Pedigree-based study to identify GOLGB1 as a risk gene for bipolar disorder

Fa-rong Liu1,2,3,4, Yunqiang Zhou1,4, Yong Wang1, Ling-ling Huang1, Xian Zhang1, Hong Luo1, Su-ying Wu2, Hai-yan Lyu2, Li-huan Huang3, Huaxi Xu* and Yun-wu Zhang*© The Author(s) 2022

Bipolar disorder (BD) is a complex psychiatric disorder with strong heritability. Identification of new BD risk genes will help determine the mechanism underlying disease pathogenesis. In the present study, we carried out whole genome sequencing for a Chinese BD family with three affected members and three unaffected members, and identified multiple candidate causal variations, including a frameshift mutation in the GOLGB1 gene. Since a GOLGB1 missense mutation was also found in another BD pedigree, we carried out functional studies by downregulating Golgb1 expression in the brain of neonatal mice. Golgb1 deficiency had no effect on anxiety, memory, and social behaviors in young adult mice. However, we found that young adult mice with Golgb1 deficiency exhibited elevated locomotor activity and decreased depressive behaviors in the tail suspension test and the sucrose preference test, but increased depressive behaviors in the forced swim test, resembling the dual character of BD patients with both mania and depression. Moreover, Golgb1 downregulation reduced PSD93 levels and Akt phosphorylation in the brain. Together, our results indicate that GOLGB1 is a strong BD risk gene candidate whose deficiency may result in BD phenotypes possibly through affecting PSD93 and PI3K/Akt signaling.

Translational Psychiatry (2022) 12:390; https://doi.org/10.1038/s41398-022-02163-x

INTRODUCTION

Bipolar disorder (BD) is a complicated neuropsychiatric disorder that shows mood changes between mania and depression. BD can be classified into type I and type II, for which type I BD is characterized by the presence of a syndromal, manic episode, and type II BD is characterized by the presence of a syndromal, hypomanic episode, and a major depressive episode [1, 2].

BD affects nearly 2% of the world’s adult population and has a heritability of about 60–85% [3–5]. Genetic studies, such as genetic linkage studies, candidate gene studies, and genome-wide association studies (GWAS), have identified multiple BD susceptibility loci [6–9]. Although some of them, such as BDNF, ANK3, and CACNA1C, may be common susceptibility loci for BD, most identified loci are rare, implicating a polygenic contribution of common and rare variations to BD susceptibility [3, 10]. Since, so far, identified loci only explain a portion of BD occurrence, further investigation in affected pedigrees may identify additional genetic loci that contribute to BD susceptibility.

In the present study, we carried out whole genome sequencing (WGS) to identify rare susceptibility variations for BD in a Chinese BD pedigree. We identified multiple variations, including a nonsense mutation in the GOLGB1 gene in affected family members but not in unaffected family members. The GOLGB1 gene encodes GOLGB1/Giantin, a protein belonging to the golgin family members that reside in the Golgi stack and modulate vesicle trafficking [11]. Although GOLGB1 has been proposed to regulate protein glycosylation [12], ciliogenesis [13, 14], and osteogenesis and/or chondrogenesis [12, 15], the exact function of GOLGB1 has yet to be further elucidated. Since a missense mutation in the GOLGB1 gene was also found in affected but not unaffected members in another BD pedigree [4], we studied mice with reduced Golgb1 expression in the brain. The results showed that Golgb1 deficiency resulted in some behavior abnormalities resembling those found in BD patients, suggesting that GOLGB1 dysregulation may contribute to certain BD phenotypes.

