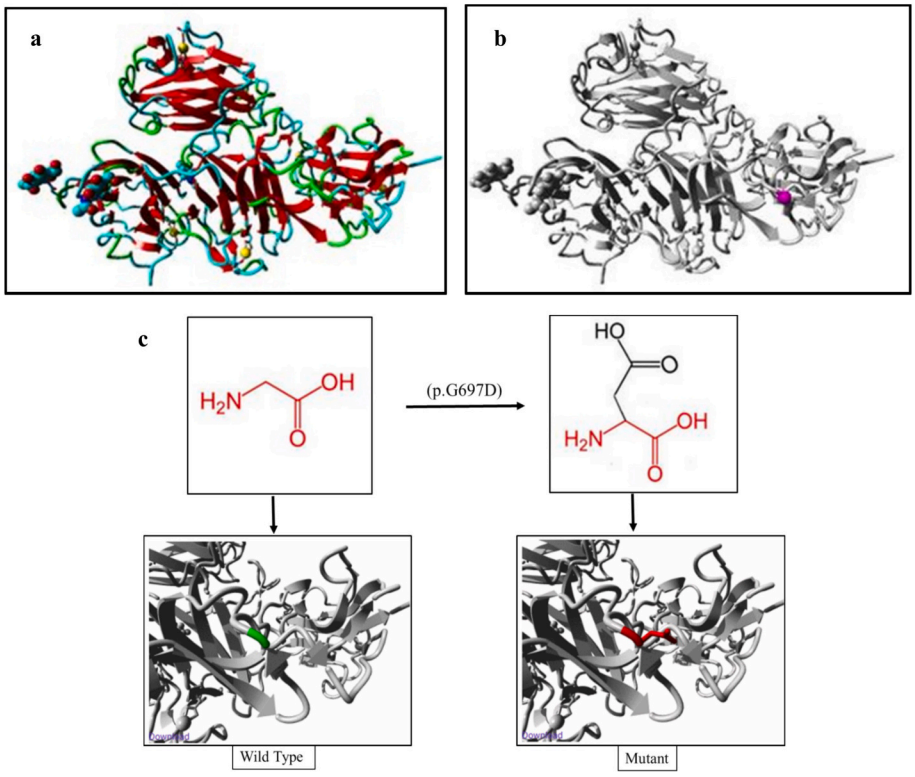

Fig. 5. 3D Structure (ribbon presentation) of Reelin protein. **(a)** Ribbon presentation of Reelin protein (normal). Blue represents the α-helix, red represents β-strands, the turns are coloured green, the 3/10 helix is coloured yellow and the random coils are in cyan. **(b)** Ribbon presentation of Reelin protein (mutant) in grey. The side chain of the mutated residue (glycine to aspartic acid) is represented as a small ball in magenta. **(c)** The figure shows the glycine (G) amino acid change into aspartic acid (D). The backbone is red (the same for each amino acid), while the side chain is unique and the change in an amino acid is black. In the ribbon structure, the protein is in grey, green represents wild-type G residue and red represents mutant (D) residue.

