Cross-Disorder Analysis of Bipolar Risk Genes: Further Evidence of \textit{DGKH} as a Risk Gene for Bipolar Disorder, but also Unipolar Depression and Adult ADHD

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Recently, several genome-wide association studies (GWAS) on bipolar disorder (BPD) suggested novel risk genes. However, only few of them were followed up and further, the specificity of these genes is even more elusive. To address these issues, we genotyped SNPs in \textit{ANK3}, \textit{CACNA1C}, \textit{CMTM8}, \textit{DGKH}, \textit{EGFR}, and \textit{NPAS3}, which were significantly associated with BPD in previous GWAS, in a sample of 380 BPD patients. Replicated SNPs were then followed up in patients suffering from unipolar depression (UPD; \(n = 387\)) or adult attention-deficit/hyperactivity disorder (aADHD; \(n = 535\)). While we could not confirm an association of \textit{ANK3}, \textit{CACNA1C}, and \textit{EGFR} with BPD, \(10\) SNPs in \textit{DGKH}, \textit{CMTM8}, and \textit{NPAS3} were nominally associated with disease, with two \textit{DGKH} markers surviving correction for multiple testing. When these were followed up in UPD and aADHD, seven \textit{DGKH} SNPs were also associated with UPD, while one SNP each in \textit{NPAS3} and \textit{CMTM8} and four in \textit{DGKH} were linked to aADHD. Furthermore, a \textit{DGKH} haplotype consisting of rs994856/rs9525580/rs9525584 GAT was associated with all disorders tested, while the complementary AGC haplotype was protective. The corresponding haplblock spans a 27-kb region covering exons coding for amino acids 65–243, and thus might include functional variants yet to be identified. We demonstrate an association of \textit{DGKH} with BPD, UPD, and aADHD by applying a two-stage design. These disorders share the feature of mood instability, so that this phenotype might be associated with genetic variation in \textit{DGKH}.

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INTRODUCTION

Bipolar disorder (BPD) is a severe psychiatric disorder and affects up to 4\% of the adult population worldwide (Bauer and Pfennig, 2005; Merikangas et al, 2007). Approximately 20\% of the patients die of suicide (Kilbane et al, 2009).

Additionally, co-morbid disorders are frequent and include adult attention-deficit/hyperactivity disorder (aADHD), anxiety disorders, and substance abuse (Kessler et al, 2006; Merikangas et al, 2007). Taken together, this results in severe psychosocial adversity and leads to serious economic burden. The etiology of BPD remains largely unknown but it is evident that genetic factors have an important role (Kieseppa et al, 2004; McGuffin et al, 2003).

Consistently replicated risk genes for BPD are still lacking. However, a variety of research tools were applied to detect susceptibility genes for BPD. This includes linkage studies, candidate gene association studies, and finally genome-wide association studies (GWAS). More than 40 linkage scans for BPD have been published to date.
Different meta-analysis found the strongest evidence for susceptibility loci on 13q and 22q (Segurado et al, 2003). In a combined analysis, 6q21–q25 and 8q24 showed genome-wide significance (McQueen et al, 2005). The underlying genes, however, have not yet been identified. With regard to candidate gene studies, several genes were shown to be associated with BPD, but none of them has been established as a specific BPD susceptibility gene. Among the best-replicated genes are DSC1, DAOA/G7Z2, BDNF, THP2, NRG1, ARNTL/CLOCK, and FAT (Barnett and Smoller, 2009). Another approach to identify genetic factors predisposing to diseases is the search for gross structural variations (Zhang et al, 2009).