RESULTS

We carried out WGS for three affected and three unaffected members in a Chinese BD family (Fig. 1). WGS analysis revealed a total of 5,472,225 single-nucleotide variations (SNVs) and 1,251,821 small insertions and deletions (INDELs) present in cases and controls combined. Among them, we filtered 940 SNVs and 142 INDELs that were potentially deleterious. Given the inheritance pattern of the pedigree, autosomal dominant genetic modifiers seem to be responsible for disease pathogenesis in this family. Therefore, from filtered variations, we further screened variations that were heterozygous in all three BD patients but not mutated in healthy members within this family as candidate causal variations. We found 23 SNVs and 3 INDELs, each of which located in one gene. We then performed PubMed (https://pubmed.ncbi.nlm.nih.gov/) literature research using each gene name and “bipolar” as keywords

1Xiamen Key Laboratory of Brain Center, The First Affiliated Hospital of Xiamen University, and Fujian Provincial Key Laboratory of Neurodegenerative Disease and Aging Research, Institute of Neuroscience, School of Medicine, Xiamen University, Xiamen, Fujian 361102, China. 2Xiamen City Xianyue Hospital, Xiamen, Fujian 361012, China. 3The Third Clinical Medical College, Fujian Medical University, Fuzhou, Fujian 350122, China. 4These authors contributed equally: Fa-rong Liu, Yunqiang Zhou. 5email: yunzhang@xmu.edu.cn

Received: 15 March 2022 Revised: 6 September 2022 Accepted: 8 September 2022
Published online: 17 September 2022
We found that NEU1 [16], TBC1D16 [17], and GOLGB1 [4] have previously been linked to BD (Table 1). Interestingly, one previous study identified a missense mutation in the GOLGB1 gene (c.983 T > C, p.V328A, in exon 9, NM_004487) in affected members but not unaffected members in a Caucasian BD family [4]. Here we identified a frameshift INDEL in the GOLGB1 gene (c.8743delC, p.H2915fs, in exon 15, NM_004487) in affected members but not in unaffected members in this Chinese BD family. Therefore, we targeted GOLGB1 for further analysis.

The human GOLGB1 gene is a big gene on chromosome 3 and has 27 exons with multiple splicing variants. Focusing on the two mutations potentially associated with BD, we sequenced entire exons 9 and 15 of GOLGB1 in 182 sporadic BD patients and 146 controls. However, we did not identify the two or other mutations in the studied subjects, suggesting that the two mutations are rare.

Because our identified GOLGB1 INDEL leads to a predicted early stop of the coding sequence and truncation of the protein, we also studied whether GOLGB1 deficiency causes BD-like phenotypes in animals. We first packaged AAVs that express different mouse Golgb1 shRNAs and tested their efficiency in down-regulating Golgb1 in mouse primary neurons. We found that all three tested Golgb1 shRNAs significantly reduced mouse Golgb1 mRNA levels, with shGOLGB1 #2 showing the most effect on reducing Golgb1 compared to the other two (Fig. 2A).

Next, we delivered AAVs expressing shGOLGB1 #2 or scrambled controls into the brain of P0 mice via bilateral intracerebroventricular (i.c.v.) injection. GFP fluorescence represented the localization of AAVs and indicated that AAVs infected mostly hippocampal and cortical regions (Fig. 2B). We confirmed that shGOLGB1 #2 expression significantly reduced Golgb1 expression in mouse hippocampal tissues (Fig. 2C). Furthermore, GFP was

![Fig. 1 Pedigree of a Chinese family with three members diagnosed with BD.](image)

