Bipolar disorder (BD) and attention deficit hyperactivity disorder (ADHD) exhibit remarkably high rates of comorbidity, as well as patterns of familial co-segregation. Epidemiological data suggests that these disorders either share a common genetic architecture or that ADHD features in BD may represent an etiologically distinct subtype. We previously used the Wender Utah Rating Scale (WURS) to assess ADHD features in BD families and identified three heritable factors relating to impulsivity, mood instability, and inattention. Linkage analysis revealed a LOD score of 1.33 for the inattention factor on 5p15.3 near the dopamine transporter gene (DAT1), which has been associated with both BD and ADHD. Pharmacological evidence also suggests a role for DAT in both disorders. We have now evaluated the association of ten DAT1 variants for the WURS total score and factors in an overlapping sample of 87 BD families. Significant associations for three SNPs were observed across the WURS measures, notably for a SNP in intron 8 with the WURS total score ($P = 0.007$) and for variants in introns 9 and 13 with mood instability ($P = 0.009$ and 0.004, respectively). Analysis of an independent sample of 52 BD cases and 46 healthy controls further supported association of the intron 8 variant with mood instability ($P = 0.005$), and a combined analysis confirmed the associations of this SNP with...
INTRODUCTION

Bipolar disorder (BD) is a mood disorder that affects approximately 1% of the population and is characterized by episodes of major depression interspersed with mania (bipolar I) or hypomania (bipolar II) [Kelsoe, 1997]. Attention deficit/hyperactivity disorder (ADHD) affects 8–12% of children [Faraone et al., 2003] and 4% of adults [Kessler et al., 2006] and is characterized by persistent patterns of impulsivity, inattention, and hyperactivity. There are similarities between the attentional impairment and hyperactivity of ADHD and the symptoms of mania in BD, such as talkativeness, distractibility, and psychomotor agitation. Mood instability is also a common feature of ADHD [Holtmann et al., 2008; Waxmonsky et al., 2008], and impaired sustained attention has been observed across all BD states, as well as in unaffected relatives of BD probands [Savitz et al., 2005; Bora et al., 2009; Brotman et al., 2009; Goldberg and Chengappa, 2009].

BD and ADHD are frequently comorbid conditions, and epidemiological studies indicate a bidirectional overlap between them [Biederman et al., 1996, 1998, 1991b; Wozniak et al., 1995]. It is estimated that 85% of children with BD have comorbid ADHD, and up to 22% of children with ADHD have comorbid BD [Singh et al., 2006]. Approximately 16% of adult patients with BD have also been diagnosed with adult ADHD [Tamam et al., 2008; Ryden et al., 2009], and approximately 10% of ADHD patients develop BD [Wilen et al., 2003]. Elevated rates of childhood ADHD have also been observed in adults with BD [Winokur et al., 1993]. In addition to the remarkably high comorbidity between ADHD and BD, higher rates of BD are found amongst the first-degree relatives of probands with ADHD, and increased rates of ADHD have been reported in the relatives of BD probands [Faraone et al., 1997]. There is also an increased risk for ADHD among children of BD parents [Biederman et al., 1991a; Carlson and Weintraub, 1993].

Both BD and ADHD are familial with strong genetic components, as evidenced by heritability estimates up to 76–80% [Thapar et al., 2000; McGuffin et al., 2003; Faraone et al., 2005]. Yet, identifying the genes underlying these high heritabilities has proven difficult with small and inconsistent effects observed in association studies. Given the high rates of comorbidity and familial segregation patterns, it is possible that these disorders share a common genetic architecture or that ADHD features in BD may represent an etiologically distinct subtype [Faraone et al., 1997, 2001].

WURS total score. Impulsivity and mood instability (P = 0.002, 0.007, and 8 × 10^{-9}, respectively). These data suggest that variants within DAT1 may predispose to a subtype of BD characterized by early prodromal features that include attentional deficits. © 2012 Wiley Periodicals, Inc.