As there are no common loci of large effect, but several genes with small effect sizes increasing the risk toward BPD, GWAS might be more fruitful. Using this rationale, several genes with small effect sizes increasing the risk toward BPD, genome-wide significance for a marker next to DCKH (diacylglycerol kinase eta). DCKH also is a promising functional candidate gene as its gene product is involved in the phosphatidyl inositol pathway, which is assumed to have an important role in lithium action. The UK Wellcome Trust Case–Control Consortium (WTCCC; WTCCC, 2007) demonstrated genome-wide significance for a marker next to PALB2, NDUFAB1, and DCTN5, and Sklar et al (2008) reported significant findings for MYO5B, TSPAN8, and EGFR. Finally, also including meta-analytic treatment of the WTCCC and Sklar data sets found strong evidence for CACNA1C (z-1 subunit of a voltage-dependent calcium channel) and ANK3 (ankyrin 3) (Ferreira et al, 2008). Taken together, these GWAS have provided risk genes that have been replicated in some cases (DCKH (Baum et al, 2008b; Ollila et al, 2009); SORCS2 and DFNB31 (Ollila et al, 2009); CACNA1C (Green et al, 2009); ANK3 (Lee et al, 2010; Schulze et al, 2009; Scott et al, 2009; Smith et al, 2009; TSPAN8 (Scholz et al, 2010)), while other replication attempts were negative. Several promising hits, however, were never attempted to replicate, and the potential impact of these candidate genes on other disorders displaying by mood disturbance has not yet been assessed. We have, therefore, picked the most promising risk genes and attempted their replication in an independent BPD sample. Confirmed risk genes thereafter were tested in samples consisting of patients suffering from unipolar depression (UPD) or aADHD, which is also characterized by severe mood dysregulation (Jacob et al, 2007).

**MATERIALS AND METHODS**

**Samples**

A detailed description of the BPD sample is available elsewhere (Lundorf et al, 2005; Reif et al, 2006b; Scholz et al, 2010). This sample consisted of 214 unrelated bipolar patients (mean age 51.7 ± 14.1 years, 65% female), from the German Lower Franconia area for whom an ICD-10 diagnosis was established by means of an extensive, semi-structured interview analogous to the AMDP interview (AMDP, 2000) carried out by two experienced psychiatrists at the University of Würzburg. Furthermore, the OPCRIT system was used in these patients (McGuffin et al, 1991). A further 166 unrelated bipolar patients (mean age 43.0 ± 11.5 years, 49% female) were ascertained according to ICD-10 diagnostic criteria for research (DCR) by means of a semi-structured interview (SCAN ver. 2.1; World Health Organization, 1998) at the Center for Psychiatric Research, Aarhus University Hospital, giving a total number of 380 patients suffering from BPD. In all, 387 unrelated patients of German origin suffered from UPD and were enrolled also in the lower Franconia region (n = 120, mean age 54.1 ± 16.3 years, 54% female) as well as at the Department of Psychiatry, University of Münster (n = 267, mean age 49.7 ± 15.4 years, 57% female) as described (Baune et al, 2008). The diagnosis for UPD was ascertained by trained psychiatrists according to the ICD-10 DCR on the basis of semi-structured interviews. Co-morbidity data for UPD and BPD, respectively, with aADHD could not be obtained as these patients were ascertained during acute disease episodes where it is not possible to reliably establish a diagnosis of aADHD.

The aADHD study sample has been described previously (Franke et al, 2010a; Jacob et al, 2007; Gross-Lesh et al, in preparation) and consisted of 535 unrelated patients (mean age 33.7 ± 10.2 years, 46% female), recruited at the University of Würzburg in the Lower Franconian region, who completed a structured interview from which diagnoses of DSM-IV aADHD were determined by two experienced psychiatrists. In all, 60% of the aADHD patients suffered from combined type ADHD, 30 of inattentive type ADHD, and 10% of hyperactive-impulsive type ADHD. In all, 57% of patients suffered from co-morbid lifetime depression, 32% of lifetime anxiety disorders, and 43% of lifetime substance abuse disorders (mainly alcohol abuse). BPD has been an exclusion criterion for these patients. None of the patients showed significant neurologic co-morbidity, mental retardation, or other somatic disorders, suggesting organic psychosis. Patients with substance-induced disorders were excluded as well.

