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Genetic Risk Score Analysis in Early-Onset Bipolar Disorder

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Disclaimers

The content of this report is solely the responsibility of the authors and does not necessarily represent the official views of the US Department of Health and Human Services, the National Institutes of Health, or the NIMH.

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Abstract

Objective—In this study, we performed a candidate genetic risk score (GRS) analysis of early-onset bipolar disorder.

Method—Treatment of Early Age Mania (TEAM) study enrollment and sample collection took place from 2003–2008. Mayo Clinic Bipolar Biobank samples were collected from 2009–2013. Genotyping and analyses for the present study took place from 2013–2014. The diagnosis of bipolar disorder was based on *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision* criteria. Eight single-nucleotide polymorphisms (SNPs), previously reported in genome-wide association studies to be associated with bipolar disorder, were chosen for GRS analysis in early-onset bipolar disease. These SNPs map to 3 genes: *CACNA1C* (calcium channel, voltage-dependent, L type, alpha 1C subunit), *ANK3* (ankyrin-3, node of Ranvier [ankyrin G]), and *ODZ4* (teneurin transmembrane protein 4 [formerly "odz, odd Oz/ten-m homolog 4 {Drosophila}, ODZ4"]). The 8 candidate SNPs were genotyped in patients from the TEAM study (n=69), adult patients with bipolar disorder (n=732) including a subset with early-onset illness [n=192]), and healthy controls (n=776). GRS analyses were performed comparing early-onset cases with controls. In addition, associations of early-onset BD with individual SNPs and haplotypes were explored.

Results—GRS analysis revealed associations of the risk score with early-onset bipolar disorder (P=.01). Gene-level haplotype analysis comparing TEAM patients with controls suggested association of early-onset bipolar disorder with a *CACNA1C* haplotype (global test, P=.01). At the level of individual SNPs, comparison of TEAM cases with healthy controls provided nominally significant evidence for association of SNP rs10848632 in *CACNA1C* with early-onset bipolar disorder (P=.017), which did not remain significant after correction for multiple comparisons.

Conclusion—These preliminary analyses suggest that previously identified bipolar disorder risk loci, especially *CACNA1C*, have a role in early-onset bipolar disorder, possibly with stronger effects than for late-onset bipolar disorder.

Keywords

ANK3; *CACNA1C*; early onset bipolar disorder; genetics; *ODZ4*

Introduction

Early-onset bipolar disorder (BD) is a devastating illness that confers significant morbidity and demonstrates continuity with adult mood disorders (1–4). Lifetime prevalence rates of bipolar disorder in children are as high as 2.1% (5). Early-onset BD may be associated with greater long-term morbidity and increased familial risk (6–9). However, the molecular underpinnings of this risk are poorly understood (10). Prior genome-wide association studies (GWAS) of adults have implicated several genes in BD, including calcium channel, voltage-dependent, L type, alpha 1C subunit (*CACNA1C*) (11–13), ankyrin-3, node of Ranvier (ankyrin G) (*ANK3*) (12,14), and teneurin transmembrane protein 4 (formerly "odz, odd Oz/ten-m homolog 4 [Drosophila], ODZ4") (*ODZ4*) (15,16). However, it is not known whether single-nucleotide polymorphisms (SNPs) in these genes have stronger associations with early-onset BD than with adult-onset BD.

Early-onset BD may represent a distinct phenotype with greater severity and genetic loading. Hence, samples of early-onset cases (EOC) present an invaluable opportunity for study of this heterogenous disease. Prior work supports an increased familial risk for BD in relatives of probands with early-onset BD (5), and neuroimaging studies suggest that children and adolescents with early-onset BD may have unique neurobiologic features (17,18). Experts have also argued that this population has a differential response to pharmacologic agents, which bolsters the contention of a discrete phenotype (1).