Key words: attention deficit hyperactivity disorder; Wender Utah Rating Scale; DAT1
Institute of Mental Health (NIMH) Genetics Initiative for Bipolar Disorder Waves 3 and 4 [Dick et al., 2003]. As WURS was collected only at the San Diego site, data was not available for the rest of the NIMH family collection. An independent set of non-familial BD cases was also recruited in San Diego. Control subjects were collected by advertising in the UCSD Mental Health Clinical Research Center and screened using the SCID for the absence of psychiatric illness.

All subjects provided written informed consent according to procedures approved by the local IRB of each university. Families were first identified through a proband diagnosed with bipolar I or bipolar II disorder (UCSD sample) or a bipolar I sibling pair (Waves 3 and 4) [Dick et al., 2003]. Each subject was interviewed and diagnosed using either a modified version of the Structured Clinical Interview for DSM-III-R (SCID) [Spitzer et al., 1992] or the Diagnostic Interview for Genetics Studies (DIGS) [Nurnberger et al., 1994]. Interviewers were extensively trained and reliability was regularly tested. A panel of clinicians reviewed the interview, medical records, and information from family informants in order to make a final DSM-IV diagnosis. A modified short form of the WURS was administered at the time of interview to retrospectively measure the severity of childhood ADHD symptoms. This self-rated scale includes 22 items, each of which is rated on a 5-point scale (0–4), and it has been shown to have good sensitivity, internal consistency, and test–retest reliability [Ward et al., 1993; McCann et al., 2000; Fossati et al., 2001]. Blood was also drawn on all subjects for the establishment of lymphoblastoid cell lines.

The final sample included 379 subjects from 60 UCSD families, 32 subjects from 8 NIMH Wave three families, and 92 subjects from 19 NIMH Wave four families. There was an average of 5.8 subjects per family, and 336 subjects had complete WURS data. Within the families, there were 139 subjects with BD, 90 subjects with major depressive disorder (MDD), 31 subjects with psychiatric conditions other than mood disorders (i.e., alcohol or substance abuse or dependence and anxiety spectrum disorders), and 70 subjects with no history of mental illness. The independent sample of 52 BD cases and 46 controls with complete WURS data was also included. All subjects were Caucasians of European ancestry.

**Genotyping**

Genotype data for 22 DAT1 SNPs was available for 61 subjects in 19 of the 87 families from prior genotyping efforts evaluating association with BD [Greenwood et al., 2001, 2006]. Based on our previous analyses of the haplotype block structure and function of DAT1, eight haplotype tagging SNPs and two additional SNPs in potentially functional regions of DAT1, as shown in Figure 1, were selected for follow-up genotyping in 259 subjects from the remaining 68 families and in the 98 cases and controls [Greenwood et al., 2002, 2006; Greenwood and Kelsoe, 2003; Guindalini et al., 2006].
DNA was extracted using QIAamp DNA Blood Kits from lymphocytes isolated from whole blood. Genotyping was performed using ABI SNPlex and an Applied Biosystems 3730 DNA Analyzer with GeneMapper Software for allele detection and identification.

### Statistical Analyses

We note that this sample overlaps with the subjects used in our previously published factor analysis of WURS measures, with 319 subjects from 81 of the families and 43 of the controls having been part of the previous analysis of 540 subjects, although only 57 of these families were available for our previous heritability and linkage analyses [Joo et al., 2009]. The distributions of all WURS scores approximated normality in this sample. We verified the factor structure of the WURS in the current sample using principal component analysis with promax rotation as previously described [Joo et al., 2009]. The Kaiser–Meyer–Olkin measure of sampling adequacy was 0.94, and the significance of Bartlett’s test of sphericity was <0.001. As expected, three correlated factors were observed, as shown in Table I, relating to impulsivity and defiant behavior, mood instability and anxiety, and inattention.