The control sample consisted of 630 healthy subjects and was composed of blood donors, staff members, and volunteers all originating from the Lower Franconia region. A total of 284 control subjects (mean age 35 ± 13 years, 47% female), consisting of healthy blood donors originating from Würzburg, were enrolled. The sample was not screened for psychiatric disorders; however, all subjects were free of medication, and the study was explained to them, so that the likelihood of severe psychiatric disorders in the control sample was low. An additional 356 subjects (mean age 33.7 ± 10.2 years, 51% female) were recruited and screened for the absence of psychiatric disorders by conducting the Structured Clinical Interview for DSM-IV (SCID-I). All case as well as control subjects were of self-reported German, or Danish, respectively, ethnicity. Only subjects who gave written informed consent were enrolled in the study, which complied with the Declaration of Helsinki and was approved by the Ethics Committees of the Universities of Würzburg, Münster, and Århus.

**SNP Selection and Genotyping**

Genes of interest were compiled from published GWAS on BPD: NPA33, ARNT2, CACNA1C, ANK3 (Ferreira et al, 2008).
2008), NXN, SLC39A3, SORCS2, DGKH (Baum et al., 2008a; Ollila et al., 2009), NALCN, SLC19A3, SLC29A3, DFN831, CMTM8 (Ollila et al., 2009; WTCC, 2007), and EGFR (Sklar et al., 2008). Genes were selected due to strength of association signals, biological rationale, and involved pathways; significant SNPs were selected from previous studies. The study focused on CACNA1C, ANK3, DGKH, CMTM8, and EGFR, as in our hands those were the most promising candidate genes. For the other genes, only one or two top SNPs were tested as pilot investigations; nevertheless, they were also included in this analysis to fully account for multiple testing. Taken together, 14 genes that contained associated SNPs were tagged with 99 SNPs. In a first step, we tested for an association of these SNPs with BPD (single marker data, Supplementary Table 1; haplotype analysis, Supplementary Table 2). Second, we genotyped the 23 SNPs that were found to be nominally associated with BPD (Table 1) in the UPD (single marker data, Supplementary Table 3; haplotype analysis, Supplementary Table 4) and aADHD (single marker data, Supplementary Table 5; haplotype analysis, Supplementary Table 6) samples to assess whether these SNP associations were specific for BPD.

SNP genotyping was performed using Sequenom’s MassArray system (Sequenom, San Diego, CA) according to the instructions supplied by the manufacturer. All PCR reactions were done using the iPlex chemistry following the manufacturer’s standard operation procedure. All primer sequences are given in Supplementary Table 7.

### Table 1: Nominally Significant Findings for Bipolar Disorder (Combined BPD Sample), Along With the Corresponding Results for Unipolar Depression (Combined UPD Sample) and Adult Attention-Deficit Hyperactivity Disorder (aADHD)

<table>
<thead>
<tr>
<th>SNP</th>
<th>Alleles</th>
<th>N = 630</th>
<th>Complete BPD sample (N = 380)</th>
<th>Complete UPD sample (N = 387)</th>
<th>Adult ADHD sample (N = 535)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Minor</td>
<td>Major</td>
<td>Controls</td>
<td>Cases</td>
<td>Nominal p-value</td>
</tr>
<tr>
<td>CMTM8, chromosome 3</td>
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<tr>
<td>rs1296256</td>
<td>G</td>
<td>A</td>
<td>0.336</td>
<td>0.282</td>
<td>0.013</td>
</tr>
<tr>
<td>rs6803740</td>
<td>G</td>
<td>A</td>
<td>0.165</td>
<td>0.210</td>
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<tr>
<td>DGKH, chromosome 13</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>rs1170191</td>
<td>A</td>
<td>G</td>
<td>0.159</td>
<td>0.197</td>
<td>0.026</td>
</tr>
<tr>
<td>rs1170169</td>
<td>G</td>
<td>C</td>
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<td>1.5 x 10^-4</td>
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<td>rs2148004</td>
<td>G</td>
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<td>0.317</td>
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<td>rs994856</td>
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<td>rs925580</td>
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<tr>
<td>rs1170101</td>
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<td>0.349</td>
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<tr>
<td>rs247405</td>
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<td>G</td>
<td>0.449</td>
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<td>NAPAS3, chromosome 14</td>
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<td>rs1745703</td>
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<td>0.296</td>
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<td>SLC39A3, chromosome 19</td>
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<tr>
<td>rs1806874</td>
<td>G</td>
<td>A</td>
<td>0.314</td>
<td>0.269</td>
<td>0.013</td>
</tr>
</tbody>
</table>

