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Suggestive linkage of the CBCL juvenile bipolar disorder phenotype to 1p21, 6p21 and 8q21

Alysa E. Doyle, PhD^{1,2,3}, Joseph Biederman, MD^{1,3}, Manuel AR Ferreira, PhD⁴, Patricia Wong, BA³, Jordan W. Smoller, MD, ScD^{1,2}, and Stephen V. Faraone, PhD⁵

¹ Drs. Doyle, Biederman and Smoller are with Harvard Medical School

² Drs. Doyle and Smoller are faculty in the Psychiatric and Neurodevelopmental Genetics Unit in the Center for Human Genetics Research at Massachusetts General Hospital (MGH)

³ Drs. Doyle and Biederman, and Ms. Wong are with the Pediatric Psychopharmacology Unit at MGH

⁴ Dr. Ferreira is in Queensland Institute of Medical Research

⁵ Dr. Faraone is with SUNY Upstate Medical University

Abstract

Objective—Several studies have documented a profile of elevated scores on the Attention Problems, Aggressive Behavior and Anxious/Depressed scales of the Child Behavior Checklist (CBCL) in youth with bipolar disorder. The sum of these scales, referred to as the CBCL Juvenile Bipolar Disorder (JBD) phenotype, has modest diagnostic utility, and high scores are associated with severity of psychopathology and poor outcome. Recently, a genomewide linkage scan of this measure in ADHD sibling pairs revealed a region of suggestive linkage on chromosome 2q21. The current study aimed to further identify quantitative trait loci that influence the CBCL-JBD phenotype by using a dense and thus, arguably, more powerful set of single nucleotide polymorphism markers in a different ADHD sibling pair sample.

Correspondence to Dr. Alysa E. Doyle, MGH WRN 705, 55 Fruit Street, Boston MA 02114; aedoyle@partners.org; Phone: 617-724-1531; Fax 617-724-1540.

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Method—Subjects were 765 individuals from 154 families with CBCL data enrolled in a linkage study of ADHD. Linkage analyses were completed using a multipoint maximum likelihood variance components approach implemented using the statistical program SOLAR.

Results—Heritability of the CBCL-JBD phenotype was estimated at .71. Although no regions of the genome surpassed empirically-derived criteria for significant linkage ($p=0.000038$), peaks on 1p21.1 ($p=0.00037$; LOD =2.76), 6p21.3 ($p=0.00054$; LOD =2.60) and 8q21.13 ($p=0.00081$; LOD =2.44) surpassed the threshold for suggestive linkage ($p=.002$). These regions have been highlighted in genomewide scans of bipolar disorder in adults, schizophrenia, autism and ADHD.

Conclusions—Findings raise the possibility that genes in these regions influence variation on the CBCL-JBD scale and the emotional and behavioral dysregulation associated with severe psychopathology.

Keywords

juvenile bipolar disorder; CBCL; genomewide linkage

Introduction

Whether to characterize youth with extreme emotional lability, aggression and behavioral dysregulation as having juvenile-onset bipolar disorder (BPD) has been a source of debate among child psychiatrists; however, there is no disagreement that understanding the etiology of such behavior is a priority for the field. Children and adolescents who carry the diagnosis of BPD share several, though not all, symptoms with the adult onset form of the condition¹ and are at risk for poor outcome, including suicide attempts² and hospitalization.³ Yet, questions remain about diagnostic boundaries, including whether youth who lack discrete episodes of euphoria/grandiosity should be considered part of the bipolar spectrum⁴ and how to account for symptomatic overlap with ADHD.^{5–7} Although recommendations have been made to assist with differential diagnoses,⁸ and a practice parameter for the disorder has been published,⁹ the lack of consensus among experts renders quantitative behavior rating scales an important tool for research into the origins of these problems.

Of particular interest is a profile from the well-studied, parent-rated Child-Behavior Checklist¹⁰ consisting of elevated scores on the Attention Problems, Aggressive Behavior and Anxious/Depressed scales. The profile was first noted by Biederman et al.¹¹ to occur in youth with BPD and to differ from scores of those without the condition, including children with ADHD who showed only an elevated Attention Problems Scale. Elevations on one or more of these three scales have been documented in youth with BPD in inpatient and outpatient settings and across different cultures,^{12–17} and the profile of elevations is supported by meta-analysis.¹⁸ The sum of the three scales, referred to as the CBCL-Juvenile Bipolar Disorder (CBCL-JBD) phenotype, has shown utility for the prediction of interview-based diagnoses of BPD in some studies,^{19,20} including one large longitudinal analysis.²¹ However, data from other samples, particularly those in which there is a low base rate of BPD,^{22,23} have not supported the profile's diagnostic utility. Nonetheless, all studies are in agreement that elevated scores characterize patients of significant clinical concern due to suicidality,^{22–24} comorbid mood and disruptive behavior disorders,²³ hospitalization,²¹ severe symptomatology²⁵ and/or functional impairment.^{23,26}

Recently, twin studies have indicated a substantial genetic contribution to the CBCL-JBD phenotype. In an ADHD-enriched Missouri birth cohort of twins, the heritability of the measure was estimated at .65²² and an association was found with the gene for the dopamine transporter though not for the dopamine 4 receptor. In a large sample of Dutch twins at ages 7, 10 and 12, Hudziak et al.²⁷ estimated the phenotype's heritability to be .6

– .7. Longitudinal data from the same sample indicated stability of CBCL-JBD scores, with 80% of the stability due to additive genetic effects.²⁸ Althoff et al.²⁹ also found a highly heritable CBCL-JBD latent class in this sample associated with suicidal ideation. Together, these estimates suggest that the measure may offer a window into the heritable biological mechanisms underlying the emotional lability, aggression and behavioral dysregulation frequently found in bipolar youth.