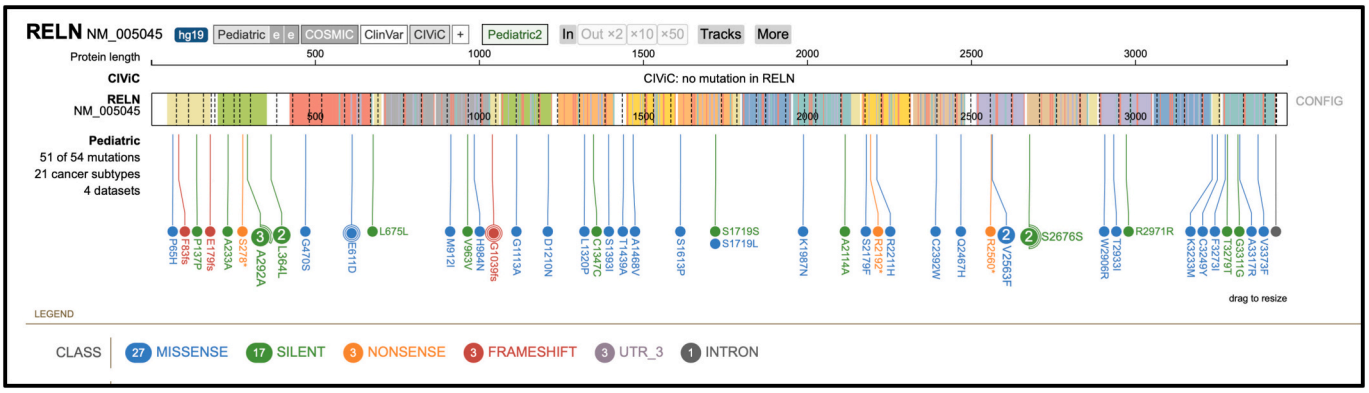

Fig. 6. The figure shows reported mutations of *Reln*. The image was obtained *via* the Protein Paint tool (PeCan Data Portal: https://proteinpaint.stjude.org).

domains, 29 amino acids long (position 673–701). The mutation was analysed using the HOPE tool, which showed that the wild-type residue (G) is fully conserved across vertebrate evolution (Supplementary Fig. S4), as are all the other GlcNAc *O*-glycan addition motif residues, critical for its interactions and binding to other molecules, suggesting that the 697Asp variant would likely be damaging to the Reelin protein function. Moreover, the differences in chemical nature and physical properties within a 3D protein structure of Asp *versus* Gly would also support the likelihood of this disruption, because the Gly (wild-type) residue is the most flexible among all residues and this flexibility is usually necessary for the protein function. Aspartic acid, however, at this position in the EGF domain could potentially disturb and abolish the domain function. Studies have indicated multiple functions of Reelin in the brain, where it plays a critical role during the development of synapses and their regulation (synaptic plasticity), as it is integral for the proper positioning of neurons, postnatally (Lammert and Howell, 2016). It is also important for neuronal migration learning, memory (Beffert et al., 2005), and the formation of spines in the adult brain (Ovadia and Shifman, 2011). Thus, the substitution of an Asp at Gly697 in the consensus motif would likely disrupt this motif and lead to preventing GlcNAc addition, subsequently disrupting the function of this EGF domain, which might result in impaired Reelin function and increase the susceptibility for BD.

Additionally, a few studies have identified other *RELN* variants; however, among the reported data, a study by Goes et al. (2010) highlighted the sex-specific transmission of an intronic SNP rs362719 (risk allele C) in *RELN,* particularly associated with BD in females. Moreover, some functional studies have also determined the lower expression of

reelin mRNA and protein levels in GABAergic neurons of patients with BD and SCZ (Fatemi et al., 2000; Guidotti et al., 2000; Veldic et al., 2007), which is influenced by epigenetic modifications, specifically the hypermethylation of CpG island upstream of the promoter sequences, in the prefrontal cortex of patients with BD and SCZ (Grayson et al., 2005), thus suggesting an association with BD and SCZ susceptibility.

Thus, the current study provides support for the association of *RELN* to BD aetiology, which is also reported by previous studies. However, the reported variant in the current study is novel. Despite this, there are some limitations to our study; first, our findings are based on only one consanguineous BD-affected family, thus establishing causality within a single family is not realistic unless supported by animal model data. Second, we were unable to fully evaluate in detail the relationship between variant and phenotype within the same family due to the unavailability of an affected female (III:2) sample. Third, WES focuses on coding regions and thus misses crucial regulatory regions of the genome, including *RELN* intronic variants that could be etiologically relevant in the case of the family BF04.