We also obtained heritability ($h^2$) estimates for the WURS scores in the 87 families via SOLAR v.4.2.0 with covariate adjustment for age at interview and sex [Almasy and Blangero, 1998]. The three factors and the WURS total score produced somewhat higher heritability estimates in this larger sample, with genes explaining 33–64% of the trait variance, as detailed in Table I.

### Table I. Description of All WURS Items, Factor Loadings, and Characteristics

<table>
<thead>
<tr>
<th>WURS Item</th>
<th>Factor 1 impulsivity</th>
<th>Factor 2 mood</th>
<th>Factor 3 inattention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temper outbursts, tantrums</td>
<td>0.91</td>
<td>0.00</td>
<td>−0.15</td>
</tr>
<tr>
<td>Disobedient, rebellious, sassy</td>
<td>0.90</td>
<td>−0.14</td>
<td>−0.03</td>
</tr>
<tr>
<td>Hot or short tempered, low boiling point</td>
<td>0.83</td>
<td>0.05</td>
<td>−0.13</td>
</tr>
<tr>
<td>Stubborn, strong willed</td>
<td>0.76</td>
<td>−0.09</td>
<td>−0.09</td>
</tr>
<tr>
<td>Trouble with authorities and school, visits to principals office</td>
<td>0.70</td>
<td>−0.34</td>
<td>0.33</td>
</tr>
<tr>
<td>Acting without thinking, impulsive</td>
<td>0.69</td>
<td>−0.04</td>
<td>0.25</td>
</tr>
<tr>
<td>Tendency to be or act irrational</td>
<td>0.65</td>
<td>0.12</td>
<td>0.13</td>
</tr>
<tr>
<td>Angry</td>
<td>0.58</td>
<td>0.46</td>
<td>−0.17</td>
</tr>
<tr>
<td>Trouble seeing things from someone else’s point of view</td>
<td>0.46</td>
<td>0.18</td>
<td>0.17</td>
</tr>
<tr>
<td>Anxious, worrying</td>
<td>−0.22</td>
<td>0.95</td>
<td>0.03</td>
</tr>
<tr>
<td>Sad or blue, depressed, unhappy</td>
<td>−0.04</td>
<td>0.94</td>
<td>−0.10</td>
</tr>
<tr>
<td>Low opinion of myself</td>
<td>−0.18</td>
<td>0.89</td>
<td>0.08</td>
</tr>
<tr>
<td>Nervous, fidgety</td>
<td>0.04</td>
<td>0.74</td>
<td>0.09</td>
</tr>
<tr>
<td>Moody, ups and downs</td>
<td>0.25</td>
<td>0.70</td>
<td>−0.05</td>
</tr>
<tr>
<td>Irritable</td>
<td>0.46</td>
<td>0.57</td>
<td>−0.14</td>
</tr>
<tr>
<td>Unpopular with other children</td>
<td>−0.08</td>
<td>0.45</td>
<td>0.34</td>
</tr>
<tr>
<td>Overall a poor student, slow learner</td>
<td>−0.05</td>
<td>−0.12</td>
<td>0.88</td>
</tr>
<tr>
<td>Not achieving up to potential</td>
<td>0.00</td>
<td>−0.01</td>
<td>0.85</td>
</tr>
<tr>
<td>Trouble with mathematics or numbers</td>
<td>−0.19</td>
<td>−0.01</td>
<td>0.76</td>
</tr>
<tr>
<td>Concentration problems, easily distracted</td>
<td>0.14</td>
<td>0.20</td>
<td>0.58</td>
</tr>
<tr>
<td>Inattentive, daydreaming</td>
<td>0.11</td>
<td>0.27</td>
<td>0.54</td>
</tr>
<tr>
<td>Trouble with stick to it-tiveness</td>
<td>0.15</td>
<td>0.22</td>
<td>0.54</td>
</tr>
<tr>
<td>Initial eigenvalues</td>
<td>10.22</td>
<td>1.98</td>
<td>1.72</td>
</tr>
<tr>
<td>Eigenvalues after rotation</td>
<td>8.50</td>
<td>8.20</td>
<td>6.40</td>
</tr>
<tr>
<td>Variance explained (%)</td>
<td>46.45</td>
<td>9.00</td>
<td>7.81</td>
</tr>
<tr>
<td>Component correlations*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Impulsivity</td>
<td>1.00</td>
<td>0.63</td>
<td>0.53</td>
</tr>
<tr>
<td>Mood</td>
<td>0.64</td>
<td>1.00</td>
<td>0.53</td>
</tr>
<tr>
<td>Inattention</td>
<td>0.51</td>
<td>0.51</td>
<td>1.00</td>
</tr>
<tr>
<td>Heritability ± standard error*</td>
<td>0.52 ± 0.13</td>
<td>0.55 ± 0.12</td>
<td>0.33 ± 0.12</td>
</tr>
</tbody>
</table>

