Brazilian Journal of Psychiatry
bjp
Revista Brasileira de Psiquiatria

Braz J Psychiatry. 2023 Jan-Feb;45(1):11-19
doi:10.47626/1516-4446-2022-2650
Brazilian Psychiatric Association

ORIGINAL ARTICLE

Heterozygosity for neuronal ceroid lipofuscinosis predisposes to bipolar disorder

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Objective: Bipolar disorder is a heritable chronic mental disorder that causes psychosocial impairment through depressive/manic episodes. Familial transmission of bipolar disorder does not follow simple Mendelian patterns of inheritance. The aim of this study was to describe a large family with 12 members affected by bipolar disorder. Whole-exome sequencing was performed for eight members, three of whom were diagnosed with bipolar disorder, and another reported as "borderline."
Methods: Whole-exome sequencing data allowed us to select variants that the affected members had in common, including and excluding the "borderline" individual with moderate anxiety and obsessive-compulsive traits.
Results: The results favored designating certain genes as predispositional to bipolar disorder: a heterozygous missense variant in *CLN6* resulted in a "borderline" phenotype that, if combined with a heterozygous missense variant in *ZNF92*, is responsible for the more severe bipolar disorder phenotype. Both rare missense changes are predicted to disrupt protein function.
Conclusions: Loss of both alleles in *CLN6* causes neuronal ceroid lipofuscinosis, a severe progressive childhood neurological disorder. Our results indicate that heterozygous *CLN6* carriers, previously reported as healthy, may be susceptible to bipolar disorder later in life if associated with additional variants in *ZNF92*.

Keywords: Bipolar disorder; WES; *ZNF92*; *CLN6*

Introduction

Bipolar disorder (BD) is a common and chronic mental disorder that causes psychosocial impairment, affecting patients with depressive/manic episodes.[1,2] Bipolar phenotypes are only defined according to clinical features and specific diagnostic tests do not yet exist.[2] Different BD subtypes have been recognized, including BD-I and BD-II, which are well described in the DSM-5.[3]

BD-I is characterized by the occurrence of at least one manic episode, which could have been preceded or followed by a hypomanic episode or major depressive disorder.[3] During a manic episode, which generally lasts about a week, mood may shift rapidly from euphoric to anger or depression, presenting psychotic features and requiring hospitalization to prevent harm to the patient or others. The lifetime risk of suicide in individuals with BD is estimated to be at least 15 times that of the general population.[3]

BD-II is characterized by the presence of at least one current or past hypomanic episode lasting approximately four days and a major depressive episode; manic episodes never occur.[2,3] During hypomanic episodes, mood disturbance is not severe enough to cause social impairment or require hospitalization; recurrent thoughts of death and suicidal ideation without a specific plan are common.[3] Bipolar-like phenomena that do not satisfy the criteria for BD-I or BD-II are classified as "unspecified bipolar and related disorders."[2,3] Several studies have demonstrated that BD has an early age of onset, with $>$ 70% of patients manifesting typical characteristics before 25 years of age.[4-6] There is also strong evidence that BD is a highly heritable phenotype.[4-10] In patients with established disease, a family history of mood or psychotic illness is common, with an estimated heritability of approximately 85%.[2,4-6] Familial transmission of BD does not follow simple Mendelian patterns of inheritance, and several analyses have shown that BD cannot be

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Submitted Feb 07 2022, accepted Jul 13 2022.

How to cite this article: Privitera F, Trusso MA, Valentino F, Doddato G, Fallerini C, Brunelli G, et al. Heterozygosity for neuronal ceroid lipofuscinosis predisposes to bipolar disorder. Braz J Psychiatry. 2023;45:11-19. http://doi.org/10.47626/1516-4446-2022-2650