To date, only one previous molecular genetic study has examined this profile using a genomewide approach. McGough and colleagues³⁰ conducted a linkage scan of the CBCL-JBD phenotype in a sample of affected ADHD sibling pairs from 270 families using 423 polymorphic markers. Although no regions yielded signals that exceeded the threshold for significant linkage, a region of suggestive linkage was found on chromosome 2q23, with a multipoint maximum LOD = 2.5. As McGough et al. point out, this region had previously emerged as a genomewide significant epistatic locus in a linkage scan of BPD in adults published in 2007.³¹ Thus, McGough et al.'s data support continued investigation into the genetic bases of the CBCL-JBD scale to find risk loci for psychiatric symptomatology associated with pediatric-onset BPD.

In the current study, we aimed to follow-up and extend findings from McGough et al.³⁰ using a more densely spaced and thus arguably more powerful set of 4885 SNP markers in a different sample of ADHD sibling pairs. Given the prior literature, we predicted that our analyses would identify quantitative trait loci (QTL) that influence scores on the CBCL-JBD phenotype.

METHOD

Sample Ascertainment

Data were from an affected sibling-pair linkage study of ADHD conducted at Massachusetts General Hospital (MGH).³² Study methods have been described previously.^{32,33} The original study included 1212 individuals from 271 families with at least one pair of full biological siblings meeting DSM-IV ADHD criteria or with one member of the pair meeting full and the other meeting subthreshold (2/3) criteria. Families were ascertained through the MGH Pediatric Psychopharmacology Program, local child psychiatry and pediatric practices, newspaper ads, an outpatient child psychiatry clinic at Boston Children's Hospital and the University of Nebraska. Potential subjects were excluded if their nuclear family was unavailable, or if they were adopted, refused a blood sample or had major sensorimotor handicaps, active psychosis or suspected IQ < 70. No ethnic/racial groups were excluded, resulting in a sample that was 95% Caucasian, 4% African-American and 1% other. After procedures and risks were described, parents provided written informed consent for themselves and their children to participate. Children and adolescents provided written assent. The study was approved by the Partners/MGH Human Research Institutional Review Board.

Clinical Assessment

For youth ages 6 to 17, DSM-IV Axis I disorders, including ADHD and BPD, were assessed using the Schedule for Affective Disorders – Child Epidemiological Version (Kiddie-SADS-E), a widely used semi-structured diagnostic interview, with established psychometric properties.³⁴ For all youth, psychiatric data were collected from the mother or primary caregiver. Youth 12 and older were also directly interviewed. Children younger than 12 were not interviewed because studies suggest limited reliability of their reports.^{35–38} Subjects 18 and older received the Structured Clinical Interview for DSM- IV (SCID)³⁹

with a supplement from the K-SADS-E to assess ADHD and other childhood-onset disorders.

Interviewers had Master's or Bachelor's degrees in psychology or a related field and were trained to high levels of reliability with one another and clinicians. Raters were blind to recruitment status because they were conducting psychiatric interviews for other MGH studies. Best-estimate diagnoses were made after blind review of data by psychiatrists and psychologists.⁴⁰ To combine discrepant parent-offspring reports, the most severe diagnosis was used, unless it was deemed unreliable.

IQ was estimated using the Wechsler Intelligence Scale for Children- Third Edition^{41,42} for subjects 6 to 17, and the Wechsler Adult Intelligence Scale- Third Edition^{41,42} for those 18 and older.

Mothers filled out a CBCL on each of their children. The CBCL is a well-validated questionnaire that assesses behavior problems and social competence in children aged 6 to 18 years, as reported by a parent or primary caregiver for the two months prior to the questionnaire's administration. The measure was normed on over 2,300 non-referred children representative of the U.S. population. Questionnaires were returned by mail on 319 youth from 154 families who were included in the current analysis. Table 1 shows the breakdown of family configurations useful for linkage analysis based on available phenotypes and genetic data.

A CBCL-JBD phenotype was constructed for youth with available data. Consistent with the literature, the score reflected the sum of the T scores from the Attention Problems, Aggressive Behavior and Anxious/Depressed Scales.

Genotyping

Genotyping was completed at the Center for Inherited Disease Research at Johns Hopkins. Markers were derived using Illumina's Linkage IVb SNP-based marker panel based on its BeadArrayTM technology on a BeadLab system. This system shows good power for mapping complex phenotypes, with a genomewide average information content of > 97.1%. The original panel consisted of 6,008 SNPs across all autosomes and sex chromosomes. Markers cover the genome with an average genetic distance of 0.64 cM and an average 482 Kb gap. The average marker heterozygosity is 0.43 in Caucasians, 0.38 in African Americans and 0.36 in Asians. Using Illumina's Gentrain software to assess the quality of genotypes, only 28 SNPs were dropped for poor performance. DNA samples from eight individuals were dropped due to poor performance. The missing data rate for all samples in the original family analysis was 0.192%. The overall missing data rate after removing Mendelian inconsistencies was 0.56%. Duplicate concordance was excellent: based on 56 experimental samples genotyped in duplicate, the blinded duplicate error rate was 0.002%. Thus, overall the genotype data are high quality.

To avoid biases in the estimation of identity-by-descent probabilities, the dataset was pruned to remove correlated SNPs ($r^2 > 0.2$), resulting in a final set of 4885 markers. Marker physical and genetic positions were obtained from the published CIDR map http://www.cidr.jhmi.edu/snp_marker.html.