Results are shown along with minor/major alleles (converted to the coding strand for the genes), the associated disease, allele frequencies for cases and controls, nominal p-values, as well as Bonferroni-corrected p-values. Bold values denote P < 0.05.
was used to estimate p-values that control the family-wise error rate (FWER). With our study population, nominal association tests have a power of 55 and 50% to detect SNPs and haplotypes, respectively, conveying an odds ratio (OR) of 1.5 (corresponding to a relative risk of 1.48) to develop BPD assuming a co-dominant model and an MAF of 0.05 (Menashe et al., 2008). Using the same parameters, the power for SNP and haplotype associations was 66 and 64% for aADHD, while for UPD, the power is 59 and 56%, respectively.

Furthermore, meta-analytic treatment of rs9315885 and rs1170191 was performed by including the studies by Baum et al. (2008a), Ollila et al. (2009), and Squassina et al. (2009) (as these SNPs were not genotyped in the study by Tesli et al. (2009), this study could not be included in the meta-analysis). ORs were calculated as a measure for effect size; thereafter, the Q-statistic was applied to assess heterogeneity. Inconsistency across studies was quantified with $I^2$ metric ($I^2 = Q - df/Q$). In the absence of heterogeneity, ORs were combined using fixed-effects models; if significant heterogeneity was detected, joint ORs were derived from random-effects models. Calculations were performed using R version 2.10 along with the package metafor version 0.5–7.

**RESULTS**

**Single Marker Analysis**

In order to replicate and assess the specificity of 14 selected genes from published GWAS on BPD, we analyzed 88 tag SNPs in the genes ANK3, ARNT2, CACNA1C, CMTM8, DFNB31, DGKH, EGF, NALCN, NPA3, NNX, SLC19A3, SLC29A3, SLC39A3, and SORCS2 in our BPD sample. After correction for multiple testing, two SNPs (rs1170169 and rs9525580) in DGKH remained significantly associated with BPD; at the nominal level, eight further significant findings were detected in the combined BPD sample, while another 13 SNPs were associated in only one of the BPD subsamples (Table 1; Supplementary Table 1). In order to examine the specificity of these associations for BPD, we further analyzed all 23 nominally associated markers (from the genes CMTM8, EGF, DFNB31, DGKH, NPA3, and SLC39A3) in UPD (Table 1; Supplementary Table 3) and aADHD (Table 1; Supplementary Table 5). SNPs from the other eight genes showed no significant association with BPD and were thus not analyzed further.

Cross-disorder genotyping revealed a total of 12 SNPs in four genes (CMTM8, DGKH, NPA3, and SLC39A3) that were associated with at least one of the three examined phenotypes at the nominal level (Table 1). Ten association p-values were nominally significant in the combined BPD sample (the remaining two had a borderline significant p = 0.056), seven in the combined UPD sample, and six in the aADHD sample, with seven SNPs being associated with two and two SNPs with all three disorders. Only those SNPs that were associated with BPD in the combined sample replicated in either UPD or aADHD (however, including rs2148004 and rs347405 with p = 0.056), but not those that were only associated in one of the subsamples (compare Supplementary Table 1 with Table 1). Most of the replicated SNPs mapped to DGKH (BPD: six SNPs; UPD: seven SNPs; aADHD: four SNPs), which was the only gene in our study that contained SNPs, which were significant following Bonferroni correction (Table 1). Noteworthy, all associations found with UPD mapped to DGKH. In CMTM8, only one SNP (rs6803740) featured an overlapping association between BPD and aADHD. The same disorders also overlapped in their association regarding the NPA3 SNP rs7455703. The SLC39A3 association of rs4806874 was found to be exclusive for BPD (Table 1).