accounted for through a single highly penetrant susceptibility gene.[2,11] Nevertheless, the biological basis of BD is still unknown.[1] Initial research for BD risk loci has chiefly focused on linkage analysis[12]; however, linkage methods do not work well for complex inheritance patterns, so this approach has failed to produce definitive, replicable findings.[12] In the past decade, large-scale genome-wide association studies of single-nucleotide polymorphisms and whole-exome sequencing (WES) of families have revealed dozens of genetic loci related to BD, even if these loci have only explained 18% of susceptibility to BD.[13-15] *ANK3* (chromosome 10q21.2), *CACNA1C* (chromosome 12p13), *TRANK1* and *DC LK3* (chromosome 3p22, both) were some of the earliest genes to be implicated in BD through genome-wide association studies[12]; WES studies of multiple families have identified promising variants in *RGS12* (chromosome 4p16.3), which has been reported in previous next-generation sequencing studies of schizophrenia.[14] No less important, at least two loci are associated with recurrent copy number variations in large, BD case-control samples: duplications in cytoband 16p11.2 and deletions in 3q29.[12] Finally, pre- and postnatal environmental risk factors have been implicated in a number of mental illnesses, including BD.[16] Prenatal infections, childhood maltreatment, and psychological stressors significantly affect the course of BD. Risk factors for BD, both genetic and environmental, are numerous, although low attributable risk, inconsistent results, the inability to identify the temporality of the relationship, the lack of a clear biological mechanism, and the nonspecific nature of many risk factors mean that causation is difficult to assign in individual patients.[16]

The aim of this study is to describe a large family with 12 members affected by BD: WES was performed for eight members, three of whom were diagnosed with BD and one reported as "borderline." The analysis revealed associations with new genes previously unreported in literature, suggesting new perspectives about the genetics of BD and expanding current knowledge of the disorder.

Materials and methods

Evaluated individuals

In the present study, we describe a family with 12 members affected by BD over five generations in an apparently Mendelian inheritance pattern. The study was performed on eight individuals: three affected by BD-I or BD-II, four with no signs of mental disease, one reported to have moderate anxiety and obsessive-compulsive personality traits. The included family members were sent to the Medical Genetics department after being evaluated at the Department of Mental Health's Psychiatry Unit (University of Siena, Policlinico "Santa Maria alle Scotte"). Their psychopathological condition was examined according to DSM-5 criteria. Each was initially evaluated using the Mood Spectrum-Self Report - Lifetime Version[17] and three psychometric scales: the Clinical Global Impression,[18] the Montgomery Asberg Depression Rating Scale[19] and the Young Mania Rating Scales.[20] The same psychometric approach was then used to evaluate improvement since the beginning of therapy. Genetic counseling was then conducted for each patient to evaluate individual phenotypes. All included individuals provided written informed consent prior to participating in the study, which was conducted according to Helsinki Declaration principles.

Genomic DNA was extracted from peripheral blood with EDTA using MagCore HF16 (Diatech Lab Line, Jesi, Ancona, Italy) according to manufacturer instructions. DNA quantity and integrity were estimated using a NanoDrop 2000/2000c spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA).

Whole-exome sequencing

The samples were prepared with Illumina DNA Prep and Exome Panel Enrichment Oligos (Illumina Inc., San Diego, CA, USA) according to manufacturer recommendations. The workflow uses a bead-based transposome complex to mediate a uniform tagmentation reaction of genomic DNA, which fragments and then tags the DNA with adapter sequences in one step. A target enrichment workflow is then applied. After pooling, the double-stranded DNA libraries are denatured and biotinylated. Illumina Exome Panel probes are hybridized to the denatured library fragments. After hybridization, streptavidin magnetic beads capture the targeted library fragments in the regions of interest. The captured and indexed libraries are eluted from the beads and further amplified before sequencing. The WES analysis was performed in an Illumina NovaSeq 6000 system according to the standard protocol. Reads were mapped against the hg19 reference genome with the Burrows-Wheeler aligner.[21] Variant calling was performed using an in-house pipeline that follows the Genome Analysis Toolkit Best Practices workflow.[22] We obtained mean coverage of 105x for targeted sequenced regions (range, 95-145x). The WES data were analyzed using enGenome-eVai (CE-IVD) software. To identify any potential pathogenic variants segregating among the family, we performed "reverse phenotyping" on all the affected individuals. In this step, no filters were used to prioritize variants. The aim was to check for any BD-related alterations in genes not previously described in association with mental disorders. After this step, the variants were prioritized according to rare variants (minor allele frequency < 0.01) found only in individuals with BD and genes previously associated with BD in genome-wide association studies and WES studies (Table S1, available as online-only supplementary material),[12,14-15,23] using the following human phenotype ontology terms: bipolar affective disorder (HP:0007302), depression (HP:0000716), obsessive-compulsive behavior (HP:0000722), mania (HP:0100754), psychosis (HP:0000709), anxiety (HP:0000739), suicide ideation (HP:0031589). Human phenotype ontology terms were specifically selected according to the symptomatology of included individuals. Missense variants were predicted to be damaging by CADD-PHRED prediction tools[24] and splice site variants by four *in silico* tools: SpliceSiteFinder-like, MaxEntScan, NNSPLICE, and GeneSplicer.