DATA ANALYSIS

Analyses were conducted using SOLAR version 4.1.5.⁴³ Heritability for the CBCL-JBD phenotype was determined by maximum-likelihood univariate variance component analysis, which decomposed phenotypic variance and covariance into components due to polygenic

additive genetic variance and environmental factors not shared between relatives. Given our sample of reared together non-twin siblings, the genetic parameter, in actuality, represents the upper bound of heritability because it also includes variance due to environmental factors shared between relatives; nonetheless, it is useful for assessing the suitability of the CBCL-JBD phenotype for inclusion in linkage analyses and comparability to heritability estimates in the literature. Age and sex were included as covariates in the analyses, and the significance of models was determined by the likelihood-ratio chi-square test.

We performed a multipoint maximum-likelihood variance components linkage analyses for the CBCL-JBD measure, adjusted for age and sex. This approach compares the maximum-likelihood estimation of the CBCL-JBD phenotype decomposed into contributions from the QTL and residual genetic and environmental components with the model based on the null hypothesis of no influence of the QTL. Consistent with McGough, we included a model with no dominance effects because twin data support predominantly additive genetic contribution to the scale. The LOD score was then converted to empirical pointwise P -values ($-\log_{10}P$) through the analysis of 1,000 genome-scan datasets simulated under the null hypothesis of no linkage. Suggestive and significant levels of significance were calculated using the 1,000 simulated genome-scans based on recognized guidelines.⁴⁴

RESULTS

Demographic features of the overall sample have been described.^{32,33} Youth with available CBCL data who were the focus of the current analyses ($N=319$) were 61% male with a mean age of 11.1 ($SD=4.0$) and an IQ in the average range ($106.9; SD=15.0$). Seventy-eight percent of youth met criteria for full DSM-IV ADHD (45% Combined, 28% Predominantly Inattentive and 6% Predominantly Hyperactive/Impulsive Types). Comorbid conditions were: oppositional defiant disorder (38.5%), conduct disorder (11.0%), major depressive disorder (14.4%), BPD (16.3%), generalized anxiety disorder (16.6%) and panic disorder (5.0%).

For purposes of comparison with other studies, means of the three T scores that were summarized to create the CBCL-JBD phenotype were: Attention Problems 62.6 ($SD=10.9$); Aggressive Behavior 59.4 ($SD=10.4$); and Anxious/Depressed 58.8 ($SD=9.3$).

Heritability of the CBCL-JBD phenotype was estimated at .71. No regions of the genome surpassed empirically-derived criteria for significant linkage ($p=0.000038$); however, regions on 1p21.1, ($p=0.00037$; $LOD=2.76$), 6p21.3 ($p=0.00054$; $LOD=2.60$) and 8q21.13 ($p=0.00081$; $LOD=2.44$) surpassed the empirically-derived threshold for suggestive linkage ($p=.002$; See Table 2 and Figure 1).

Table 3 lists published genomewide studies of psychiatric disorders that have highlighted the regions of interest from our linkage scan. Notably, all three regions have been highlighted previously in studies of major psychiatric illnesses, including schizophrenia (1p21.1, 6p21.3 and 8q21.13), autism (1p21.1, 6p21.3 and 8q21.13) and bipolar disorder (6p21.3 and 8q21.13).

DISCUSSION

The CBCL-JBD phenotype is a parent-rated measure based on a profile of CBCL scale-elevations that have been replicated in bipolar youth. The three scales that contribute to the overall score (ie Attention Problems, Aggressive Behavior and Anxious/Depressed) contain items that reflect symptoms of cognitive, behavioral and emotional dysregulation. We conducted a genomewide linkage scan of this measure in 154 families ascertained through ADHD sibling pairs. Based on a panel of 4885 SNP markers, our scan identified three

regions of the genome (1p21.1, 6p21.3 and 8q21.13) that surpassed empirically-defined criteria for suggestive linkage. These results extend support for the suitability of the CBCL-JBD phenotype for molecular genetic analyses and raise the possibility that QTL under these peaks influence psychiatric symptomatology in youth.

Prior to this analysis, only one study had examined the CBCL-JBD phenotype using a genome-wide approach. McGough et al.³⁰ found a region of suggestive linkage on 2q23, a location at which genome-wide significance had been achieved in a two-dimensional linkage scan of adult bipolar disorder in 52 families. Our current study did not yield a linkage peak in this region. Inconsistent patterns of replication are often found in linkage studies of complex phenotypes, most likely due to genetic heterogeneity and the low power of linkage studies to find genes of small effect.⁴⁵ In the current case, it is possible that this lack of convergence is due to differences in methods or sample characteristics, e.g. the UCLA study required that two parents be available for study, used a different diagnostic interview (i.e. KSADS-PL), had a lower rate of BPD and larger percentage of non-Caucasian subjects and used a microsatellite-based genotyping platform with less dense coverage.

Although not overlapping with McGough et al.³⁰, the suggestive linkage peaks from the current scan include regions highlighted by prior genome scans of major psychiatric disorders. Like McGough et al., our data show convergence with risk loci identified by prior genome-wide studies of BPD in adults. As shown in Table 3, our peaks on 6p21.3 and 8q21.13 were highlighted in a meta-analysis of genome-wide expression studies of bipolar disorder.⁴⁶ Additionally, the region on 6p21 included a SNP ranked in the top 200 results from the STEP-UCL GWAS of BPD in adults⁴⁷, and the region on 8q21 overlapped with secondary-level associations in the bipolar sample from the WTCCC GWAS (1×10^{-5} < p value $< 1 \times 10^{-4}$).⁴⁸

Moreover, all three regions overlap with genome-wide scans of autism and schizophrenia. Our region on 1p21.1 overlapped with the primary findings in two previous linkage studies of schizophrenia and one of autism. For example, in 236 affected sibling pair families from Japan⁴⁹, this region was the site of the primary (genome-wide significant) linkage peak for schizophrenia in the study. In a smaller sample of 22 Canadian families with a heavy schizophrenia loading, this region harbored one of 18 peaks with a LOD score of > 1.5 in.⁵⁰ This region also included the peak marker in an initial genome-wide scan of 90 multiplex autism families⁵¹ that showed allele sharing in an additional 49 families, though the combined sample fell short of genome-wide significance.