The strongest factor loading for each item is indicated in bold. All heritability estimates have been adjusted as necessary for age at interview and sex. Heritability of the WURS total score was 0.64 ± 0.13 (P < 0.001).

*All P < 0.001.
associations with respect to specificity for particular ADHD features. The 98 BD cases and controls served as an independent sample for replication. Since UNPHASED can accommodate familial and unrelated individuals simultaneously, we also performed a combined analysis of all families, cases, and controls for the one SNP in intron 8 (rs27048) that was found to be independently associated in both samples. We performed 1,000–10,000 permutations with UNPHASED for all analyses to estimate the empirical significance of our results.

RESULTS

Association analyses of the ten DAT1 SNPs in the family sample identified significant associations for three SNPs with the WURS total score, as shown in Table II. The A allele of rs27048 in intron 8 was found to be strongly associated with higher WURS total scores ($P = 0.007$), while the T allele of rs2550936 in intron 9 and the G allele of rs11133767 in intron 13 revealed more moderate associations ($P < 0.05$). To assess the specificity of DAT1 SNPs for particular ADHD features, we evaluated association with the three WURS factors. The A allele of rs27048 in intron 8 was found to be moderately associated with higher inattention scores ($P = 0.031$). The T allele of rs2550936 in intron 9 and the G allele of rs11133767 in intron 13 were both strongly associated with higher mood scores ($P = 0.009$ and 0.004, respectively), with rs2550936 revealing moderate associations with higher impulsivity and inattention scores ($P < 0.05$).

Analysis of the independent case–control sample revealed a strong association for the A allele of rs27048 in intron 8 with higher mood scores ($P = 0.005$) and more moderate associations with the total and impulsivity scores ($P < 0.05$), as shown in Table III. No other association observed in the family sample was replicated in the case–control sample. Based on these observations, we performed a combined analysis of rs27048 in the family and case–control samples. This analysis produced even stronger associations with the total score ($P = 0.002$), impulsivity ($P = 0.007$), and mood ($P = 8 \times 10^{-4}$) than were seen in either sample individually, all in the same direction as the previously observed associations.