Herein, we report on a study that utilized samples from the Treatment of Early Age Mania (TEAM) study (19), from adults with BD, and from healthy control adults to perform a candidate gene study of early-onset BD. The primary aim was to perform a genetic risk score (GRS) analysis of early-onset BD using SNPs previously implicated as risk factors for BD. Eight SNPs in 3 genes were selected: 4 in *CACNA1C* (rs1006737 [12], rs1024582 [12], rs4765913 [15], and rs10848632 [20]); 3 in *ANK3* (rs1938526 [12], rs9804190 [15], and rs10994336 [12]); and 1 in *ODZ4* (rs12576775 [15]). We hypothesized that these 8 SNPs would be associated with a risk for BD and that the associations might be greater for patients with early-onset BD.

Methods

Patients

The study protocol and procedures were approved by the Mayo Clinic Institutional Review Board. Research clinicians obtained informed consent from adult participants, assent from child participants, and informed consent from primary caretakers. The protocol and procedures related to the execution of the TEAM study (19) and sample collections were approved through each local institutional review board (Washington University School of Medicine, St. Louis, Missouri; Children's National Medical Center, Washington, DC; The University of Texas Medical Branch, Galveston, Texas; and the Johns Hopkins Medical Institutions, Baltimore, Maryland).

The present study included samples from patients with early-onset BD from the TEAM study (n=82), samples from adult BD patients (n=855), and samples from healthy controls (n=857) (21). TEAM study enrollment and sample collection took place from 2003–2008. Mayo Clinic Bipolar Biobank samples were collected from 2009–2013. Genotyping and analyses for the present study took place from 2013–2014. After quality control and removal of non-Caucasian samples (to avoid confounding by population structure), we analyzed a total of 69 TEAM samples, 732 samples from adults with BD (192 with early-onset BD, 256 with late-onset BD, and 284 with an undetermined age of onset), and 776 control samples. Demographics and comorbidities of the cohorts are described in Table 1.

TEAM Cohort—The ascertainment and assessment procedures for the TEAM study are described elsewhere (19). Briefly, TEAM participants were child and adolescent outpatients aged 6 to 15 years with a diagnosis of BD and currently in a manic or mixed episode for a minimum of 4 weeks preceding enrollment. Diagnosis of BD was based on a semistructured interview using the Washington University in St. Louis Kiddie Schedule for Affective Disorders and Schizophrenia (22) to establish BD criteria as described in the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision* (DSM-IV-TR) (23). A diagnosis of mania required a threshold number of symptoms of at least moderate severity for a concurrent period. All participants had a Children's Global Assessment Scale score of 60 or less, good health, and an intelligence quotient of 70 or greater. Those with pervasive developmental disorders, schizophrenia, and major medical comorbidities were excluded (19).

Adult Bipolar Disorder Cohorts—Adult samples were from 855 patients with a confirmed diagnosis of BD based on criteria from DSM-IV-TR (23). The diagnosis was confirmed by a senior psychiatrist upon review of a Structured Clinical Interview for DSM-IV-TR (SCID) (24). Data were also collected using a patient questionnaire, a clinical questionnaire, and, if available, the electronic health record (EHR). The ages of first manic and first depressive episodes were determined on the basis of a review of the SCID, a clinical questionnaire, and the EHR by a research coordinator. After this review, a subset of adult patients (n=192) was classified as having early-onset BD. Patients were classified as having early-onset BD if their first manic or depressive episode occurred at age 19 years or younger (3,25,26). This early-onset characterization was independently confirmed for each patient by 2 child and adolescent psychiatrists (P.E.C. and M.V.), who also reviewed the SCID, the clinical questionnaire, and the EHR. The overall demographics of the adult bipolar biobank study, from which samples in the present study were obtained, have been reported previously (27).

Adult Controls—The control group comprised 857 participants from a biobank (21). Patients were excluded from the control group if they reported having a first-degree relative with BD or a prior diagnosis of a psychiatric condition such as BD, schizophrenia, major depression, Down syndrome, autism, or attention-deficit/hyperactivity disorder. Eligible control patients were matched to adult cases by age, sex, and race/ethnicity.