**Table 1.** Potential gene variations associated with bipolar disorder identified by WGS.

| Potential casual genes | Mutation types | Transcript | Exon | Coding | Protein | Association with BD in other studies |
|------------------------|----------------|------------|------|--------|---------|-------------------------------------|
| RAB3GAP1               | SNV-missense   | NM_001172435 | 25   | C2821G | P941A   | -                                   |
| GPD2                   | SNV-missense   | NM_001083112 | 9    | A1096G | I366V   | -                                   |
| CCR4                   | SNV-missense   | NM_005508    | 2    | G424A  | A142T   | -                                   |
| SCN10A                 | SNV-missense   | NM_006514    | 13   | C2015T | T672I   | -                                   |
| GOLIM4                 | SNV-missense   | NM_014498    | 4    | A334G  | S112G   | -                                   |
| ADH5                   | SNV-missense   | NM_000671    | 4    | C328T  | L110F   | -                                   |
| PELO                   | SNV-missense   | NM_015946    | 2    | A716G  | K239R   | -                                   |
| TTC37                  | SNV-missense   | NM_014639    | 11   | G829A  | G277S   | -                                   |
| NEU1                   | SNV-missense   | NM_000434    | 4    | C640T  | R214C   | [16]                                |
| MDC1                   | SNV-missense   | NM_014611    | 63   | C10551A| D3517E  | -                                   |
| SPA5G1                 | SNV-missense   | NM_172218    | 9    | C844T  | R282C   | -                                   |
| APX1                   | SNV-missense   | NM_175073    | 9    | C952A  | R318S   | -                                   |
| AKNA                   | SNV-missense   | NM_030767    | 3    | G577C  | V193L   | -                                   |
| COG6                   | SNV-missense   | NM_020751    | 12   | A1145G | K382R   | -                                   |
| MAP3K9                 | SNV-missense   | NM_031141    | 13   | A2936G | N979S   | -                                   |
| YLPM1                  | SNV-missense   | NM_019589    | 5    | T3638C | M1213T  | -                                   |
| PKD1L2*                | SNV-unknown    | unknown      | 16   | unknown| unknown | -                                   |
| CCDC40                 | SNV-missense   | NM_017950    | 20   | C3355T | P1119S  | -                                   |
| TBCD                   | SNV-missense   | NM_005993    | 20   | C1810T | P604S   | -                                   |
| CYS5A                  | SNV-missense   | NM_148923    | 1    | G2ST   | V9L     | -                                   |
| ELANE                  | SNV-missense   | NM_001972    | 2    | C100T  | R34W    | -                                   |
| DOT1L                  | SNV-missense   | NM_032482    | 20   | T2250G | C750W   | -                                   |
| TMRPSS15               | SNV-missense   | NM_002772    | 4    | G428T  | G143V   | -                                   |
| GOLGB1                 | INDEL-frameshift deletion | NM_004487 | 15 | 8743delC | H2915fs | [4]                               |
| EYS                    | INDEL-nonframeshift deletion | NM_001292009 | 19 | 2953_2961del | TDG085_987del | - |
| TBC1D16                | INDEL-frameshift deletion | NM_019020 | 5 | 1015delC | H339fs | [17] |

*The representative PKD1L2 transcript (NM_052892) is present in some human individuals but absent from the reference genome. Therefore, the effects of the identified SNV on its coding and protein sequences are unknown.
found to colocalize with the neuron marker NeuN (Fig. 2D) but not with the microglia marker Iba1 (Fig. 2E) or the astrocyte marker GFAP (Fig. 2F), suggesting that AAVs mostly infected neurons, i.e., Golgb1 was mostly downregulated in neurons.

We next investigated whether Golgb1 knockdown (KD) affects mouse behaviors. Two months after AAV infection, both male and female mice were subjected to various behavioral tests. Since both sexes are affected in this BD family, we combined data from mice with both sexes for comparisons. In the open field test, we found that Golgb1 KD mice were more active than control mice, as they exhibited significantly increased total travel distance and numbers of center entries (Fig. 3A). In the tail suspension test, we found that Golgb1 KD mice had less immobility time than control mice (Fig. 3B), implying a decrease of depression in Golgb1 KD mice. Consistently, Golgb1 KD mice had increased sucrose preference indicative of decreased depression compared control mice in the two-bottle choice sucrose preference test (Fig. 3C). Surprisingly, in the forced swim test, Golgb1 KD mice showed significantly
elevated immobility time, implying increased depression compared to controls (Fig. 3D). Together, these behaviors may resemble those found in BD patients, who exhibit mood change between mania and depression.