Peaks on 6p21.3 and 8q21.13 also overlap with putative risk loci identified through genome-wide scans of schizophrenia and autism. A meta-analysis of genome-wide linkage scans from 2003⁵² identified the 6p region as one of twelve (out of 120) bins considered strong schizophrenia candidate regions with $P_{\text{AvgRnk}} < .05$ in weighted and unweighted analysis. Additionally, one of the top 25 SNPs from the first genome-wide association scan of schizophrenia⁵³ fell in this region, which also overlaps with 3 of 12 clusters of interest in the same study. The region identified on 8q21.13 overlapped with a secondary level association in the CATIE GWAS of schizophrenia.⁵³ And, both the 6p and 8q regions were sites of autism-specific copy number variants.⁵⁴

The current results are intriguing given data supporting a genetic overlap between schizophrenia and BPD,⁵⁵ schizophrenia and autism⁵⁶ and growing evidence for a three-way genetic overlap between schizophrenia, BPD and autism.⁵⁷ Although a small number of candidate genes that may influence this cluster of disorders have been explored in other regions (e.g. DISC1),⁵⁸ more variants are expected to emerge to explain the familial and

genetic overlap of these conditions. Our data raise the possibility that such variants lie within our highlighted regions and are indexed by the CBCL-JBD scale.

Determining precisely what construct is measured by the CBCL-JBD phenotype goes beyond the current study. Yet, convergence with genomewide scans of a range of conditions lends support to the hypothesis that the measure reflects a heritable trait relevant to a range of severe psychopathology rather than a specific measure of pediatric BPD per se. The items on the three scales that contribute to the CBCL-JBD profile reflect emotional and behavioral lability and distractability, i.e. items that index the capacity for self-regulation across a wide range of domains (i.e. cognitive, behavioral and affective). Further evidence for this conceptualization comes from Ayer and colleagues⁵⁹ who found that the CBCL-JBD phenotype can be modeled as sharing a single latent trait with a different secondary CBCL scale purported to measure post-traumatic stress problems (PTSP). Like the CBCL-JBD phenotype, the PTSP scale is associated with suicidality and poor outcome and features a number of items overlapping with the CBCL-JBD that relate to self-regulation. Based on this analysis, the authors suggest both scales index a single dysregulatory syndrome.

The fact that the CBCL-JBD phenotype taps into a trait relevant to a range of psychiatric disorders may help to explain the profile's lack of diagnostic specificity to juvenile-onset BPD in clinical studies.²⁶ Yet, the empirical literature supports the relevance of the profile to the emotional and behavioral dysregulation that is characteristic of BPD in youth, thereby leaving open the possibility that genes that influence variation on the measure are relevant to this disorder. The profile of elevations that form the basis of the phenotype has been repeatedly replicated in affected children and adolescents.¹⁸ Faraone et al.¹⁹ also showed that the profile has excellent diagnostic efficiency for concurrent interview-based bipolar diagnoses in children and adolescents (AUC's = .82 – .97) from an ADHD family study, and Diler et al.²⁶ showed moderate diagnostic efficiency in retrospective ratings of individuals <12 years from a study of bipolar children (AUCs: .70–.77). A recent study²¹ further demonstrated good longitudinal prediction of BPD and associated features such as hospitalization, major depression and conduct disorder. Even in studies that have indicated poor diagnostic utility for the measure,^{22–24,26} the association of high CBCL-JBD scores with suicidality and hospitalization^{22–24,26} supports its relevance to a severe mood disorder.

Further work is needed to investigate susceptibility genes that may exist in these regions and their relationship to different forms of psychopathology. Given that genomewide association studies will have greater power and resolution than standard linkage scans to identify common variants of small effect, such studies provide the next logical step to follow-up these findings. Still, genomewide linkage scans such as this remain useful for identifying rare variants with strong effects in a small number of families or multiple mutations in the same gene that may differ across families. Thus, a lack of replication of the current results in a standard GWAS study of the CBCL-JBD phenotype would suggest that these other possibilities should be considered.

Such work is needed given how little is currently known about the genetic risk factors for mood disorders in children. As Mick and Faraone⁶⁰ have recently reviewed, family studies and secondary analyses in adult bipolar linkage studies support the hypothesis that an early age of onset is associated with a greater genetic risk than adult-onset BPD. Nonetheless, no regions that have emerged in adult linkage studies of early-onset cases have been replicated or overlap with the current findings. A handful of candidate gene studies have found interesting associations with pediatric BPD for BDNF, SLC6A3 and GAD1, but contradictory findings and failures to replicate have not yielded definitive conclusions, and new candidates from the adult bipolar literature have yet to be tested in affected pediatric samples.