5. Conclusion

To sum up, we identified the rare variant NM_005045; c.2090G>A; p.(Gly697Asp) in the gene *RELN*, which has not previously been reported in association with BD. While a range of phenotypes, including lissencephaly, polymicrogyria, myoclonic dystonia, epilepsy, as well as autism and schizophrenia have been reported in association with rare damaging heterozygous *RELN* variants, to date only lissencephaly, cerebellar hypoplasia, and pachygyria have been reported for biallelic variants. The BF04 family with a homozygous missense *RELN* variant show no signs of these brain anomalies, and instead present with either BD or psychosis, more akin to some of the reported heterozygous variants. However, based on the study limitations, future functional studies are needed to expand this study for a better understanding of the nature of the identified novel genetic variant. Identifying the *RELN*-associated pathways in BD would also be useful and may provide valuable insights into the role of *RELN* in BD, with implications for diagnosis, and as Reelin-based treatments are currently being developed for Alzheimer's disease (Sandberg et al., 2025) therapeutic optimisation.

CRediT authorship contribution statement

Aisha Nasir Hashmi: Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation, Conceptualization. **Ricardo S. Harripaul:** Writing – review & editing, Formal analysis. **Tahir Muhammad:** Methodology, Investigation, Conceptualization. **Benjamin J. Lowther:** Methodology, Investigation. **Anna Mikhailov:** Validation, Investigation. **Zehra Agha:** Validation. **Raheel Qamar:** Writing – review & editing, Formal analysis. **John B. Vincent:** Writing – review & editing, Visualization, Validation, Project administration, Formal analysis, Conceptualization. **Maleeha Azam:** Writing – review & editing, Resources, Project administration, Conceptualization.

Funding source

The study was supported by the International Research Support Fellowship (IRSIP), awarded to Aisha Nasir Hashmi by the Higher Education Commission (HEC) of Pakistan.

Declaration of competing interest

None.

Acknowledgments

We thank all the subjects for their cooperation and participation in the study. We are also grateful to Dr. Rizwan Taj, head of the psychiatry department, and the staff members of the Pakistan Institute of Medical Sciences (PIMS), Islamabad, for their valuable support in sample collection. We are also thankful to the Higher Education Commission of Pakistan for providing an International Research Support Fellowship to Aisha Nasir Hashmi.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jad.2026.121554.

Data availability

All the relevant data is either mentioned in the manuscript, or mentioned as supplementary data in an additional file available with the manuscript. The written consent forms of the participating subjects or their legal representatives are available upon request. The raw whole-exome sequencing data are not publicly available due to privacy or ethical restrictions. Genotype and DNA sequencing data generated or analysed within this study are available upon an email request from the corresponding author. All authors read and approved the final manuscript.

References

Aadil, M., Munir, A., Arshad, H., Tariq, F., Anwar, M.J., Amjad, N., Akhlaq, A., 2017. Consanguinity associated with increased prevalence and severity of bipolar disorder in Pakistan: a case report highlighting the genetic link. Cureus. https://doi.org/10.7759/cureus.1467.

American Psychiatric Association, 2013. Diagnostic and Statistical Manual of Mental Disorders (DSM-5®). American Psychiatric Association Publishing, Washington D.C.

Badner, J.A., Gershon, E.S., 2002. Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. Mol. Psychiatry 7 (4). https://doi.org/10.1038/sj.mp.4001012.

Beffert, U., Weeber, E.J., Durudas, A., Qiu, S., Masiulis, I., Sweatt, J.D., Li, W.P., Adelmann, G., Frotscher, M., Hammer, R.E., Herz, J., 2005. Modulation of synaptic plasticity and memory by Reelin involves differential splicing of the lipoprotein receptor Apoer2. Neuron 47 (4). https://doi.org/10.1016/j.neuron.2005.07.007.

Bhamani, S.S., Farooq, S., Parpio, Y., Karmaliani, R., Asad, N., Azam, I., Pasha, O., 2016. Factors affecting depression among married women living in urban squatter settlements of Karachi, Pakistan. Open J. Epidemiol. 06 (01). https://doi.org/10.4236/ojepi.2016.61008.

Carta, M.G., Zimbrean, P.C., Fantini, M.C., Primavera, D., 2025. Addressing psychiatric symptoms in Wilson's disease: translational overlap with bipolar disorder and emerging therapeutic strategies. J. Clin. Med. 14 (16). https://doi.org/10.3390/jcm14165866.

Copley, R.R., Russell, R.B., Ponting, C.P., 2001. Sialidase-like asp-boxes: sequence-similar structures within different protein folds. Protein Sci. 10 (2). https://doi.org/10.1110/ps.31901.