We also examined anxiety-like behaviors in Golgb1 KD mice. In the open field test, although Golgb1 KD mice exhibited increased numbers of center entries, their time spent in the center square was not different from that of controls (Fig. 3A). In the light/dark box test, both Golgb1 KD and control mice showed comparable time spent in the light box and similar light box entry numbers (Fig. 4A). In the elevated O-maze test (Fig. 4B) and the elevated plus-maze test (Fig. 4C), both Golgb1 KD and control mice had comparable time spent in the open arm and similar open arm entry numbers. Together, these results suggest that Golgb1 downregulation has no effect on anxiety-like behaviors in mice.

In the Y-maze test, Golgb1 KD mice showed no differences in their spontaneous alternation percentage compared to controls (Fig. 4D). In the novel object recognition test, Golgb1 KD mice also showed no differences in their discrimination on the novel and familiar objects compared to controls (Fig. 4E), suggesting that PSD93 protein reduction upon Golgb1 downregulation is not attributed to its gene expression alternation.

DISCUSSION

BD has been demonstrated to have a strong heritability [1, 18, 19]. However, although multiple genes have been identified as susceptibility loci for BD, they only account for a portion of BD occurrence. Additional studies in affected pedigrees shall help identify new disease-causing mutations and provide new insight into disease mechanisms.

In the present study, we carried out WGS for a Chinese BD family that shows an autosomal dominant mode of inheritance. From filtered variants, we identified 23 SNVs and three INDELs as candidate causal variations, as they were potentially malignant and heterozygous in all affected members but not mutated in unaffected members within this family. Among the 26 genes carrying SNVs or INDELS potentially associated with BD, we found that GOLGB1, NEU1, and TBC1D16 were previously linked to BD to some extent.

The GOLGB1 gene encodes GOLGB1/Giantin, a protein belonging to the golgin family that resides and modulates the vesicle trafficking network within the Golgi stack [11]. The exact function of GOLGB1 remains largely unclear, though several studies found that GOLGB1 could regulate protein glycosylation [12] and

Fig. 3  Downregulation of Golgb1 causes BD-like behaviors in mice. Mice of both sexes injected with AAVs expressing shGOLGB1 or a scrambled control shRNA were subjected to behavioral tests at 2 months of age. A In the open field test, mice were studied for their total travel distance, their numbers of center entries, and their duration time in the center. B In the tail suspension test, mice were studied for their immobility time during the tail suspension. C In the 2-bottle choice sucrose preference test, mice were studied for their preference for sucrose. D In the forced swim test, mice were studied for their immobility duration in water. n = 27 (13 females and 14 males) for shGOLGB1 mice, n = 19 (ten females and nine males) for scrambled control mice; ns not significant, *p < 0.05, **p < 0.01, ***p < 0.001; two-tailed Student's t-test.
modulate ciliogenesis by controlling dynein-2 localization [13, 14]. In addition, homozygous loss of function mutation of \textit{Golgb1} leads to several osteochondrodysplasia and late embryonic lethality in rats but only cleft palate in mice [12, 15], suggesting that GOLGB1 may regulate osteogenesis and/or chondrogenesis. Herein, we identified a frameshift INDEL in the \textit{GOLGB1} gene in this Chinese BD family. Previously a \textit{GOLGB1} missense mutation was also identified in a Caucasian BD family [4]. Therefore, we further studied the potential contribution of GOLGB1 deficiency to BD. We did not identify the two mutations or any other mutations in exons 9 and 15 of the \textit{GOLGB1} gene in 182 sporadic BD patients and 146 controls, implying that BD-associated \textit{GOLGB1} mutations may be rare. We then used AAV infection to downregulate \textit{Golgb1} expression in the mouse brain. Although mice with \textit{Golgb1} downregulation were morphologically normal, they exhibited elevated locomotor activity in the open field test and anti-depressive activities in the tail suspension test and the 2-bottle choice sucrose preference test, but depressive behavior in the forced swim test. These behaviors resemble the dual character of BD patients with both mania and depression.