The UCSC genome browser (https://genome.ucsc.edu; December 2021), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/; January 2022), Clinical Interpretation of genetic variants by ACMG/AMP 2015 guidelines (https://wintervar.wglab.org/; December 2021), VarSome (https://varsome.com/; January 2022), and the Combined Annotation-Dependent Depletion (https://cadd.gs.washington.edu/; January 2022) databases, in addition to Alamut Visual v.2.11 (Jan 2018 Interactive Biosoftware, Rouen, France) were used to evaluate the genomic coordinates, probable deleterious effects on the protein, allele frequency, possible splicing effects, and the American College of Medical Genetics pathological classification of each variant. Online Mendelian Inheritance in Man (https://www.MIM.org/; December 2021), the Database of Chromosomal Imbalances and Phenotypes using Ensemble Resources (https://www.deciphergenomics.org/, February 2022), and the Bipolar Exosome Consortium (https://bipex.broadinstitute.org/, February 2022) were used to evaluate gene involvement in BD and whether they had been previously identified in affected individuals.

Ethics statement

The family members provided informed consent to participate in the study and for diagnostic testing. The study was performed and the samples obtained in accordance with the 1964 Helsinki Declaration (Fortaleza 2013 update).

Results

Clinical description

The clinical findings of the family members are described below. The family pedigree is shown in Figure 1. Psychometric evaluations were performed for each patient by the Department of Mental Health's Psychiatry Unit.

Patient 1 (Figure 1; V;5)

Patient 1 is a 27-year-old woman. She was born at term by spontaneous delivery. She experienced sleep disturbances until the age of six, which were treated with niaprazine. She manifested her first psychopathological symptoms when she started college at 18 years of age. She developed symptoms of demoralization, obsessive-compulsive behavior, anxiety, mood swings, and irritability. The family doctor prescribed her an unspecified antidepressant. After three days of this medication, her mood expanded, she developed sub-total insomnia, logorrhea, tangential thought processes, strong behavioral disinhibition, episodes of dysphoria and impulsiveness, and mystical and megalomaniac delusions regarding paranormal powers. Hospitalization followed this event, during which BD-I was diagnosed. Over the years her psychiatric history has alternated between satisfying and symptomatic conditions. At 24 years old, the patient experienced a severe manic episode followed by reduced energy, hypersomnia, and lethargy (side effects of the therapy), such that a second hospitalization was necessary. Currently, the patient is in good psychopathological condition. She is taking valproic acid (600 mg per day), lorazepam (1 mg once per day), cariprazine (1.5 mg twice per day), and lamotrigine (50 mg once per day).

Patient 2 (Figure 1; IV;10)

Patient 2 is the 55-year-old mother of the proband and is affected by BD-II. Her first signs of depression arose following the death of the mother, although the age of her