Genotyping and Quality Control

Because of the small number of available early-onset cases from the TEAM study, this study focused on candidate loci previously reported to be associated with bipolar disorder. On the basis of the results of prior GWAS, we selected 8 candidate SNPs for study: 4 in *CACNAIC* (rs1006737 [12], rs1024582 [12], rs4765913 [15], and rs10848632 [20]); 3 in *ANK3* (rs1938526 [12], rs9804190 [15], and rs10994336 [12]); and 1 in *ODZ4* (rs12576775 [15]). TEAM samples were genotyped with the TaqMan genotyping assay (Life Technologies) at Washington University. Adult samples were genotyped at Mayo Clinic genotyping core facility using the Illumina GoldenGate Genotyping Assay (Illumina Inc) as part of a larger candidate gene study (28). For quality control, 10 Mayo Clinic samples were also genotyped on the Illumina platform, obtaining 100% concordant genotype calls between Mayo Clinic and Washington University. To reduce the likelihood of confounding by population structure, we restricted analyses to patients of self-reported Caucasian ancestry. SNP genotype distributions were tested for departure from Hardy-Weinberg equilibrium. No departures from Hardy-Weinberg equilibrium were detected in the full set of Caucasian subjects (all $P>.05$). Although 1 SNP (rs1938526) in the subset of controls showed marginal evidence for departure from Hardy-Weinberg equilibrium ($P=.047$), this evidence of disequilibrium is not significant, given the number of tests performed. After quality control, 69 TEAM early-onset BD cases, 732 adult BD cases (192 with early-onset BD), and 776 controls—all of European ancestry—were included in the analyses.

Statistical Analysis

To determine whether previously identified BD risk associated SNPs have a role in early-onset illness that might be greater than their effect on general BD risk, we performed global tests of association of the genotyped SNPs with BD and early-onset BD using GRS analysis. For the GRS analyses, we excluded the *CACNA1C* SNP rs1024582 from the risk scores because rs1006737 and rs1024582 are in very high linkage disequilibrium ($r^2=.9$; $D'=1$), and thus, including both SNPs would weigh the effect of these SNPs too heavily by double-counting the risk from that locus. We used 2 methods to calculate a GRS: 1) a simple count GRS (SC-GRS) obtained by summing the total number of risk alleles in the 7 remaining SNPs and 2) an odds ratio–weighted GRS (OR-GRS), with risk alleles weighted by their odds ratios (ORs) from prior studies (29). Thus, for each patient, the SC-GRS and OR-GRS were calculated as:

$$\text{SC- GRS}=\text{sum}(\text{G}_\text{i}) \text{ and } \text{OR- GRS}=\text{sum}(\log[\text{OR}_\text{i}] \times \text{G}_\text{i})$$

where G_i = genotype at SNP i coded as the number of risk alleles and OR_i is the risk allele OR for SNP i. Although the SNPs were selected on the basis of several prior GWAS (12,15,16,20), for consistency, the risk alleles and ORs used in the GRS calculations were based on the large analysis performed by the Psychiatric GWAS Consortium (15). Logistic regression models were used to test for association of the risk scores with BD or early-onset BD. Results of the GRS analysis were considered statistically significant if $P<.05$ because all SNPs were included in a single analysis (ie, the risk score calculated from all SNPs formed a single predictor variable that was tested in the analysis).

To determine whether particular SNPs may be driving the results of the GRS analysis, genetic data were also analyzed at the individual SNP and haplotype levels. We first used the adult patient (n=732) and control samples (n=776) to determine whether each SNP was associated with BD. We then tested whether the SNPs were associated with early-onset BD by comparing the TEAM early-onset samples (n=69) with the controls. We subsequently divided the adult BD sample into EOC and late-onset cases, and we performed analyses comparing the EOC (n=192) with controls and all available EOC (adult plus TEAM cases, n=261) with controls (n=776). Analyses were based on logistic regression models with SNP genotypes coded as 0, 1, or 2 copies of the minor allele. Haplotype analyses used all genotyped SNPs in each gene (for *CACNA1C* and *ANK3* genes) and were performed using the score statistic implemented in the R package Haplo.stats (Mayo Foundation for Medical Education and Research) (30), comparing the same groups as in the individual SNP analysis. Linkage disequilibrium plots for *ANK3* and *CACNA1C* were generated in Haploview (Broad Institute) (31) using 1,000-genome Caucasian ancestry data (32) (Figure).