DISCUSSION

While the WURS and its three factor structure has been validated in several studies of patients with ADHD and control subjects, in addition to families and subjects with BD [McCann et al., 2000; Fossati et al., 2001; Joo et al., 2009, 2012], the relationship of WURS scores to early onset ADHD in BD or a subtype of BD with comorbid ADHD features has not been fully explored. We have shown here and elsewhere [Joo et al., 2009] that the WURS total score and factors are significantly heritable in BD families, suggesting that the first-degree relatives of BD subjects with high WURS scores and are also more likely to have high WURS scores and show evidence of childhood ADHD symptoms. Since BD subjects score higher across all WURS measures as compared to their unaffected relatives or controls [Joo et al., 2009], it is possible that the WURS may simply be detecting an early age at onset of BD or aspects of a BD prodrome characterized by mild mood and attentional symptoms. In our sample, we find that age at onset of BD is in fact significantly and negatively correlated with all WURS scores ($r = -0.4$, $P < 0.001$), except inattention, suggesting that subjects with more attentional deficits in childhood also tend to have an earlier age of onset of mood symptoms.
We have also explored the possible relationship of DAT1 variants to childhood ADHD features in BD. We observed strong associations between a variant in intron 8 (rs27048) and WURS scores in the BD family sample, the case-control sample, and the combined analysis. We have previously found evidence for association of this variant to BD (allelic \( P = 0.036 \)), as well as several haplotypes extending from intron 5 through intron 9 (peak genotypic \( P = 1 \times 10^{-5} \)), in an independent sample of 70 BD trios obtained from the NIMH bipolar collection [Greenwood et al., 2006]. The same allele (A) that was found to be associated with higher WURS scores in this study was also over-transmitted or part of an over-transmitted haplotype in our previous analyses of BD. This SNP is in strong LD with a 30 bp VNTR in intron 8 (\( D' = 0.82 \)), with the A allele of rs27048 segregating primarily with the 6-repeat allele of the VNTR. This VNTR appears to play a role in DAT1 expression, with the 6-repeat risk allele having reduced functional activity compared to the 5-repeat allele and showing evidence of allele specific induction in the presence of cocaine [Guindalini et al., 2006; Hill et al., 2010]. The 6-repeat allele has also shown associations with both cocaine abuse and adult ADHD [Guindalini et al., 2006; Silva et al., 2009]. Thus, the association with rs27048 observed here for childhood ADHD symptoms in BD and in our previous studies of BD may be a reflection of the intron 8 VNTR as a functional variant.

In addition to localizing a susceptibility variant near intron 8, we observed associations for variants in introns 9 (rs2550936) and 13 (rs1133767) with the WURS scores in the family sample. This is consistent with our previous studies of BD in 50 trios, which revealed an association with BD for rs1133767 (allelic \( P = 0.024 \)) [Greenwood et al., 2006], as well as significant associations to 3’ haplotypes extending from exon 9 through exon 15 (allelic \( P = 0.001 \), genotypic \( P = 4 \times 10^{-4} \)) [Greenwood et al., 2001]. It should be noted that 19 of the 50 trios overlap with the current BD family sample, which otherwise consists of larger, extended families. Again, the same allele of each variant (T for rs2550936 and G for rs1133767) that was found to be associated with higher WURS scores in this study was also over-transmitted or part of an over-transmitted haplotype in our previous analyses of BD. Linkage disequilibrium (LD) is well preserved in the 3’ region of DAT1 (see Fig. 1), and these alleles segregate together almost exclusively as part of a common ancestral haplotype that spans exons 9 through 15 and accounts for 63% of all 3’ haplotypes, with rs1133767 in intron 13 defining the 3’ clades [Greenwood et al., 2006]. The 10-repeat allele of the widely studied 40 bp VNTR in the 3’ UTR, which has been repeatedly associated with ADHD [Cook et al., 1995; Gill et al., 1997; Waldman et al., 1998; Daly et al., 1999; Barr et al., 2001; Curran et al., 2001; Chen et al., 2003], also segregates with this haplotype and is in strong LD (\( D' > 0.9 \); see Fig. 1) with rs1809393 in exon 15 [Greenwood et al., 2002, 2006]. This SNP revealed only a trend towards significance for mood instability in the family sample (\( P = 0.095 \)). While there is some data to suggest a functional role for the 3’ UTR VNTR, reports are conflicting as to which allele, the 9-repeat or 10-repeat, is associated with increased expression, with some studies finding no functional activity for either allele [Heinz et al., 2000; Jacobsen et al., 2000; Fuke et al., 2001; Michelaugh et al., 2001; Mill et al., 2002; Miller and Madras, 2002; Greenwood and Kelsoe, 2003; Hill et al., 2010]. It is therefore likely that the observed associations to the 3’ region of DAT1 with BD, ADHD, and childhood ADHD symptoms in BD reflect the presence of one or more undetected, possibly rare, functional variants that are in strong LD with the associated variants.