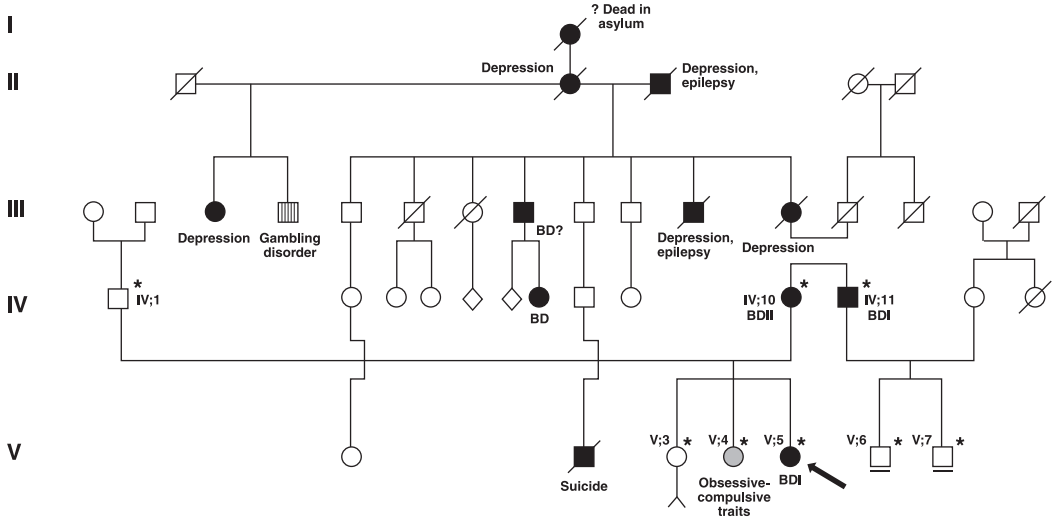

Figure 1 Family pedigree. * Whole-exome sequencing analysis was performed for this individual. The black arrow indicates the index subject, Patient 1(V;5). Squares = males; circles = females; rhombi = undetermined sex. Black = family members affected by BD symptomatology; striped = family members affected by various disorders; gray = family member with obsessive-compulsive traits. Genetic counseling was conducted with the family (internal code #17949).

first symptoms is unavailable. After being treated with a diuretic due to Ménière's syndrome, she began experiencing depression, anxiety, sleep disturbances, labile mood, irritability, inner tension and polarization about her daughter's health problems. These symptoms were followed by sadness, apathy, and abulia. Over the years her psychiatric history has alternated between satisfying and symptomatic conditions. She is currently showing significant clinical improvement and is taking valproic acid 300 mg and citalopram 10 mg.

Patient 3 (Figure 1; IV;11)

Patient 3 is the maternal uncle of the index subject. He is a 63-year-old male affected by BD-I, having manifested subclinical depression since he was young. At 57 years of age, he was hospitalized for episodes of depression (including lack of volition, insomnia, delusions of failure, and partial insight that he was experiencing psychological troubles), as well as further manic episodes and behaviors in line with the DSM-5 criteria for BD-I. Over the years, his psychiatric history has alternated between satisfying and symptomatic conditions. He was released in good psychopathological condition after being treated with olanzapine 5 mg, lamotrigine 150 mg, sertraline 50 mg, and lithium carbonate 900 mg.

Patient 4 (Figure 1; V;4)

Patient 4 is the middle sister of Patient 1. She is 30 years old. Psychiatrists reported that she has moderate anxiety and obsessive-compulsive personality traits. No other relevant clinical data were described and she was reported to be in good health.

Molecular characterization

The WES results are summarized in Table 1. All variants refer to the human genome assembly GRCh37/hg19. We searched for common variants among the affected individuals and found heterozygous missense and synonymous variants in 10 genes: *PDE1C*, *HERC2*, *CHD7*, *MRC2*, *SPON1*, *ZNF92*, *TTC19*, *RBM12*, *CLN6*, and *WDR62* (Table 1). However, since variants in *PDE1C*, *HERC2*, *CHD7*, *MRC2* and *TTC19* were observed in both healthy and affected family members (Figure S1, available as online-only supplementary material), they were excluded from the analysis. Due to their low CADD PHRED scores (< 15),[24] variants in *SPON1*, *RBM12*, and *WDR62* were also excluded (Table 1).

In the initial analysis, we considered mutated genes only in individuals with BD, finding that the common variant was in *ZNF92* (Figure 2). As a second step, we considered the mutated genes in common with the "borderline" family member, obtaining the missense variant in *CLN6* (Figure 2). Based on these data, we hypothesize that the *CLN6* variant alone predisposes to the obsessive-compulsive phenotype, but combined with the *ZNF92* variant generates the BD phenotype through oligogenic inheritance.