All data cleaning and statistical analyses were performed using R statistical computing software (The R Foundation for Statistical Computing) or SAS version 9.2 (SAS Institute Inc).

Results

The results of risk score analyses are presented in Table 2. Analysis of adult cases compared with controls provided no evidence of association of the SC-GRS or the OR-GRS with BD (SC-GRS, P=.71; OR-GRS, P=.56). Further, comparison of the adult late-onset group with controls did not demonstrate significant associations of risk scores and BD (SC-GRS, P=.96; OR-GRS, P=.85). The associations with GRS were also not significant in comparisons of TEAM EOC vs controls, although the OR point estimates for risk score analysis of TEAM EOC compared with controls were larger than in the adult case–control comparison. Analyses comparing the adult EOC with controls did not show significant differences with a simple count approach, but when using an OR-weighted approach, significant evidence of association of risk scores with early-onset BD was observed (SC-GRS, P=.06; OR-GRS, P=.02). Furthermore, analysis of the combined group of EOC (TEAM EOC plus adult EOC) vs controls also demonstrated association of risk scores with early-onset BD (SC-GRS, P=.02; OR-GRS, P=.01). The OR in the SC-GRS analysis (OR, 1.08) provides an estimate of the mean impact on the risk for early-onset BD of a risk allele at the 7 SNPs included in the risk score. Results were similar when the combined EOC group was compared with the late-onset cases, demonstrating that the loci investigated appear to primarily contribute to risk of early-onset, rather than late-onset, BD.

Haplotype analyses demonstrated no significant associations between *ANK3* and *CACNA1C* haplotypes when adult BD cases were compared with healthy controls. However, the comparison of TEAM EOC with controls revealed significant associations of early-onset BD with *CACNA1C* haplotypes composed of the SNPs rs10848632, rs1006737, rs1024582, and rs4765913 (global test, P=.01 in analysis of haplotypes with frequencies ≥5%) (Table 3); the result of the *CACNA1C* global haplotype test is marginally significant after correction for the number of genes (3) that were examined at the gene level. The

CACNA1C haplotype analysis results in Table 3 show that the TGGT haplotype was associated with an increased risk for early-onset BD (maximum statistic simulation, *P*=.009) in the comparison of TEAM cases vs controls. A similar *CACNA1C* haplotype analysis found no significant difference between adult EOC and controls or between the TEAM EOC and the adult EOC compared with controls (global statistic, *P*>.10) (Table 3). *ANK3* haplotypes were not significantly associated with early-onset BD.

No statistically significant associations with the 8 candidate SNPs were observed when adult cases were compared with controls (Table 4). The comparison of TEAM EOC with controls (Table 5) showed nominally significant evidence of association of the minor allele at rs10848632 in *CACNA1C* with early-onset BD (OR, 1.54; *P*=.017), which is not significant after correction for multiple comparisons. Comparison of adult EOC with controls provided nominally significant evidence of association of rs10994336 in *ANK3* with early-onset BD (OR, 1.49; *P*=.049) (Table 5), which was also not significant after correction for multiple testing.

Discussion

This study of a relatively small sample of early-onset BD cases is, to our knowledge, the first investigation of *CACNA1C*, *ANK3*, and *ODZ4* genetic variations in early-onset BD. On the basis of prior GWAS studies, we selected 8 SNPs, including rs10848632, rs1006737, rs1024582, and rs4765913 in *CACNA1C*; rs1938526, rs9804190, and rs10994336 in *ANK3*; and rs12576775 in *ODZ4* (12,15,16). Our GRS analyses suggested an association between a risk score composed of the genotyped candidate SNPs and early-onset BD, but not with BD in general. Furthermore, our results suggest a *CACNA1C* haplotype may be associated with early-onset BD. Contrary to our expectations, the individual SNPs of interest were not significantly associated with risk of adult-onset BD (with the index episode of depression or mania occurring after age 19 years).