PSD93 is a scaffold protein in the post-synaptic density of excitatory neurons and regulates synaptic plasticity. Mutations affecting \textit{DLG2}, the gene encoding PSD93, have been associated with a series of neurodevelopmental psychiatric disorders, including schizophrenia and potentially BD [20, 21]. Dysregulation of the PI3K/Akt signaling pathway has been found and proposed as an important cause of BD [22]. Here we found that down-regulation of \textit{Golgb1} in the brain also reduced PSD93 protein levels and Akt phosphorylation. Therefore, our results suggest that GOLGB1 deficiency may cause the occurrence of certain BD phenotypes, possibly through altering multiple pathways such as PSD93 and PI3K/Akt signaling.

Interestingly, we also identified candidate causal variations in another two Golgi-related genes, \textit{GOLIM4} and \textit{COG6}. GOLIM4...
two-tailed Student’s t-test. Protein levels of phosphorylated Akt (pAkt) were quantified and normalized to those of Akt for comparison. n = 6 for each group (B). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001; two-tailed Student’s t-test. C RNAs were extracted from cortical and hippocampal tissues of treated mice. The mRNA levels of PSD93 and PSD95 were determined by qRT-PCR and normalized to those of β-actin for comparison. n = 11 for shGOLGB1 mice, n = 8 for scrambled control mice. ns: not significant, two-tailed Student’s t-test.

Fig. 5 Downregulation of GOLGB1 reduces PSD93 protein levels and Akt phosphorylation. A, B Cortical (Cor) and hippocampal (Hip) tissues from mice injected with AAVs expressing shGOLGB1 or a scrambled control shRNA were collected. Equal amounts of protein lysates were subjected to western blot to study indicated proteins (A). Protein levels of PSD93 were quantified and normalized to those of GAPDH for comparison. n = 8 for shGOLGB1 mice, n = 5 for scrambled control mice. Protein levels of phosphorylated Akt (pAkt) were quantified and normalized to those of Akt for comparison. n = 6 for each group (B). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001; two-tailed Student’s t-test.

In summary, herein, we have identified several candidate causal variations in a Chinese BD family. Through combining functional investigation, we have demonstrated that GOLGB1 is a strong BD risk gene candidate whose deficiency may result in BD phenotypes possibly by affecting PSD93 and PI3K/Akt signaling. Further corroboration in large patient cohorts and additional functional studies shall help conclude the causality of GOLGB1 and other risk gene variations identified in this family.

MATERIALS AND METHODS
Human samples
Three BD patients who met the ICD-10 criteria of bipolar disorders in a Chinese family, including the grandfather, the father, and the daughter were diagnosed by two senior psychiatrists, and treated at Xiamen City Xianyue Hospital (Fig. 1). Other family members recruited in this study, including the mother, the uncle, and the grandmother were reportedly healthy. Details of the clinical features of the three affected members used in this study are compiled in Supplementary Table 1. Moreover, 182 sporadic BD patients and 146 healthy controls were recruited and tested for GOLGB1 mutations. This study was approved by the Medical Ethics Committee of Xiamen City Xianyue Hospital. Informed consent was obtained from participants.

Whole genome sequencing (WGS)
WGS was carried out at Novogene Bioinformatics Technology Co., Ltd (Beijing, China). Briefly, genomic DNA was extracted from peripheral blood and fragmented to an average size of ~350 bp. DNA library was created using established Illumina paired-end protocols and subjected to WGS using the Illumina Novaseq 6000 platform (Illumina Inc., San Diego, CA, USA) to generate 150-bp paired-end reads with a minimum coverage of 10x for ~98.5% of the genome (average sequencing depth over 30x).
The pairwise identity-by-descent (IBD) calculation in PLINK [42]. The IBD sharing was used to perform a wide scan for linkage in this family. This linkage analysis using merlin tools included minor allele frequencies from public control data sets as well as deleteriousness and conservation scores, enabling further filtering and assessment of the likely pathogenicity of variations.