Finally, assuming that the effect of some identified variants could be aggravated by the concomitant presence of structural variants, we analyzed raw WES data from the affected individuals using the read count-based tool EXCAVATOR2.[25] The tool uses BAM files of WES experiments to extract the depth of sequencing coverage, correct it for technical biases, and identify regions with altered copy numbers.[26] By running the tool in "pooling" mode, each affected or "borderline" individual was compared with the same global control sample that results from pooling all unaffected samples. Copy number variations common to affected individuals (with or without the "borderline" case) were then merged into copy-number variable regions and annotated using AnnotSV v2.2 (https://lbgi.fr/AnnotSV; Supplementary Material 2, available online only). This tool uses BED or SV files to produce a tab-separated values file, scoring and ranking structural variants into five classes from pathogenic to benign.[27] The results are summarized in Table 2. The analysis showed the presence of 12 copy-number variable regions in two out of three family members affected by BD, plus the borderline member. Probably due to insufficient coverage, the WES data from patient V;5 were excluded from the shared structural variants comparison to prevent biased results. Although the sample passed EXCAVATOR2 pipeline quality control requirements, there was a distortion in the results for all chromosomes, which could lead to erroneous interpretations (Figure S2, available as online-only supplementary material). None of the 12 observed structural variants were confirmed among healthy individuals. Some deletions were scored as likely pathogenic (score 4), but none of them involve genes previously associated with BD.

Discussion

BD is a very heterogeneous condition due to dozens of completely unexplained genetic factors.[1] Over the years, up to 50 significant loci have been found in genome-wide association studies,[7,12,13,15] and WES analyses performed on families have allowed the characterization of up to 100 candidate genes for BD, including *de novo* or segregating variants over generations.[8,10,14] The present study describes WES results for a family affected by BD: three affected members, four healthy members, and one member with obsessive-compulsive traits. The analysis revealed missense heterozygous variants in ten relevant genes: *PDE1C*, *HERC2*, *CHD7*, *MRC2*, *SPON1*, *ZNF92*, *TTC19*, *RBM12*, *CLN6*, and *WDR62*. To identify the variants involved in phenotypic manifestation, we focused initially on those shared by the affected individuals, and then on those in common with obsessive-compulsive traits. Based on the results, *CLN6* was selected as the predisposing gene for obsessive-compulsive disorder, manifesting the BD phenotype when amplified by concomitant variants in other genes, such as *ZNF92*.

CLN6 (MIM#606725), a gene involved in a group of lysosomal storage disorders, has a predominantly autosomal recessive inheritance.[28] Neuronal ceroid lipofuscinosis, which is caused by biallelic variants in the *CLN6* gene, usually presents in early to late childhood (1.5 to 8

Table 1 Variants in common among individuals with bipolar disorder according to whole-exome sequencing analysis

Gene symbol (#OMIM)	Transcript	HGVS genomic	HGVS_coding	HGVS_protein	Variant effect	Classification (ClinVar/InterVar)	dbSNP	gnomAD	Splicing effect	CADD/ PHRED
PDE1C (#602987)	Chr 7, NM_001191057.4	g.31904581 A>G	c.725 A>G	p.Tyr242Cys	Missense	Unreported/uncertain	N/A	N/A	N/A	26.5
HERC2 (#605837)	Chr15, NM_004667.6	g.28386774 G>A	c.11819 G>A	p.Arg3940Gln	Missense	Unreported/uncertain	rs370282963	0.0012%	N/A	27.6
CHD7 (#608892)	Chr 8, NM_017780.4	g.61735198 G>C	c.3094 G>C	p.Glu1032Gln	Missense	Unreported/uncertain	rs1355615827	0.00040%	N/A	23
MRC2 (#612264)	Chr 17, NM_006039.5	g.60769687 C>T	c.4315 C>T	p.Arg1439Cys	Missense	Unreported/uncertain	rs776632141	0.0013%	N/A	29.2
SPON1 (#604989)	Chr 11, NM_006108.4	g.13963049 C>A	c.145 C>A	p.Arg49Ser	Missense	N/A	N/A	N/A	N/A	1.98
ZNF92 (#603974)	Chr 7, NM_152626.4	g.64864204 G>A g.64864203 G>T	c.1177 G>A c.1176 G>T	p.Gly393Arg p.Thr392Thr	Missense Synonymous	Unreported/uncertain Unreported/ Likely Benign	rs764034193 rs566012007	0.0024% 0.00037%	N/AN/A	15.95
TTC19 (#613814)	Chr 17, NM_017775.4	g.15928441G>T	c.787 G>T	p.Ala263Ser	Missense	Uncertain	rs141892030	0.029%	It creates an alternative splicing site at 3' in exon 8 for two predictive *in silico* tools (SpliceSiteFinder-like; MaxEntScan)	27
RBM12 (#607179)	Chr 20, NM_006047.6	g.34242641 A>G	c.604 A>G	p.Met202Val	Missense	Unreported/ Likely Benign	rs146171962	0.0040%	It creates an alternative splicing site at 3' in exon 3 for two predictive *in silico* tools (SpliceSiteFinder-like; MaxEntScan)	13.23
CLN6 (#606725)	Chr 15, NM_017882.3	g.68504183 C>T	c.316 C>T	p.Arg106Cys	Missense	Uncertain	rs202226970	0.0067%	N/A	24.3
WDR62 (#606725)	Chr 19, NM_001083961.2	g.36595687 G>A	c.4329 G>A	p.Gln1443Gln	Missense	Uncertain/Likely Benign	rs771131709	0.0028%	N/A	0.10