The haplotype analysis of TEAM EOCs vs controls provided marginally significant evidence of association of early-onset BD with *CACNA1C* genetic variation. However, the T-G-G-T haplotype that was associated with increased risk of early-onset BD had a relatively low frequency, and thus the results should be interpreted cautiously, given the small sample size. Nevertheless, the preliminary results, which suggest that *CACNA1C* may have a more prominent role in early-onset BD, are interesting and warrant further investigation. Genes that regulate calcium channel functioning, such as *CACNA1C,* may have widespread effects on the development and manifestations of psychiatric illness (33–36). Analyses of genome-wide SNP data, from autism spectrum disorder, attention-deficit/hyperactivity disorder, BD, unipolar depression, and schizophrenia, have implicated risk loci in the *CACNA1C* and *CACNB2* L-type voltage-gated calcium channel subunits across a range of psychiatric illnesses. Hence, genotypic variations in calcium channel genes may have broad implications for psychiatric phenotypes. These findings also underscore the potential limitations of traditional, descriptive approaches to psychiatric diagnosis (37). The National Institute of Mental Health Research Domain Criteria (RDoC) program is shifting how research hypotheses are structured (38). For example, recent studies of biomarker panels have characterized neurobiologically distinct categories among patients with bipolar

disorder, psychosis, and schizoaffective disorder that are not congruent with clinical diagnostic categories (39). Further research using the RDoC framework could inform studies of children and adolescents with bipolar disorder.

The pleomorphic nature of BD and challenges in its phenotypic classification have likely hindered the progress of genetic risk studies of this lifelong disease, which produces considerable individual and societal burden (38). Clinical characteristics (eg, psychotic symptoms, panic attacks, and alcohol misuse) have shown promise as refined phenotypic characterizations with familial aggregation in BD (40,41). Early-onset BD, in particular, is an important subgroup for study, because this characteristic confers greater familial risk and likely presents a more homogenous population to study (42,43). The diagnostic stability and adult continuity of childhood bipolar disorder is poorly understood and understudied (44). A recent systematic review describing the dearth of positive findings in genetic research of early-onset bipolar disorder concluded that the genetic heterogeneity of childhood bipolar disorder is a substantial barrier (45). Emotional dysregulation and irritability in childhood are important targets of contemporary research efforts (46). The SNPs of interest in the present study may have broader relationships with dimensional symptoms, as opposed to a DSM-IV-TR (23) diagnosis of bipolar disorder.

It is widely recognized that genetic risk for complex psychiatric diseases such as BD is conferred by many alleles both within and across genes. For example, an 8-locus *CACNA1C* haplotype has been shown to be associated with risk for BD (47). Polygenic risk scores have been successfully utilized in recent studies, for instance to show genetic overlap between BD and the clinical dimensions of mania in schizophrenia (48) and to identify new associations in schizophrenia (49). Although the small number of SNPs examined in the present study is not suitable for developing GRS for the purpose of clinical risk prediction, we applied the GRS methodology to facilitate investigation of the overall impact of well-established BD loci in the risk of a subphenotype (early-onset illness). Our results support the notion that polygenic methodologies may be important for future work examining genetic risk for early-onset BD.

Strengths of our study include its focus on a narrow and decidedly heritable phenotype in well-characterized samples. Patients in the TEAM EOC with DSM-IV-TR BD I were rigorously characterized by child and adolescent psychiatrists with a high level of expertise in early-age mania (19). Patients in the adult EOC were also rigorously characterized on the basis of a review of SCID and EHR data, with independent confirmation by 2 child and adolescent psychiatrists. These factors are critical, given the heterogeneity of BD and of early-onset mood disorders in general.