**Haplotype Analysis**

Haplotype analysis was then performed with all cross-disorder genotyped SNPs (BPD, significant findings: Table 2, complete data are given in Supplementary Table 2; UPD significant findings: Table 2, complete data are given in Supplementary Table 4; aADHD significant findings: Table 2, complete data are given in Supplementary Table 6). The strongest association found in all analyzed disorders was in DGKH block 2 (rs994856–rs9525580–rs9525584; Figure 1) haplotype GAT, which is exclusively composed of each single marker’s risk alleles; this was consistent in all three examined phenotypes. Accordingly, GAT frequency was increased in all case groups as compared with controls; although this was nominally significant in all three disorders, the FWER was below 5% only in BPD and aADHD, but slightly above this threshold in UPD (permutation p = 0.056). The GAT haplotype can, therefore, be assumed to predispose to at least two, but possibly to any of the three disorders (see Table 2). In terms of frequency, GAT follows its ‘complementary’ haplotype AGC, which is composed of those alleles that have a higher MAF in controls. The expected protective effect conveyed by AGC, however, was only significant in UPD following FWER correction, and nominally also in BPD (Table 2). A similar phenomenon was seen in DGKH block 1 (rs1170191–rs1170169–rs2148004) haplotype GCC, whose frequency was lower in all case groups as compared with controls, but the presumed protective effect was nominally significant only in BPD and UPD. The haplotype GGA in turn was enriched in all cases; following FWER, this was significant in BPD, whereas nominally it was also associated with aADHD and UPD, respectively (p = 0.051; Table 2). Two further risk haplotypes were exclusively found to be associated with UPD (Table 2).

Haplotype associations in genes other than DGKH were found to be restricted to specific psychiatric disorders and did not withstand correction for multiple testing. In CMTM8, block 1 (rs6550109–rs12496256; Figure 2) haplotype TG was significantly protective, while block 3 (rs4276227–rs6803740) haplotype CG was associated with risk for BPD (Table 2). The CMTM8 block 2 (rs4955272–rs7644602–rs7632109) haplotype GGG was the only significant haplotype association in aADHD and presumably to be protective (Table 2). NPA3 rs8015959–rs17455703 had two alleles associated with BPD, the protective CG and the risk haplotype CA (Table 2).

**Meta-Analysis**

To compare our findings with previous studies, we have subjected the significant SNPs rs9315885 and rs1170191
from the studies by Baum et al (2008a), Ollila et al (2009), and Squassina et al (2009) to a formal meta-analysis (Figure 3; Supplementary Table 8). While rs9315885, which was significant in the three other studies but not our data set, proved to be highly significantly associated in the meta-analysis (Figure 3a), this was not the case for rs1170191, as the effect direction was reversed in our as compared with other studies (Figure 3b).

**DISCUSSION**

The present study had two major aims: (1) to replicate risk genes for BPD obtained through GWAS and (2) to assess the specificity of associated risk genes by testing all nominally associated SNPs in UPD and aADHD as well. We chose to specifically test risk variants in CACNA1C, ANK3, DGKH, CMTM8, and EGFR, while for several other genes only a few SNPs were tested, which we report here as well to fully account for multiple testing. In the following discussion, we will elaborate on a gene-by-gene wise manner, yet genes where only a few SNPs were tested and not found to be associated will not be commented further upon (ARNT2, DFNB31, NALCN, NNN, SLC19A3, SLC29A3, SLC39A3, and SORC2). An important caveat that has to be considered in the interpretation of our data is the use of a single control group, which has been compared against all three diagnostic groups. Significant deviation of our control group from the population's allele distribution, therefore, would bias our association data. We have thus compared the MAFs of our

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Association of Haplotypes With the Complete BPD, UPD, and aADHD Samples Containing At Least One Significant Haplotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMTM8</td>
<td>Complete BPD sample (N=380)</td>
</tr>
<tr>
<td>rs6550109</td>
<td>rs12496256</td>
</tr>
<tr>
<td>T G</td>
<td>0.274/0.336</td>
</tr>
<tr>
<td>T A</td>
<td>0.133/0.110</td>
</tr>
<tr>
<td>C A</td>
<td>0.593/0.554</td>
</tr>
</tbody>
</table>

Chromosome 3; block 2

rs4955272 rs7644602 rs7632109

| G G | 0.239/0.23 | 0.667 | I | 0.199/0.23 | 0.134 | 0.973 | 0.185/0.23 | 0.016 | 0.251 |
| A G | 0.299/0.287 | 0.573 | I | 0.285/0.287 | 0.916 | I | 0.290/0.287 | 0.923 | I |
| C A | 0.279/0.265 | 0.537 | I | 0.305/0.265 | 0.068 | 0.674 | 0.281/0.265 | 0.313 | 0.997 |