The current study should be considered in the context of its limitations. Our analyses, like those of McGough et al.³⁰, were conducted in families originally ascertained through ADHD sibling pairs. Such a sample is relevant to the etiology of pediatric BPD given evidence from multiple samples of shared familial influences on ADHD and the disorder⁶¹ and the high rate of ADHD in youth with bipolar diagnoses. Additionally, the heritability of the phenotype in the current sample was comparable to the heritability estimated from large population-based twin studies. Moreover, the QTL identified in these two studies do not overlap with loci identified through genomewide studies of ADHD, with the exception of the finding on 6p21.3 which included one of the top 25 SNPs from a genomewide association scan of ADHD.⁶² Thus, the current findings do not appear solely driven by ADHD. Still, further studies are needed to replicate and extend our findings in non-ADHD samples.

A second limitation is the low rate at which subjects in the sample returned the CBCL questionnaires. Our analysis is based on only 54% of the original families in our linkage study. Yet the comparability of subject characteristics, including IQ and rates of comorbid diagnoses in this analysis, to those in the original study suggests that the current sample is not skewed in terms of high functioning families.

A small number of additional factors are worthy of consideration. For example, we note that the CBCL-JBD phenotype was positively skewed. However, the evidence for linkage at each marker was estimated through simulations (ie. we generated empirical p-values rather than rely on asymptotic theory). Thus, any potential bias introduced by normality deviations would have been accounted for (i.e. no increase in false-positive rates would be expected because of the normality deviation). We further note that this is the third paper based on this same linkage sample, following published papers examining the ADHD phenotype specifically³² and a multivariate analysis of neurocognitive traits and ADHD symptoms³³. Any single study can only realistically attempt to control its own study-wide error rate, and this and previous papers addressed specific, separate a priori hypotheses. Ultimately, though, issues of multiple testing should be acknowledged and our results require confirmation in independent samples. We also acknowledge that evidence for linkage to the identified regions is only suggestive. This finding, however, generates a very specific set of hypotheses (that loci regulating this phenotype are located in three defined chromosomal regions) that can be addressed by follow-up association studies.

Despite these concerns, our data raise the possibility that QTL on 1p21 6p21 and 8q21 contribute to variation on the CBCL-JBD phenotype. Further work is needed to confirm these findings, explore genes under these peaks and investigate the compelling hypothesis that risk variants in these regions contribute to severe, early-onset psychopathology and/or difficulties with self-regulation that overlap genetically with the shared risk for bipolar disorder, schizophrenia and autism.

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References

1. Coyle JT, Pine DS, Charney DS, et al. Depression and Bipolar Support Alliance Consensus Statement on the Unmet Needs in Diagnosis and Treatment of Mood Disorders in Children and Adolescents. *Journal of the American Academy of Child and Adolescent Psychiatry* 2003;42(12): 1494–1503. [PubMed: 14627885]

2. Goldstein TR, Birmaher B, Axelson D, et al. History of suicide attempts in pediatric bipolar disorder: factors associated with increased risk. *Bipolar Disord* 2005 Dec;7(6):525–535. [PubMed: 16403178]
3. Meyer SE, Carlson GA, Wiggs EA, et al. A prospective study of the association among impaired executive functioning, childhood attentional problems, and the development of bipolar disorder. *Dev Psychopathol* 2004 Spring;16(2):461–476. [PubMed: 15487606]
4. Demeter CA, Townsend LD, Wilson M, Findling RL. Current research in child and adolescent bipolar disorder. *Dialogues Clin Neurosci* 2008;10(2):215–228. [PubMed: 18689291]
5. Borchardt CM, Bernstein GA. Comorbid disorders in hospitalized bipolar adolescents compared with unipolar depressed adolescents. *Child Psychiatry and Human Development* 1995;26(1):11–18. [PubMed: 7587476]
6. West S, McElroy S, Strakowski S, Keck P, McConville B. Attention deficit hyperactivity disorder in adolescent mania. *American Journal of Psychiatry* 1995;152(2):271–273. [PubMed: 7840364]
7. Wozniak J, Biederman J, Kiely K, et al. Mania-like symptoms suggestive of childhood onset bipolar disorder in clinically referred children. *Journal of the American Academy of Child and Adolescent Psychiatry* 1995;34(7):867–876. [PubMed: 7649957]
8. Baroni A, Lunsford JR, Luckenbaugh DA, Towbin KE, Leibenluft E. Practitioner review: the assessment of bipolar disorder in children and adolescents. *J Child Psychol Psychiatry* 2009 Mar;50(3):203–215. [PubMed: 19309325]
9. McClellan J, Kowatch R, Findling RL. Practice parameter for the assessment and treatment of children and adolescents with bipolar disorder. *J Am Acad Child Adolesc Psychiatry* 2007 Jan;46(1):107–125. [PubMed: 17195735]
10. Achenbach, TM. Manual for the Child Behavior Checklist/4–18 and the 1991 Profile. Burlington, VT: University of Vermont, Department of Psychiatry; 1991.
11. Biederman J. Developmental subtypes of juvenile bipolar disorder. *Harvard Review of Psychiatry* 1995;3(4):227–230. [PubMed: 9384951]
12. Carlson G, Loney J, Salisbury H, Volpe R. Young referred boys with DICA-P manic symptoms vs. two comparison groups. *Journal of Affective Disorders* 1998;51(2):113–122. [PubMed: 10743844]
13. Dienes KA, Chang KD, Blasey CM, Adleman NE, Steiner H. Characterization of children of bipolar parents by parent report CBCL. *J Psychiatr Res* 2002;36(5):337–345. [PubMed: 12127602]
14. Geller B, Warner K, Williams M, Zimmerman B. Prepubertal and young adolescent bipolarity versus ADHD: assessment and validity using the WASH-U-KSADS, CBCL and TRF. *Journal of Affective Disorders* 1998;51:93–100. [PubMed: 10743842]
15. Hazell P, Lewin T, Carr V. Confirmation that Child Behavior Checklist clinical scales discriminate juvenile mania from attention deficit hyperactivity disorder. *Journal of Paediatrics and Child Health* 1999;35:199–203. [PubMed: 10365361]
16. Wals M, Hillegers MH, Reichart CG, Ormel J, Nolen WA, Verhulst FC. Prevalence of psychopathology in children of a bipolar parent. *J Am Acad Child Adolesc Psychiatry* 2001;40(9):1094–1102. [PubMed: 11556634]
17. Giles LL, DelBello MP, Stanford KE, Strakowski SM. Child behavior checklist profiles of children and adolescents with and at high risk for developing bipolar disorder. *Child Psychiatry Hum Dev* 2007 Jun;38(1):47–55. [PubMed: 17160586]
18. Mick E, Biederman J, Pandina G, Faraone SV. A preliminary meta-analysis of the child behavior checklist in pediatric bipolar disorder. *Biological Psychiatry* 2003;53(11):1021–1027. [PubMed: 12788247]
19. Faraone SV, Althoff RR, Hudziak JJ, Monuteaux MC, Biederman J. The CBCL predicts DSM bipolar disorder in children: A receiver operating characteristic curve analysis. *Bipolar Disorders* 2005;7(6):518–524. [PubMed: 16403177]
20. Kahana SY, Youngstrom E, Findling RL, Calabrese J. Employing parent, teacher, and youth self-report checklists in identifying pediatric bipolar spectrum disorders: An examination of diagnostic accuracy and clinical utility. *Journal of Child and Adolescent Psychopharmacology* 2003;13(4):471–488. [PubMed: 14977460]