However, it is important to note the limitations of this exploratory work. First, the sample size was small and the TEAM sample was particularly small, limiting the power of the study. Although no statistically significant associations were observed for individual SNPs (after correction for multiple testing), the OR estimates, particularly in the comparisons of early-onset BD cases with controls, indicated the same direction of effect as in the original reports of association with BD (12,15). Thus, low study power likely had a role in the failure to identify significant associations at the SNP level. Second, the analyses were restricted to

patients of European ancestry, which limits the generalizability of our results; however, this approach has the important advantage of reducing the likelihood of confounding by population structure. Reliance on self-reported race/ethnicity is also a limitation, in that we cannot exclude the possibility of residual population stratification. Because genome-wide SNP data were not available for these samples, more robust methods such as principal components could not be applied to control for population stratification. Nevertheless, prior research has shown that self-reported ancestry is usually a reliable approach for accounting for population structure in candidate gene studies (50). Third, in most cases, the classification of adult samples as early-onset vs late-onset BD was based on a retrospective review of records and patient report, which may have been biased or inexact. Also, it was not possible to characterize numerous, potentially important factors such as co-occurring disorders, treatment history, and functional impairment. Finally, this study was limited to a small number of SNPs. It was not meant to fully characterize the genetics of early-onset BD; rather, we aimed to explore whether previously identified risk loci might have a more prominent role in early-onset illness. Other important limitations include the potential differences in comorbidity among cohorts. We were unable to fully characterize and control for comorbidities in the present study because of differences between the case groups and data collection methods. This may explain the lack of specificity among some of our findings. Finally, as we discussed above, the poor longitudinal diagnostic stability of bipolar disorder in childhood presents inherent limitations in the interpretation of our findings.

In conclusion, this study used GRS analysis to demonstrate that the selected candidate loci contribute primarily to the risk of early-onset BD rather than to the risk of late-onset BD. Our results also suggested an association of early-onset BD with a *CACNA1C* haplotype. Future studies with larger, ethnically diverse samples and more detailed phenotypic characterizations will refine the understanding of genetic risk profiles in early-onset BD.

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Abbreviations

ANK3	ankyrin-3, node of Ranvier (ankyrin G)
BD	bipolar disorder
CACNA1C	calcium channel, voltage-dependent, L type, alpha 1C subunit

DSM-IV-TR *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision*

EHR electronic health record

EOC early-onset cases

GRS genetic risk score

GWAS genome-wide association studies

ODZ4 teneurin transmembrane protein 4 (formerly “odz, odd Oz/ten-m homolog 4 [Drosophila], ODZ4”)

OR odds ratio

OR-GRS odds ratio–weighted genetic risk score

RDoC National Institute of Mental Health Research Domain Criteria

SC-GRS simple count genetic risk score

SCID Structured Clinical Interview for DSM-IV-TR

SNPs single-nucleotide polymorphisms

TEAM Treatment of Early Age Mania

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Clinical Points

1. Early-onset bipolar disorder may represent a distinct phenotype with greater severity and genetic loading.
2. Despite considerable prior work, the genetics of early-onset bipolar disorder are poorly understood.
3. A candidate genetic risk score with 8 single-nucleotide polymorphisms showed an association with early-onset bipolar disorder.

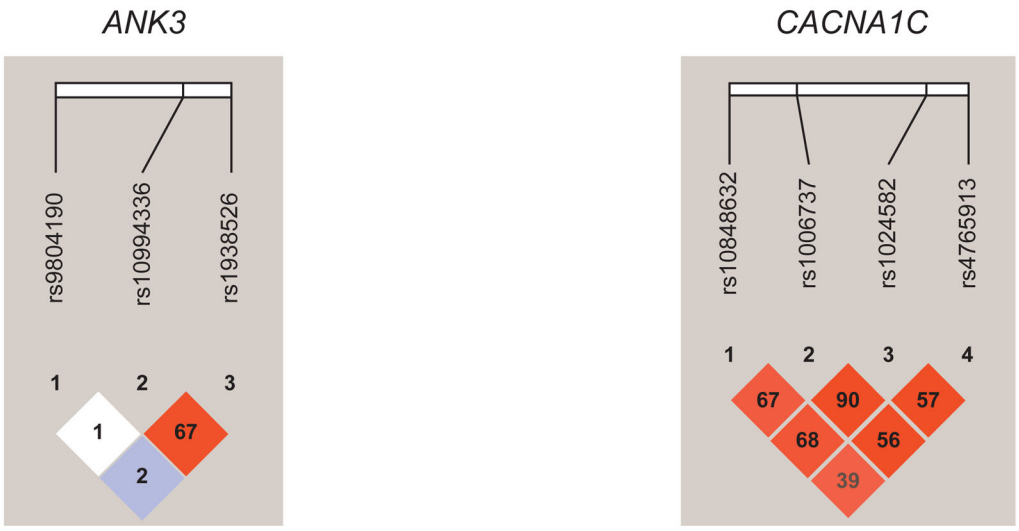

Figure.