Chromosome 3; block 3

rs4276227 rs6803740

| C G | 0.210/0.165 | 0.013 | 0.519 | 0.186/0.165 | 0.225 | 0.952 | 0.194/0.165 | 0.070 | 0.691 |
| T A | 0.306/0.325 | 0.393 | I | 0.335/0.325 | 0.627 | I | 0.328/0.325 | 0.870 | I |
| C A | 0.484/0.510 | 0.270 | I | 0.479/0.510 | 0.170 | 0.853 | 0.478/0.510 | 0.086 | 0.768 |

DGKH

Chromosome 13; block 1

rs1170191 rs170169 rs2148004

| G C | 0.269/0.318 | 0.021 | 0.677 | 0.257/0.318 | 0.005 | 0.197 | 0.286/0.318 | 0.107 | 0.835 |
| A T | 0.189/0.161 | 0.112 | 0.998 | 0.211/0.161 | 0.006 | 0.140 | 0.168/0.161 | 0.706 | I |
| G G | 0.218/0.159 | 5.4 x 10^-4 | 0.030 | 0.188/0.159 | 0.051 | 0.563 | 0.192/0.159 | 0.009 | 0.151 |
| C A | 0.324/0.362 | 0.051 | 0.941 | 0.343/0.362 | 0.288 | 0.990 | 0.354/0.362 | 0.579 | I |

Chromosome 13; block 2

rs994856 rs9525580 rs9525584

| A G | 0.210/0.48 | 0.009 | 0.406 | 0.390/0.48 | 1.6 x 10^-4 | 0.021 | 0.445/0.48 | 0.120 | 0.873 |
| G A | 0.312/0.228 | 2.9 x 10^-10 | 0.001 | 0.288/0.228 | 0.004 | 0.056 | 0.296/0.228 | 3.0 x 10^-4 | 0.004 |
| G T | 0.145/0.17 | 0.153 | I | 0.166/0.17 | 0.810 | I | 0.159/0.17 | 0.479 | I |
| A A | 0.127/0.122 | 0.737 | I | 0.155/0.122 | 0.039 | 0.867 | 0.101/0.122 | 0.132 | 0.899 |

NPAS3

Chromosome 14; block 1

rs8015959 rs17455703

| C G | 0.253/0.296 | 0.039 | 0.883 | — | — | — | — | — | — |
| T A | 0.024/0.026 | 0.823 | I | — | — | — | — | — | — |
| C A | 0.723/0.678 | 0.038 | 0.877 | — | — | — | — | — | — |

Bold values denote p < 0.05.
control sample to a set of German population based controls, which have been ascertained via the HNR/KORA studies and genotyped on an Illumina microarray (n = 795; M Mattheisen and S Cichon, personal communication). From the 86 SNPs entering our analysis, 52 could be retrieved from the microarray. Only one of these 52 SNPs however significantly differed from our control sample (nominal p-value p = 0.017, rs1370717; all other p-values were > 0.15, data not shown), which argues against the assumption of a systematic bias due to the use of an unusual control group.

ANK3 and CACNA1C

In our panel of candidate genes, we have also included the top SNPs of the most replicated BPD risk genes so far: ANK3 (Ferreira et al, 2008; Lee et al, 2010; Schulze et al, 2009; Scott et al, 2009; Smith et al, 2009), which was also associated with schizophrenia (Athanasiu et al, 2010) and CACNA1C (Ferreira et al, 2008; Keers et al, 2009; Sklar et al, 2008; WTCCC, 2007), which was as well demonstrated to be associated with schizophrenia (Green et al, 2009; Moskvina et al, 2009; Nyegaard et al, 2010), and its endophenotypes (Casamassima et al, 2010). CACNA1C was shown to exert effects on verbal fluency and functional (Erk et al, 2010; Krug et al, 2010; Wessa et al, 2010) and structural (Franke et al, 2010b; Kempton et al, 2009) neuroimaging. We have aimed to replicate these genes in our bipolar sample, yet there was no significant association of either ANK3 or CACNA1C so that we abstained from testing them further. Several reasons for this lack of replication have to be considered: (1) lack of power owing to the sample size of n = 380 bipolar patients, as compared with the huge number of patients tested in current GWAS; (2) missed common variants, as we did not tag the whole gene but rather focused on previously associated SNPs however, including CACNA1C rs1006737, which was tested in the genomic imaging studies outlined above; (3) missed rare variants causing an association of common variants in the discovery samples (Dickson et al, 2010), thus escaping replication attempts due to differing LD substructures in the examined population; and (4) genetic heterogeneity of BPD, resulting in an association of risk genes in some, but not all populations (which might be very likely as associations were hitherto restricted to US American, UK, and Irish populations in the case of CACNA1C).