21. Biederman J, Petty CR, Monuteaux MC, et al. The child behavior checklist-pediatric bipolar disorder profile predicts a subsequent diagnosis of bipolar disorder and associated impairments in ADHD youth growing up: a longitudinal analysis. *J Clin Psychiatry* 2009 Apr 21;70(5):732–740. [PubMed: 19389330]
22. Volk HE, Todd RD. Does the child behavior checklist juvenile bipolar disorder phenotype identify bipolar disorder? *Biol Psychiatry* 2007 Jul 15;62(2):115–120. [PubMed: 16950211]
23. Meyer SE, Carlson GA, Youngstrom E, et al. Long-term outcomes of youth who manifested the CBCL-Pediatric Bipolar Disorder phenotype during childhood and/or adolescence. *J Affect Disord*. 2008
24. Holtmann M, Bolte S, Goth K, et al. Prevalence of the Child Behavior Checklist-pediatric bipolar disorder phenotype in a German general population sample. *Bipolar Disord* 2007 Dec;9(8):895–900. [PubMed: 18076540]
25. Youngstrom E, Youngstrom JK, Starr M. Bipolar diagnoses in community mental health: Achenbach Child Behavior Checklist profiles and patterns of comorbidity. *Biol Psychiatry* 2005 Oct 1;58(7):569–575. [PubMed: 15950197]
26. Diler RS, Birmaher B, Axelson D, et al. The Child Behavior Checklist (CBCL) and the CBCL-bipolar phenotype are not useful in diagnosing pediatric bipolar disorder. *J Child Adolesc Psychopharmacol* 2009 Feb;19(1):23–30. [PubMed: 19232020]
27. Hudziak J, Althoff RR, Rettew DC, Derks EM, Faraone SV. The prevalence and genetic architecture of CBCL-juvenile bipolar disorder. *Biological Psychiatry* 2005;58(7):562–568. [PubMed: 16239161]
28. Boomsma DI, Rebollo I, Derks EM, et al. Longitudinal stability of the CBCL-juvenile bipolar disorder phenotype: A study in Dutch twins. *Biol Psychiatry* 2006 Nov 1;60(9):912–920. [PubMed: 16735031]
29. Althoff RR, Rettew DC, Faraone SV, Boomsma DI, Hudziak JJ. Latent class analysis shows strong heritability of the CBCL-juvenile bipolar phenotype. *Biol Psychiatry*. 2006
30. McGough JJ, Loo SK, McCracken JT, et al. CBCL Pediatric Bipolar Disorder Profile and ADHD: Comorbidity and Quantitative Trait Loci Analysis. *J Am Acad Child Adolesc Psychiatry* 2008 Aug 21;47(10)
31. Abou Jamra R, Fuerst R, Kaneva R, et al. The first genomewide interaction and locus-heterogeneity linkage scan in bipolar affective disorder: strong evidence of epistatic effects between loci on chromosomes 2q and 6q. *Am J Hum Genet* 2007 Nov;81(5):974–986. [PubMed: 17924339]
32. Faraone SV, Doyle AE, Lasky-Su J, et al. Linkage analysis of attention deficit hyperactivity disorder. *Am J Med Genet B Neuropsychiatr Genet* 2007 Dec 14;147B(8):1387–1391. [PubMed: 18081027]
33. Doyle AE, Ferreira MA, Sklar PB, et al. Multivariate genomewide linkage scan of neurocognitive traits and ADHD symptoms: Suggestive linkage to 3q13. *Am J Med Genet B Neuropsychiatr Genet* 2008 30;Oct 30;147B(8):1399–1411. [PubMed: 18973233]
34. Orvaschel, H. Schedule for Affective Disorder and Schizophrenia for School-Age Children Epidemiologic Version. 5. Ft. Lauderdale: Nova Southeastern University, Center for Psychological Studies; 1994.
35. Achenbach, TM.; McConaughy, SH. Empirically Based Assessment of Child and Adolescent Psychopathology: Practical Applications. Newbury Park: Sage Publications, Inc; 1987.
36. Breton J, Bergeron L, Valla J, Lepine S, Houde L, Gaudet N. Do children aged 9 through 11 years understand the DISC version 2.25 questions? *Journal of the American Academy of Child and Adolescent Psychiatry* 1995;34(7):946–956. [PubMed: 7649966]
37. Edelbrock C, Costello AJ, Dulcan MK, Kalas R, Conover NC. Age differences in the reliability of the psychiatric interview of the child. *Child Development* 1985;56:265–275. [PubMed: 3987406]
38. Schwab-Stone M, Fallon T, Briggs M, Crowther B. Reliability of diagnostic reporting for children aged 6–11 years: a test-retest study of the Diagnostic Interview Schedule for Children- Revised. *Am J Psychiatry* 1994;151(7):1048–1054. [PubMed: 8010363]