To filter rare variations, we first selected variations with a MAF less than 0.01 in 1000 Genomic data (1000g_all) [35], esp6500siv2_all [9], gnomAD data (gnomAD_ALL and gnomAD_EAS) [10], and in-house Novo-Zhonghua exome database from Novogene. After discarding synonymous SNVs and small fragment nonframeshift (<10 bp) INDELs in the repeat region defined by RepeatMasker, we analyzed only nonsynonymous SNVs and INDELs occurring in exons or splice sites (splicing junction 10 bp). Variations were screened according to scores of SIFT [36], Polyphen [37], MutationTaster [38], and RepeatMasker, we analyzed only nonsynonymous SNVs and INDELs occurring in the repeat region.

The relationship between proband and parents was estimated using the pairwise identity-by-descent (IBD) calculation in PLINK [42]. The IBD sharing between the proband and parents in all trios is between 45 and 55%.

To better predict the harmfulness of variation, the American Committee of Xiamen University.

#### GOLOB1 sequencing
Genomic DNAs were extracted from BD patients and controls and used as PCR templates to amplify exons 9 and 15 of the Golgb1 cation system was analyzed at 10 DIV.

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#### Animal behavioral tests
Treated mice at 2 months of age, including ten female and nine male mice injected with AAVs expressing scrambled control shRNA and 13 female and 14 male mice injected with AAVs expressing shGOLGB1 #2 were subjected to various behavioral tests. All behavioral analyses were carried out in a two-bottle choice sucrose preference test, the Y-maze test, and the novel object recognition test were reported previously [43, 44]. Procedures for other behavioral tests were as the following.

For the two-bottle choice sucrose preference test, mice were first acclimated to two identical bottles, one filled with water and the other one filled with water containing 1% sucrose, for 36 h. Mice were then fasted overnight without food and water for 12 h. After fasting, mice were presented with water and 1% sucrose again. The two bottles were weighed and exchanged positions every 12 h. Three times. Sucrose preference was determined as the ratio of total sucrose to water consumption.

For the forced swim test, mice were placed in the water with a temperature of 25 °C in a cylinder (21 cm in diameter and 30 cm in height) for 7 min. Total immobility duration during the last 6 min was recorded for comparison.

The dark/light box consisted of one black/dark (15 cm × 20 cm × 25 cm) and one light (30 cm × 20 cm × 25 cm) plexiglass compartment that were connected by a tunnel. Mice were placed into the light box and allowed to move freely for 10 min. The time spent in the light box and the number of entries into the light box were recorded for comparison.

Elevated O-maze consisted of an elevated circular platform with two opposite quadrants enclosed and two open arms. Animals were placed in the center of one open arm and let explore open and closed arms for 5 min. The time spent in open arms and numbers of open arm entries were analyzed.

#### Western blot
Samples were lysed in TEN buffer containing 50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 2 mM EDTA, and 1% NP-40, supplemented with protease inhibitors and phosphatase inhibitors. Protein concentration was determined by BCA assay (BCA Protein Assay Kit, Thermo Fisher Scientific). Equal amounts of protein samples were subjected to SDS-polyacrylamide gel electrophoresis and PVDF membrane transfer. Proteins were identified by incubating with indicated primary antibodies and then with appropriate HRP-conjugated secondary antibodies. Protein band intensities were determined using the Image J software [45]. Antibodies used were: anti-GAPDH (Abways, ab0037), anti-mActin-F, 5′-GCCTCATTCAAGAGCATGTCAT-3′, and anti-mActin-R, 5′-GGAGAGAAACTGGGTGAAGGGTA-3′. The scrambled control shRNA sequence is 5′-CTTAAAGTTAACTGGCCCTCG-3′. Cultured mouse primary neurons were infected with AAV for 3 days in vitro (DIV) and analyzed at 10 DIV.

For in vivo injection, one microliter of AAV containing shGOLGB1 #2 or scrambled control shRNA (5 × 10**12** V.G/ml) was slowly injected into lateral ventricles (2 mm distance from ventral to skin and 2.5 from lambda suture to the eye) of 14 male mice injected with AAVs expressing scrambled control shRNA and 13 female and 14 male mice injected with AAVs expressing shGOLGB1 #2 were subjected to various behavioral tests. All behavioral analyses were carried out in a two-bottle choice sucrose preference test, the Y-maze test, and the novel object recognition test were reported previously [43, 44]. Procedures for other behavioral tests were as the following.