Fang, X.Q., Zhang, R.R., Liu, X.W., 2022. Heterozygous missense mutation of the RELN gene is one of the causes of epilepsy. Neurol. Res. 44 (3). https://doi.org/10.1080/01616412.2021.1979748.

Fatemi, S.H., Earle, J.A., McMenomy, T., 2000. Reduction in Reelin immunoreactivity in hippocampus of subjects with schizophrenia, bipolar disorder and major depression. Mol. Psychiatry 5 (6). https://doi.org/10.1038/sj.mp.4000783.

Goes, F.S., Willour, V.L., Zandi, P.P., Belmonte, P.L., MacKinnon, D.F., Mondimore, F.M., Schweizer, B., DePaulo, J.R., Gershon, E.S., McMahon, F.J., Potash, J.B., 2010. Sex-specific association of the reelin gene with bipolar disorder. Am. J. Med. Genet. B Neuropsychiatr. Genet. 153 (2). https://doi.org/10.1002/ajmg.b.31018.

Goes, F.S., Pirooznia, M., Parla, J.S., Kramer, M., Ghiban, E., Mavruk, S., Chen, Y.C., Monson, E.T., Willour, V.L., Karchin, R., Flickinger, M., Locke, A.E., Levy, S.E., Scott, L.J., Boehnke, M., Stahl, E., Moran, J.L., Hultman, C.M., Landén, M., Potash, J. B., 2016. Exome sequencing of familial bipolar disorder. JAMA Psychiatry 73 (6). https://doi.org/10.1001/jamapsychiatry.2016.0251.

Grayson, D.R., Jia, X., Chen, Y., Sharma, R.P., Mitchell, C.P., Guidotti, A., Costa, E., 2005. Reelin promoter hypermethylation in schizophrenia. Proc. Natl. Acad. Sci. USA 102 (26). https://doi.org/10.1073/pnas.0503736102.

Guerrini, R., Parrini, E., 2010. Neuronal migration disorders. In *Neurobiology of Disease* 38 (2). https://doi.org/10.1016/j.nbd.2009.02.008.

Guidotti, A., Auta, J., Davis, J.M., Gerevini, V.D., Dwivedi, Y., Grayson, D.R., Impagnatiello, F., Pandey, G., Pesold, C., Sharma, R., Uzunov, D., Costa, E., 2000. Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: a postmortem brain study. Arch. Gen. Psychiatry 57 (11). https://doi.org/10.1001/archpsyc.57.11.1061.

Haltom, A.R., Jafar-Nejad, H., 2015. The multiple roles of epidermal growth factor repeat O-glycans in animal development. In Glycobiology 25 (10). https://doi.org/10.1093/glycob/cwv052.

Harripaul, R., Vasli, N., Mikhailov, A., Rafiq, M.A., Mittal, K., Windpassinger, C., Sheikh, T.I., Noor, A., Mahmood, H., Downey, S., Johnson, M., Vleuten, K., Bell, L., Ilyas, M., Khan, F.S., Khan, V., Moradi, M., Ayaz, M., Naeem, F., Vincent, J.B., 2018. Mapping autosomal recessive intellectual disability: combined microarray and exome sequencing identifies 26 novel candidate genes in 192 consanguineous families. Mol. Psychiatry 23 (4). https://doi.org/10.1038/mp.2017.60.

Hussain, R., Bittles, A.H., 1998. The prevalence and demographic characteristics of consanguineous marriages in Pakistan. J. Biosoc. Sci. 30 (2). https://doi.org/10.1017/S0021932098002612.

Ichihara, H., Jingami, H., Toh, H., 2001. Three novel repetitive units of reelin. Mol. Brain Res. 97 (2). https://doi.org/10.1016/S0169-328X(01)00307-2.

Kataoka, M., Matoba, N., Sawada, T., Kazuno, A.A., Ishiwata, M., Fujii, K., Matsuo, K., Takata, A., Kato, T., 2016. Exome sequencing for bipolar disorder points to roles of de novo loss-of-function and protein-altering mutations. Mol. Psychiatry 21 (7). https://doi.org/10.1038/mp.2016.69.