39. First, MB.; Spitzer, RL.; Gibbon, M.; Williams, JBW. Structured Clinical Interview for DSM-IV Axis I Disorders-Clinician Version (SCID-CV). Washington, DC: American Psychiatric Press; 1997.
40. Leckman JF, Sholomskas D, Thompson D, Belanger A, Weissman MM. Best estimate of lifetime psychiatric diagnosis: A methodological study. *Arch Gen Psychiatry* 1982;39:879–883. [PubMed: 7103676]
41. Wechsler, D. Manual for the Wechsler Intelligence Scale for Children. 3. San Antonio: The Psychological Corporation, Harcourt Brace Jovanovich, Inc; 1991.
42. Wechsler, D. Wechsler Adult Intelligence Scale III [manual]. 3. San Antonio, TX: The Psychological Corporation; 1997.
43. Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. *American Journal of Human Genetics* 1998;62:1198–1211. [PubMed: 9545414]
44. Kruglyak L, Lander ES. High-resolution genetic mapping of complex traits. *American Journal of Human Genetics* 1995;56:1212–1223. [PubMed: 7726179]
45. Suarez, BK.; Hampe, CL.; Van Eerdewegh, P. Problems of replicating linkage claims in psychiatry. In: Gershon, ES.; Cloninger, CR.; Barrett, JE., editors. *Genetic Approaches in Mental Disorders*. Washington, D.C: American Psychiatric Press; 1994. p. 23-46.
46. Elashoff M, Higgs BW, Yolken RH, et al. Meta-analysis of 12 genomic studies in bipolar disorder. *J Mol Neurosci* 2007;31(3):221–243. [PubMed: 17726228]
47. Sklar P, Smoller JW, Fan J, et al. Whole-genome association study of bipolar disorder. *Mol Psychiatry* 2008 Jun;13(6):558–569. [PubMed: 18317468]
48. Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared controls. *Nature* 2007 Jun 7;447(7145):661–678. [PubMed: 17554300]
49. Arinami T, Ohtsuki T, Ishiguro H, et al. Genomewide high-density SNP linkage analysis of 236 Japanese families supports the existence of schizophrenia susceptibility loci on chromosomes 1p, 14q, and 20p. *Am J Hum Genet* 2005 Dec;77(6):937–944. [PubMed: 16380906]
50. Brzustowicz LM, Hodgkinson KA, Chow EW, Honer WG, Bassett AS. Location of a major susceptibility locus for familial schizophrenia on chromosome 1q21–q22. *Science* 2000;288(5466):678–682. [PubMed: 10784452]
51. Risch N, Spiker D, Lotspeich L, et al. A genomic screen of autism: evidence for a multilocus etiology. *Am J Hum Genet* 1999 Aug;65(2):493–507. [PubMed: 10417292]
52. Lewis CM, Levinson DF, Wise LH, et al. Genome Scan Meta-Analysis of Schizophrenia and Bipolar Disorder, Part II: Schizophrenia. *Am J Hum Genet* 2003;73(1):34–48. [PubMed: 12802786]
53. Sullivan PF, Lin D, Tzeng JY, et al. Genomewide association for schizophrenia in the CATIE study: results of stage 1. *Mol Psychiatry* 2008 Jun;13(6):570–584. [PubMed: 18347602]
54. Marshall CR, Noor A, Vincent JB, et al. Structural variation of chromosomes in autism spectrum disorder. *Am J Hum Genet* 2008 Feb;82(2):477–488. [PubMed: 18252227]
55. Moskvina V, Craddock N, Holmans P, et al. Gene-wide analyses of genome-wide association data sets: evidence for multiple common risk alleles for schizophrenia and bipolar disorder and for overlap in genetic risk. *Mol Psychiatry* 2009 Mar;14(3):252–260. [PubMed: 19065143]
56. Burbach JP, van der Zwaag B. Contact in the genetics of autism and schizophrenia. *Trends Neurosci* 2009 Feb;32(2):69–72. [PubMed: 19135727]
57. Rzhetsky A, Wajngurt D, Park N, Zheng T. Probing genetic overlap among complex human phenotypes. *Proc Natl Acad Sci U S A* 2007 Jul 10;104(28):11694–11699. [PubMed: 17609372]
58. Tomppo L, Hennah W, Lahermo P, et al. Association Between Genes of Disrupted in Schizophrenia 1 (DISC1) Interactors and Schizophrenia Supports the Role of the DISC1 Pathway in the Etiology of Major Mental Illnesses. *Biol Psychiatry*. 2009 Feb 27;
59. Ayer L, Althoff R, Ivanova M, et al. Child Behavior Checklist Juvenile Bipolar Disorder (CBCL-JBD) and CBCL Posttraumatic Stress Problems (CBCL-PTSP) scales are measures of a single dysregulatory syndrome. *J Child Psychol Psychiatry*. 2009 May 22;
60. Mick E, Faraone SV. Family and genetic association studies of bipolar disorder in children. *Child Adolesc Psychiatr Clin N Am* 2009 Apr;18(2):441–453. x. [PubMed: 19264272]