Linkage Disequilibrium Plots for *ANK3* and *CACNA1C* Single-Nucleotide Polymorphisms. Colors are based on D′, whereas numbers represent r^2 measures of linkage disequilibrium.

Table 1

Demographic Characteristics

Characteristic	TEAM (n=69)	Mayo, All Cases (n=732)	Mayo, Early-Onset Cases (n=192)	Mayo, Late-Onset Cases (n=256)	Controls (n=776)
Age at enrollment, mean (SD), y	10.5 (2.9)	42.0 (15.1)	35.9 (12.8)	47.3 (15.2)	52.5 (21.9)
Body mass index at enrollment, mean (SD), kg/m^2	19.4 (4.2)	30.1 (7.0)	30.2 (7.3)	29.6 (6.7)	26.0 (8.5)
Sex, male, No. (%)	30 (43.5)	303 (41.4)	65 (33.9)	122 (47.7)	318 (41.0)
Race, Caucasian, No. (%)	69 (100)	732 (100)	192 (100)	256 (100)	776 (100)
Age of onset, mean (SD)	4.9 (2.5)	24.8 (13.9)	13.7 (3.7)	33.2 (12.8)	...
Attention-deficit/hyperactivity disorder, No. (%)	67 (97.1)	183/691 (26.5)	55/184 (29.9)	54/244 (22.1)	...
Psychosis, No. (%)	57 (82.6)	323/716 (45.1)	109/187 (58.3)	140/254 (55.1)	...
Mixed episodes, No. (%)	66 (95.7)	92/575 (16.0)	27/186 (14.5)	38/244 (15.6)	...
Separation anxiety disorder, No. (%)	25 (36.2)	...	...	...	...
Panic disorder, No. (%)	0 (0)	224/700 (32.0)	74/185 (40.0)	66/249 (26.5)	...
Obsessive-compulsive disorder, No. (%)	14 (20.3)	109/703 (15.5)	38/184 (20.7)	34/246 (13.8)	...
Social phobia, No. (%)	20 (29.0)	...	...	...	...
Social anxiety disorder, No. (%)	…	179/696 (25.7)	50/182 (27.5)	68/247 (27.5)	...
Generalized anxiety disorder, No. (%)	23 (33.3)	353/706 (50.0)	87/183 (47.5)	124/249 (49.8)	...
Any substance dependence or abuse, No. (%)	1 (1.4)	411/722 (56.9)	112/189 (59.3)	138/255 (54.1)	...

Abbreviation: TEAM, Treatment of Early Age Mania study.

Table 2

Genetic Risk Score Analysis of Patients With Early-onset Bipolar Disorder Compared With Controls

		Mean Risk Score			
Groups Compared	**Risk Score**	**Cases**	**Controls**	**OR**	***P* Value**
Adult cases vs controls	SC-GRS	4.10	4.06	1.01	.71
	OR-GRS	0.27	0.26	1.10	.56
TEAM vs controls	SC-GRS	4.48	4.06	1.10	.11
	OR-GRS	0.30	0.26	1.58	.24
Adult EOC vs controls	SC-GRS	4.38	4.06	1.08	.06
	OR-GRS	0.32	0.26	1.77	.02
TEAM+adult EOC vs controls	SC-GRS	4.41	4.06	1.08	.02
	OR-GRS	0.31	0.26	1.74	.01
TEAM+adult EOC vs adult LOC	SC-GRS	4.41	4.05	1.09	.047
	OR-GRS	0.31	0.26	1.74	.06
Adult LOC vs controls	SC-GRS	4.05	4.06	0.99	.96
	OR-GRS	0.26	0.26	1.05	.85

Abbreviations: EOC, early-onset cases; LOC, late-onset cases; OR, odds ratio; OR-GRS, odds ratio–weighted genetic risk score; SC-GRS, simple count genetic risk score; TEAM, Treatment of Early Age Mania.