Kerner, B., 2014. Genetics of bipolar disorder. Appl. Clin. Genet. 7. https://doi.org/10.2147/TACG.S39297.

Lammert, D.B., Howell, B.W., 2016. RELN mutations in autism spectrum disorder. In: Frontiers in Cellular Neuroscience, 10. https://doi.org/10.3389/fncel.2016.00084. Number MAR2016.

Lan, T.H., Beaty, T.H., Raymond DePaulo, J., McInnis, M.G., 2007. Parent-of-origin effect in the segregation analysis of bipolar affective disorder families. Psychiatr. Genet. 17 (2). https://doi.org/10.1097/YPG.0b013e328013e604.

Luo, X., Chen, S., Xue, L., Chen, J.H., Shi, Y.W., Zhao, H., 2019. Snp variation of RELN gene and schizophrenia in a Chinese population: a hospital-based case-control study. Front. Genet. 10. https://doi.org/10.3389/fgene.2019.00175.

Michelucci, R., Pulitano, P., Di Bonaventura, C., Binelli, S., Luisi, C., Pasini, E., Striano, S., Striano, P., Coppola, G., La Neve, A., Giallonardo, A.T., Mecarelli, O., Serioli, E., Dazzo, E., Fanciulli, M., Nobile, C., 2017. The clinical phenotype of autosomal dominant lateral temporal lobe epilepsy related to reelin mutations. Epilepsy Behav. 68. https://doi.org/10.1016/j.yebeh.2016.12.003.

Monson, E.T., Pirooznia, M., Parla, J., Kramer, M., Goes, F.S., Gaine, M.E., Gaynor, S.C., de Klerk, K., Jancic, D., Karchin, R., McCombie, W.R., Zandi, P.P., Potash, J.B., Willour, V.L., 2017. Assessment of whole-exome sequence data in attempted suicide within a bipolar disorder cohort. Complex Psychiatry 3 (1). https://doi.org/10.1159/000454773.

Ovadia, G., Shifman, S., 2011. The genetic variation of RELN expression in schizophrenia and bipolar disorder. PloS One 6 (5). https://doi.org/10.1371/journal.pone.0019955.

Ranaivoson, F.M., von Daake, S., Comoletti, D., 2016. Structural insights into reelin function: Present and future. In: Frontiers in Cellular Neuroscience, 10. https://doi.org/10.3389/fncel.2016.00137. Number MAY.

Rao, A.R., Yourshaw, M., Christensen, B., Nelson, S.F., Kerner, B., 2017. Rare deleterious mutations are associated with disease in bipolar disorder families. Mol. Psychiatry 22 (7). https://doi.org/10.1038/mp.2016.181.

Rice, J., Cloninger, C.R., Reich, T., 1978. Multifactorial inheritance with cultural transmission and assortative mating. I. Description and basic properties of the unitary models. Am. J. Hum. Genet. 30 (6).

Salehi, B., Khoz, S., Sadeghi, B., Amanat, M., Salehi, M., 2017. Genealogy study of three generations of patients with bipolar mood disorder type i. Indian J. Psychol. Med. 39 (4). https://doi.org/10.4103/IJPSYM.IJPSYM_300_16.

Sambrook, J., Russell, D.W., 2001. Molecular cloning: a laboratory manual (3-volume set). In: Molecular Cloning: A Laboratory Manual.

Sandberg, A., Puttagunta, S., Duval, N., Fleming, H., Koza, L., Hieber, K., Holsopple, J., Reyna, M., Paredes, D., Linseman, D.A., 2025. Immunocal®, a cysteine-rich whey protein, rescues reelin and reduces amyloid plaque burden in a transgenic amyloid-β protein precursor (hAβPPSweInd) mouse model of Alzheimer's disease. Journal of Alzheimer's Disease : JAD 106 (1). https://doi.org/10.1177/13872877251338323.