CADD = combined annotation-dependent depletion; Chr = chromosome; HGVS = Human Genome Variation Society (https://www.hgvs.org); N/A = not available.
In "Classification," a unique output correlates to both ClinVar and InterVar interpretations.
CADD PHRED was calculated for missense variants only.[24]

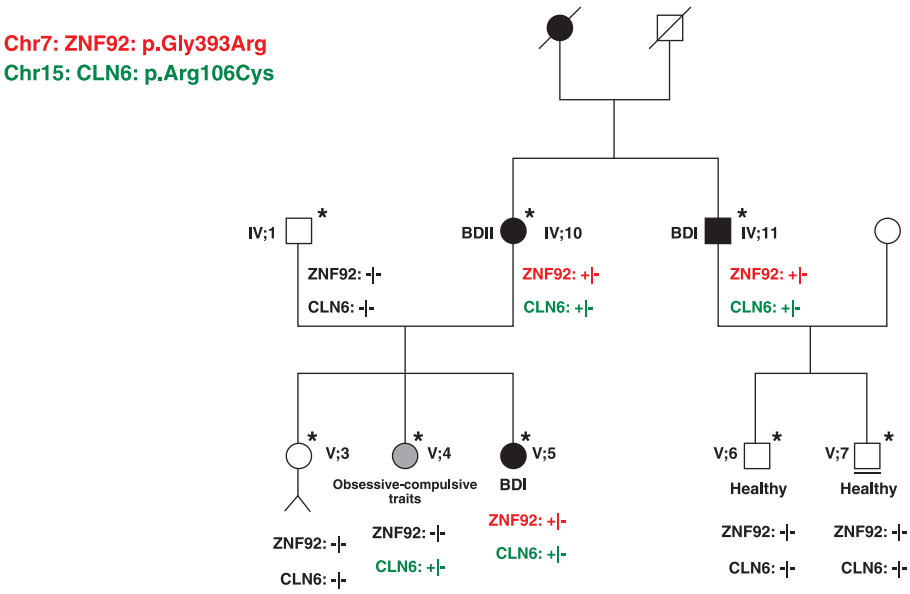

Figure 2 Genes in common between affected and borderline family members. Squares = males; circles = females; + and - refer to the presence or the absence, respectively, of the heterozygous variant in the indicated gene. The missense variant in *ZNF92* was shared exclusively by family members with BD; the missense variant in CLN6 was only found in the "borderline" individual.

years of age) and involves slow motor degeneration, ataxia, vision loss, epilepsy, and mental disabilities.[29] Early death by 12-15 years of age is reported.[29] Variants in *CLN6* may also lead to a rare adult-onset form of neuronal ceroid lipofuscinosis in which the symptoms present in adulthood, typically between 24 and 38 years of age.[30,31] The symptomatology includes ataxia, tremor, and cognitive impairment that appear at approximately 41 years of age.[31] Homozygous variants in *CLN6* have also been reported in association with depression and anxiety, and some evidence supports a link with psychiatric problems and obsessive-compulsive behavior.[30,31] Although, to date, heterozygous *CLN6* carriers do not seem to show any distinct clinical phenotype or association with psychiatric conditions,[31,32] we have described an individual with different phenotypic features and propose that special attention should be paid to the implications that these variants could have in the development of "borderline" phenotypes, in the context of a multifactorial disease worsened by concomitant variants in other genes, such as that identified in *ZNF92*.