Table 3

CACNA1C

Haplotype Analysis for TEAM and Adult Early-onset Cases of Bipolar Disorder Compared With Controls

Analysis	Haplotype[a]	Case Freq	Control Freq	*P* Value	Max-stat Sim *P* Value	Global-stat *P* Value
TEAM vs controls	CGGT	0.48	0.59	.01		.01
	TAAT	0.14	0.13	.63		
	TAAA	0.22	0.20	.46		
	TGGT	0.11	0.05	.002	.009	
Adult EOC vs controls	CGGT	0.56	0.59	.33	.76	.79
	TGGT	0.05	0.05	.66		
	TAAT	0.13	0.13	.63		
	TAAA	0.22	0.20	.37		
TEAM+adult EOC vs controls	CGGT	0.54	0.59	.048	.15	.21
	TAAT	0.14	0.13	.54		
	TAAA	0.22	0.20	.28		
	TGGT	0.07	0.05	.07		

Abbreviations: EOC, early-onset cases; Freq, frequency; Global-stat, global statistic; Max-stat Sim, maximum statistic simulation; TEAM, Treatment of Early Age Mania.

[a]Haplotype consists of rs10848632, rs1006737, rs1024582, and rs4765913.

Table 4

Association of Single-Nucleotide Polymorphisms With Bipolar Disorder Based on an Analysis of Adult Cases Compared With Controls

SNP	Gene	MAF Cases	MAF Controls	OR	*P* Value
rs10848632	*CACNA1C*	0.39	0.39	1.02	.77
rs1006737	*CACNA1C*	0.34	0.35	0.99	.92
rs1024582	*CACNA1C*	0.35	0.35	0.99	.94
rs4765913	*CACNA1C*	0.22	0.23	0.98	.79
rs1938526	*ANK3*	0.07	0.07	1.04	.76
rs9804190	*ANK3*	0.22	0.23	0.98	.77
rs10994336	*ANK3*	0.07	0.06	1.10	.53
rs12576775	*ODZ4*	0.17	0.16	1.07	.48

Abbreviations: MAF, minor allele frequency; OR, odds ratio; SNP, single-nucleotide polymorphism.

Table 5

Association of Candidate Single-Nucleotide Polymorphisms With Early-onset Bipolar Disorder

			TEAM Cases vs Controls			Adult EOC vs Controls s			TEAM+Adult EOC vs Controls s	
SNP	**Gene**	**Control MAF**	**MAF TEAM**	**OR**	***P* Value**	**MAF Mayo EOC**	**OR**	***P* Value**	**OR**	***P* Value**
rs10848632	*CACNA1C*	0.39	0.49	1.54	.017	0.42	1.12	.32	1.22	.056
rs1006737	*CACNA1C*	0.35	0.39	1.20	.34	0.36	1.08	.52	1.11	.33
rs1024582	*CACNA1C*	0.35	0.40	1.23	.26	0.38	1.12	.34	1.15	.19
rs4765913	*CACNA1C*	0.23	0.27	1.28	.24	0.24	1.08	.59	1.13	.32
rs1938526	*ANK3*	0.07	0.07	1.02	.95	0.10	1.37	.10	1.28	.16
rs9804190	*ANK3*	0.23	0.24	1.07	.76	0.20	0.85	.26	0.91	.43
rs10994336	*ANK3*	0.06	0.07	1.06	.88	0.09	1.49	.049	1.37	.086
rs12576775	*ODZ4*	0.16	0.20	1.25	.34	0.18	1.14	.38	1.17	.24

Abbreviations: EOC, early-onset cases; MAF, minor allele frequency; OR, odds ratio; SNP, single-nucleotide polymorphism; TEAM, Treatment of Early Age Mania.