61. Doyle AE, Faraone S. Familial Links Between ADHD, Conduct Disorder and Bipolar Disorder. *Current Psychiatry Reports* 2002;4(2):146–152. [PubMed: 11914177]
62. Neale BM, Lasky-Su J, Anney R, et al. Genome-wide association scan of attention deficit hyperactivity disorder. *Am J Med Genet B Neuropsychiatr Genet* 2008 Nov 3;147B(8):1337–1344. [PubMed: 18980221]
63. Schwab SG, Hallmayer J, Albus M, et al. A genome-wide autosomal screen for schizophrenia susceptibility loci in 71 families with affected siblings: support for loci on chromosome 10p and 6. *Mol Psychiatry* 2000 Nov;5(6):638–649. [PubMed: 11126394]
64. Bierut LJ, Stitzel JA, Wang JC, et al. Variants in nicotinic receptors and risk for nicotine dependence. *Am J Psychiatry* 2008 Sep;165(9):1163–1171. [PubMed: 18519524]

Figure 1.

Results of the genomewide linkage scan of the Child Behavior Checklist (CBCL) juvenile bipolar phenotype. Values on the Y-axis reflect the $-\log_{10}$ empirical point-wise p-value, with lines indicating thresholds for significant ($p=0.000038$) and suggestive ($p=.002$) linkage. Three regions surpassed the empirically-derived genomewide threshold for suggestive linkage: 1p21.1 ($p=0.00037$; LOD =2.76), 6p21.3 ($p=0.00054$; LOD =2.60) and 8q21.13 ($p=0.00081$; LOD =2.44).

Table 1

Families included in analyses based on available genotype and Child Behavior Checklist data

Main family configuration ^a	# Families	Total subjects ^b
2 parents, 3+ siblings	24	125
2 parents, 2 siblings	46	185
1 parent, 3+ siblings	6	25
1 parent, 2 siblings	23	70
0 parents, 3+ siblings	1	3
0 parents, 2 siblings	2	7

^aNumbers consider individuals in a family if they were genotyped (founders) or both genotyped and phenotyped (non-founders).

^bFor a small number of families data were included from other relatives (eg. children of siblings) other than those part of the main family configuration. For this reason, the total number of subjects may differ from that expected from the main family configuration and number of families with data.

Table 2

Detail of regions surpassing threshold for suggestive linkage.

Chromosomal Region	Top SNP	position	Empirical p-value	LOD	1-LOD interval
1p21.1	rs987243	105.75	0.00037	2.76	102.88 – 105.75
6p21.3	rs11908	33.05	0.00054	2.60	19.28 – 33.22
8q21.13	rs2162229	81.21	0.00081	2.44	76.64–89.36

SNP = Single nucleotide polymorphism; LOD= logarithm (base 10) of odds

Table 3

Overlap of linkage peaks with regions of interest in genomewide analyses of psychiatric disorders

Current Study		Overlapping study			Primary finding from paper?
Chromosome	Study	Study Type	Phenotype	Peak Marker/SNP (position)	
1p21.1	Arinami 2005 ⁴⁹	GLA	Schizophrenia	rs2048839 (105.7 Mb)	Yes (LOD 3.39) *
	Brzustowicz 2000 ⁵⁰	GLA	Schizophrenia	DIS1631	No (LOD 2.19)
	Risch 1999 ⁵¹	GLA	Autism	DIS1631	Yes (LOD 2.15)
	Sklar 2008 ⁴⁷	GWAS	Bipolar	rs176461 (28934384)	No- (OR 1.43)
6p21.3	Sullivan 2008 ⁵³	GWAS	Schizophrenia	rs9295938 (31061084; 31.06 Mb)	No- (OR 1.64)
	Schwab 2000 ⁶³	GLA	Schizophrenia	HLA-DQB1	Yes -NPL=3.3
	Lewis 2003 ⁵²	GSMA	Schizophrenia	Bin 6.2 (32.6 – 65.1 cM)	No- had P AvgRnk < 0.5 in weighted and unweighted analysis
	Neale 2008 ⁶²	GWAS	ADHD	rs10807124 (among top 25 SNPs – 6p21.32)	Yes-among top 25
8q21.13	Bierut 2007 ⁶⁴	GWAS two stage design	Nicotine Dependence	Rs999	Yes- among top 35 SNPs w/p < 10 ⁻⁴
	Marshall 2008 ⁵⁴	CNV	Autism		No
	WTCCC 2007 ⁴⁸	GWAS	Bipolar	rs6926599	No
	WTCCC 2007 ⁴⁸	GWAS	Bipolar	rs16919670; rs9643449	1 × 10 ⁻⁵ < p value < 1 × 10 ⁻⁴
	Sullivan 2008 ⁵³	GWAS	Schizophrenia		No
	Marshall 2008 ⁵⁴	CNV	Autism		No

* Reached genomewide significance

GLA = Genomewide Linkage Analysis; GWAS= Genomewide Association Study; GSMA = Meta-Analysis; ADHD = Attention-Deficit/hyperactivity Disorder; SNP = single nucleotide polymorphism; CNV = Copy number variant; LOD= logarithm (base 10) of odds; OR = Odds Ratio