Seelow, D., Schuelke, M., Hildebrandt, F., Nürnberg, P., 2009. HomozygosityMapper - an interactive approach to homozygosity mapping. Nucleic Acids Res. 37(SUPPL, 2. https://doi.org/10.1093/nar/gkp369.

Smoller, J.W., Finn, C.T., 2003. Family, twin, and adoption studies of bipolar disorder. In American Journal of Medical Genetics - Seminars in edical Genetics 123. https://doi.org/10.1002/ajmg.c.20013. C (Number 1).

Sobue, A., Kushima, I., Nagai, T., Shan, W., Kohno, T., Aleksic, B., Aoyama, Y., Mori, D., Arioka, Y., Kawano, N., Yamamoto, M., Hattori, M., Nabeshima, T., Yamada, K., Ozaki, N., 2018. Genetic and animal model analyses reveal the pathogenic role of a novel deletion of RELN in schizophrenia. Sci. Rep. 8 (1). https://doi.org/10.1038/s41598-018-31390-w.

Toma, C., Shaw, A.D., Allcock, R.J.N., Heath, A., Pierce, K.D., Mitchell, P.B., Schofield, P. R., Fullerton, J.M., 2018. An examination of multiple classes of rare variants in extended families with bipolar disorder. Transl. Psychiatry 8 (1). https://doi.org/10.1038/s41398-018-0113-y.

Van Snellenberg, J.X., De Candia, T., 2009. Meta-analytic evidence for familial coaggregation of schizophrenia and bipolar disorder. Arch. Gen. Psychiatry 66 (7). https://doi.org/10.1001/archgenpsychiatry.2009.64.

Veldic, M., Kadriu, B., Maloku, E., Agis-Balboa, R.C., Guidotti, A., Davis, J.M., Costa, E., 2007. Epigenetic mechanisms expressed in basal ganglia GABAergic neurons differentiate schizophrenia from bipolar disorder. Schizophr. Res. 91 (1–3). https://doi.org/10.1016/j.schres.2006.11.029.

Venselaar, H., te Beek, T.A.H., Kuipers, R.K.P., Hekkelman, M.L., Vriend, G., 2010. Protein structure analysis of mutations causing inheritable diseases. An e-science approach with life scientist friendly interfaces. BMC Bioinformatics 11. https://doi.org/10.1186/1471-2105-11-548.

Vieta, E., Berk, M., Schulze, T.G., Carvalho, A.F., Suppes, T., Calabrese, J.R., Gao, K., Miskowiak, K.W., Grande, I., 2018. Bipolar disorders. Nat. Rev. Dis. Primers 4. https://doi.org/10.1038/nrdp.2018.8.

Wray, N.R., Visscher, P.M., 2010. Narrowing the boundaries of the genetic architecture of schizophrenia. Schizophr. Bull. 36 (1). https://doi.org/10.1093/schbul/sbp137.

Xue, S., Asif, M., Khoso, A.B., Shakoor, S., Umer, M., Burgos, L.A., Jones, B.D.M., Ortiz, A., Chaudhry, N., Chaudhry, I.B., Husain, N., Mulsant, B.H., Husain, M.I., 2026. Sociodemographic and clinical characteristics among individuals with bipolar disorder in Pakistan – a gender-focused analysis. J. Affect. Disord. 392. https://doi.org/10.1016/j.jad.2025.120146.

Yang, J., Manolio, T.A., Pasquale, L.R., Boerwinkle, E., Caporaso, N., Cunningham, J.M., De Andrade, M., Feenstra, B., Feingold, E., Hayes, M.G., Hill, W.G., Landi, M.T., Alonso, A., Lettre, G., Lin, P., Ling, H., Lowe, W., Mathias, R.A., Melbye, M., Visscher, P.M., 2011. Genome partitioning of genetic variation for complex traits using common SNPs. Nat. Genet. 43 (6). https://doi.org/10.1038/ng.823.

Yin, J., Lu, Y., Yu, S., Dai, Z., Zhang, F., Yuan, J., 2020. Exploring the mRNA expression level of RELN in peripheral blood of schizophrenia patients before and after antipsychotic treatment. Hereditas 157 (1). https://doi.org/10.1186/s41065-020-00158-6.