ZNF92 (MIM#603974) belongs to the ZNF family of transcription factors, which contains the Kruppel-associated box domain.[33] The ZNF family includes motifs in DNA- and RNA-binding proteins and appears to be involved in many cellular functions, such as DNA recognition, RNA packaging, transcriptional activation, apoptosis regulation, and protein folding and integration.[34] Increasing evidence suggests that genetic variants in ZNFs influence susceptibility to psychiatric disease, especially BD.[12,34] Here, we propose *ZNF92* as a new gene involved in the pathogenesis of bipolar phenotype.

Despite the small sample size (8 individuals), this study provides new molecular information regarding the genetics of BD. We cannot completely define the role that the observed variants may have in the onset of psychiatric conditions, since they have not yet been reported in the ClinVar database or other BD-specific databases, such as the Bipolar Exome collaboration. In the latter browser, a dataset of 14,210 cases and 14,422 negative controls (https://bipex.broadinstitute.org/), there is no evidence that these two variants are associated with BD. In a subset of 2726 samples analyzed with enGenome-eVai (CE-IVD) software, only one sample, already tested and reported negative for Alport syndrome, presented the same *CLN6* variants; the *ZNF92* variant was reported only in the individuals affected with BD in the present study. None of these individuals had both variants simultaneously. Given its rarity in the general population and its recurrence within this family, it is highly probable that this combination is idiosyncratic. The bipolar phenotype could be due to impairment of the protein structure encoded by *CLN6*, previously reported as a gene involved in mental disorders. Nevertheless, a multifactorial pathway should be considered in which additional genes or the family environment could contribute to the final phenotype. Actually, there is no strong basis for the assumption that any of the shared variants are fully penetrant, or that those found in common with the healthy individuals play a role in the family's illness. For instance, it is well known that mutations in *HERC2* produce clinical syndromes in which key neurodevelopmental events are altered, resulting in intellectual disability and other neurological disorders.[35] Genetic variation in *PDE1C* is associated with multiple measures of human cognitive function[36]; *CHD7* has a critical role in the epigenetic regulation of neuronal differentiation, disruptions of which are believed to be associated with

Table 2 Analysis of copy number variations from EXCAVATOR2 and AnnotSV

AnnotSV ID/SV type	SV Chr	SV Start	SV End	SV size (Kb)	Gene name	Transcript	GnomAD_ID	AnnotSV ranking	Association with bipolar phenotypes
1_2181795_2185513_DEL	1	2181795	2185513	3.71 Kb	*SKI*	NM_003036	gnomAD_v2_DEL_1_331	4	No
2_168811559_168855462_DEL	2	168811559	168855462	43.9 Kb	*STK39*	NM_013233	N/A		No
3_6786589_6863041_DEL	3	6786589	6863041	76.4 Kb	*GRM7-AS3*	NR_110123	gnomAD_v2_DEL_3_34441	2	No
4_56436502_56446416_DEL	4	56436502	56446416	9.9 Kb	*PDCL2*	NM_152401	N/A	2	No
7_5121870_5182726_DEL	7	5121870	5182726	60.8 kb	*ZNF890P*	NR_034163	gnomAD_v2_DEL_7_90969; gnomAD_v2_DEL_7_90970; gnomAD_v2_DEL_7_90981	2	No
8_144111099_144116293_DEL	8	144111099	144116293	5.19 Kb	No gene	-	gnomAD_v2_DEL_8_114898	2	No
10_133456235_133465931_DEL	10	133456235	133465931	9.96 Kb	No gene	-	N/A	2	No
11_117833717_117838299_DEL	11	117833717	117838299	4.58 Kb	No gene	-	gnomAD_v2_DEL_11_144738	2	No
12_113206065_113246063_DEL	12	113206065	113246063	39.9 Kb	*RPH3A*	NM_001347952	N/A	2	No
17_81444368_81463817_DEL	17	81444368	81463817	19 Kb	No gene	-	-	2	No
19_4719845_4770779_DEL	19	4719845	4770779	50.9 Kb	*DPP9*	NM_139159	N/A	2	No
19_4719845_4770779_DEL	19	4719845	4770779	50.9 Kb	*MIR7-3*	NR_029607	N/A	2	No
19_4719845_4770779_DEL	19	4719845	4770779	50.9 Kb	*MIR7-3HG*		N/A	2	No
19_15238361_15244668_DEL	19	15238361	15244668	6.3 Kb	No gene	-	gnomAD_v2_DEL_19_200694	2	No

Chr = chromosome; N/A = not available; SV = structural variant.
AnnotSv ranking scores span from 1-5 according to the American College of Medical Genetics technical standards for the interpretation of constitutional copy number. Class 5 = pathogenic copy number variations; class 4 = likely pathogenic; class 3 = variant of uncertain significance; class 2 = likely benign; class 1= benign. The analysis was conducted for Patients IV;10, IV;11, and V;4 (Figure 1). Probably due to the low coverage of WES data from Patient V, 5 analyses could not be completed.