This study complements previous studies in suggesting the presence of multiple functional variants, each contributing a small effect towards disease susceptibility. For example, a haplotype comprised of the 10-repeat allele of the 3’ UTR VNTR and the 6-repeat allele of the intron 8 VNTR has shown consistent evidence for increased risk of ADHD [Brookes et al., 2006a,b; Asherson et al., 2007; Hawi et al., 2010]. Other regions of potential regulatory function have also been identified in DAT1, including a 15 bp insertion/deletion polymorphism in intron 14. While the deletion allele segregates with the 3’ BD risk haplotype described above and has been found to enhance transcriptional activity, the insertion allele segregates exclusively with a non-transmitted haplotype and may thus exert a protective effect against BD [Greenwood and Kelsoe, 2003; Greenwood et al., 2006]. Most associations with BD and ADHD have involved the 3’ region of the gene, and we have previously shown that increased recombination in the middle of the gene has effectively unlinked the 5’ (promoter through intron 2) and 3’ (exons 9 through 15) regions, while maintaining high levels of LD within the two regions (average \( D' = 0.92 \) and 0.84, respectively; see Fig. 1) [Greenwood et al., 2002, 2006]. However, the associated variants in intron 8 fall within this region of high recombination. Associations with 5’ variants have also been reported for ADHD, suggesting that additive or interactive effects of loci in both the 5’ and 3’ regions of the gene may be involved in susceptibility [Brookes et al., 2008].

The use of the WURS to measure the severity of childhood ADHD features in our sample may have resulted in possible limitations to this study. First, the WURS is a retrospective, self-rated scale based on a subject’s own subjective evaluation of childhood characteristics and is therefore vulnerable to recall bias. While we have tried to temper the effect of age on recall bias for all subjects by covarying for age at interview in our analyses, we were unable to control for current mood status in this study. Since only subjects meeting criteria for BD or MDD have data regarding current state, using state measures as covariates in the analyses would effectively eliminate all subjects with WURS scores but no history of mood disorders (i.e., healthy relatives and controls). However, we did assess possible state effects in two ways. A comparison of WURS scores between subjects who were in full or partial remission at the time of interview with those who met full criteria or were currently symptomatic at the time of interview found no significant group differences (\( P > 0.05 \)). A further analysis of Global Assessment of Functioning (GAF) scores for all subjects with available data also failed to reveal significant correlations with any of the WURS scores. Second, the exclusive use of the WURS may not be sufficient to
accurately diagnose childhood ADHD. Therefore, we have only assessed childhood ADHD features in our BD families, cases, and controls. In the absence of subjects with confirmed childhood ADHD diagnoses with and without adult BD, we cannot conclusively determine whether DAT1 contributes susceptibility to a subtype of BD with comorbid ADHD or whether it represents a joint susceptibility locus for both BD and ADHD. Finally, with the analysis of ten SNPs and four phenotypes the issue multiple comparisons must be addressed. While the SNPs are relatively independent as a result of the selection of haplotype tagging SNPs, the four WURS measures are highly correlated and not at all independent. If we apply the Bonferroni correction, which assumes independence, to adjust for the multiple comparisons of ten SNPs, the associations of mood instability with rs11133767 in intron 13 in the case–control sample remain significant with \( P < 0.005 \), as do the associations of rs27048 with the total score and mood instability in the combined analysis.

\( \text{DAT1} \) may be just one of many genes that predispose to ADHD or BD susceptibility. The association of \( \text{DAT1} \) with WURS scores in this study suggests that variants of this gene may predispose to a particular subtype of BD with childhood ADHD, although it is possible that the WURS simply detects early prodromal symptoms of BD in this context. Our results and those of others provide evidence for allelic heterogeneity at the \( \text{DAT1} \) locus and suggest a complex interplay of several functional variants in regulating gene expression, and therefore dopamine availability in the synapse. Further study will be required to elucidate the functional variants and their mechanisms of action.

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