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#### Quantitative real-time PCR (qRT-PCR)
Total RNAs were isolated using TRIzol reagent (Life Technologies). After reverse-transcription using Superscript III transcriptase (Invitrogen), samples were analyzed on a LightCycler® 480 Real-Time PCR System (Roche Applied Science, Basel, Switzerland). PCR primers used are the following: mGOLGB1-F, 5′-GCTCTACAAGAGCATGTCAT-3′; mGOLGB1-R, 5′-GCTGATCTTGAGCATGAC-3′; mPSD93-F, 5′-CAAAGGGTCTGAGTACGACA-3′; mPSD93-R, 5′-CCCTGATCATTGACCTTTC-3′; mPSD95-F, 5′-TGAGAGAAACTGGGTGAAGGGTA-3′; mPSD95-R, 5′-CCCTGATCATTGACCTTTC-3′; mActin-F, 5′-GGGTATGGTACCTTCACTC-3′; mActin-R, 5′-CCAGAGAAACTGGGTGAAGGGTA-3′.
Immunostaining
Brain samples of mice were fixed in 4% paraformaldehyde, sequentially dehydrated in 20, 25, and 30% sucrose solution, frozen in OCT compound, and then prepared as 15-µm slices. Slices were incubated with indicated primary antibodies overnight at 4 °C, followed by incubation with appropriate secondary antibodies conjugated with fluorescein and DAPI for 60 min at room temperature. The fluorescence microscope images were acquired by an A1R (Nikon) confocal microscope. Antibodies used were: anti-NeuN (Cell Signaling Technology, #9404S), anti-GFAP (ProteinTech, #16825-1-AP), anti-IL-1β (Wako, #019-79471), and Alexa fluor 594-conjugated goat anti-rabbit IgG (H+C L) secondary antibody (Thermo Fisher Scientific, #A-11012).

Statistical analyses
Statistical analyses were performed using GraphPad Prism 8.3 software (GraphPad Software). Sample sizes were determined based on the assumption of a normal distribution and similar variability between experimental groups. No animals or samples were excluded from or randomized in the analyses. The normality distribution was corroborated using the Kolmogorov–Smirnov test. Two-tailed Student’s t-test was used for the comparison of two independent groups. The variances were similar between groups. Data represent mean ± standard error of the mean (SEM). p < 0.05 was considered to be statistically significant.

DATA AVAILABILITY
The data sets generated in this study are available from the corresponding author upon reasonable request.

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ACKNOWLEDGEMENTS
The authors would like to thank members of this BD family and other participants who provided their genetic data. This work was supported by grants from the National Natural Science Foundation of China (82130039 and U21A20361 to Y-wZ), the National Key Research and Development Program of China (2018YFC2000400 to Y-wZ), The Guideline Project of Xiamen Science and Technology Plan (3502Z2014ZD1276 to F-rL), Fujian Clinical Research Center for Mental Disorders (No. 2021Y2020 to F-rL), and The Guideline Project of Xiamen Medical Hygiene (3502Z20199091 to S-yW).

AUTHOR CONTRIBUTIONS
F-rL, YZ, and Y-wZ designed the research. F-rL identified this Chinese BD family, collected BD patient and control samples, and performed WGS analysis. YZ, YW, and L-lH carried out animal and biochemical studies. XZ and HL provided technical support. S-yW, H-yL, and L-hH helped on BD patient sample collection and WGS analysis. HX helped with data interpretation. Y-wZ supervised the project and performed WGS analysis. F-rL, YZ, and Y-wZ drafted the manuscript. All authors reviewed and proved the final paper.

COMPETING INTERESTS
The authors declare no competing interests.

ADDITIONAL INFORMATION
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41398-022-02163-x.

Correspondence and requests for materials should be addressed to Yun-wu Zhang.

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