Table 3 Predictive scores for each gene identified from whole-exome sequencing analysis according to the DECIPHER database

Genes	pLI[†]	%HI[‡]
PDE1C	0.00	22.08
HERC2	1.00	50.09
CHD7	1.00	2.39
MRC2	0.67	43.17
SPON1	-	-
ZNF92	0.00	92.19
TTC19	0.00	51.13
RBM12	0.00	14.22
CLN6	0.00	41.58
WDR62	0.00	51.07

%HI = probability of haploinsufficiency; pLI = probability of loss-of-function intolerance index.
[†] The pLI score is the probability that a given gene falls into the haploinsufficient category, and is thus extremely intolerant of loss-of-function variation. Genes with high pLI scores ($\geqslant$0.9) are extremely intolerant to loss-of-function, whereby genes with low pLI scores (0.1) are more tolerant.[40]
[‡] A haploinsufficient gene requires two functional copies to produce the standard phenotype. The probability that a gene is haploinsufficient is based on a set of functional, evolutionary, and network properties. High ranks (e.g., 0-10%) indicate a gene is more likely to be haploinsufficient, while low ranks (e.g., 90-100%) indicate that a gene is more likely not to be haploinsufficient.[41]

schizophrenia and autism[37]; *TTC19* mutations have been identified in a family with severe psychiatric manifestations[38]; and, finally, truncating mutations in *RBM12* are associated with psychosis.[39] Based on specific prediction scores reported in DECIPHER database (Table 3),[40,41] some of these genes, such as *CHD7* and *HERC2,* seem to be intolerant to loss-of-function variations. It is thus possible that variants in these genes could contribute to full manifestation of the disorder, even if they are also present in unaffected individuals. We cannot even be certain that individuals identified as ''healthy'' may not manifest psychiatric disorders in the future. Although it would be interesting to calculate a polygenic risk score for each genotyped individual, our group of individuals was unfortunately too small to provide sufficient data for comparison with an extended set of controls. Although no polygenic risk score for BD has been reported in the Polygenic Score Catalog (https://www.pgscatalog.org/), it would not exportable to a different population even if did exist.

Based on these scores, *HERC2* and *CHD7* seem to be intolerant to loss-of-function variations, although the rest of the genes, including *CLN6* and *ZNF92,* seem to be more tolerant to variations. It is possible that *HERC2* and *CHD7*, although present in healthy individuals, could contribute to the observed phenotype to a lesser extent.

Finally, none of the twelve identified copy number variations showed a clinical correlation with BD; some were classified as likely pathogenic, but the lack of clear associations with psychiatric disorders does not help in their interpretation. This study further reinforces the role of the two genes, *ZNF92* and *CLN6*, found by WES in the manifestation of the disease.

In conclusion, this study suggests new perspectives regarding the genetics of BD. It was based on a smaller group of individuals than other WES studies and lacks reproducibility in other families. However, all investigations, including the analysis of structural variants in WES and raw data from EXCAVATOR2 (not reported in BD studies so far), led to a unique result: the involvement of *CLN6* and *ZNF92* in BD and obsessive-compulsive behavior. Further analysis should be performed in larger cohorts to clarify the roles of these genes and the impact of the selected variants on the manifestation of the bipolar phenotype. If additional variants in *ZNF92* and *CLN6* are detected in psychiatric disorders, such analysis could support their correlation to the disorders, thus helping identify at-risk individuals and improving clinical management by recommending appropriate therapies and monitoring patient health over time.

Acknowledgements

The authors would like to thank all of the family members for participating in the study, as well as ''Cell lines and DNA bank of Rett Syndrome, X-linked mental retardation, and other genetic diseases,'' member of the Telethon Network of Genetic Biobanks (project no. GTB18001), which is funded by Telethon Italy, the EuroBioBank network, and RD-Connect, for providing us with specimens.

Disclosure

The authors report no conflicts of interest.

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