CMTM8 and EGFR

These two genes are considered together, as CMTM8 (CKLF-like MARVEL transmembrane domain containing 8) appears to be a negative regulator of EGF-induced signaling (Jin et al, 2005, 2007), which is mediated by the EGF receptor EGFR (previously termed ErbB). Thus, a common pathway of EGFR and CMTM8 seems reasonable. While the first evidence for an involvement of EGFR in BPD came from the GWAS by Sklar et al (2008), CMTM8 was identified in the WTCCC data set (WTCCC, 2007) yet not replicated later (Olliila et al, 2009). While there is almost no information on CMTM8, there is a vast body of literature on EGFR. This receptor kinase signals through PI3K/Akt as well as RAS/RAF/MEK/ERK (Wong and Guillaud, 2004), leading to downstream mechanisms including cell proliferation and survival. Accordingly, EGFR was shown to regulate neural stem cell proliferation (Cesetti et al, 2009; Grimm et al, 2009; Suh et al, 2009) and migration (Kim et al, 2009). Most interestingly, NO exerts its effect on neural stem cell proliferation by preventing EGFR-induced Akt phosphorylation (Torroglosa et al, 2007). Thus, EGFR and it regulators are excellent candidate molecules for neuropsychiatric disorders. Although other ErbB isoforms have gained much interest due to their interaction with neuregulin-1 (Birchmeier, 2009), there are almost no studies on EGFR/ErbB1 and its pathway. In the present study, we...
could however not find support for an involvement of $EGFR$ variation in BPD, in contrast to Sklar et al (2008). Again, this might be due to population-specific associations, yet the independent finding on $CMTM8$ in the WTCCC GWAS underscores the notion that GWAS, when combined, can indentify novel pathways and thereby provide a starting point for more mechanistic studies. As we could replicate $CMTM8$ as a bipolar $F$ and, with borderline significance, aADHD $F$ risk gene, we consider further studies worthwhile. Interestingly, when looking up $CMTM8$ in our pooled GWAS on aADHD (Lesch et al., 2008), rs9833771 which is just 18 kb away from our most significant $CMTM8$ finding rs6803740 was associated with aADHD at $p = 0.0002$, adding further support for our notion that it is involved in aADHD.

NPAS3

Several lines of evidence link the transcription factor neuronal PAS domain protein 3 (NPAS3) to psychiatric disorders (Pickard et al., 2006). Kamnasaran et al (2003) reported on a family in which a disruption of NPAS3 segregates with schizophrenia and was also associated with learning disability (Pickard et al., 2005). This finding was picked up soon thereafter in animal studies, demonstrating that $Npas3$ deletion mutant mice display schizophrenia-like behavioral abnormalities (Erbel-Sieler et al., 2004). Most interestingly, this was paralleled by a marked reduction of hippocampal adult neurogenesis (Pieper et al., 2005), which is suggested to have a role in schizophrenia (Reif et al., 2006a). Later, Pickard et al (2009) could show that common genetic variation in the NPAS3 gene is associated with both schizophrenia and BPD. Furthermore, coding non-synonymous variants were identified and demonstrated to be associated with schizophrenia (MacIntyre et al., 2010), which might well underlie the association of common, intronic variants (Dickson et al., 2010). Additionally, NPAS3 was identified in two GWAS to be associated with iloperidone response (Lavedan et al, 2009) and, interestingly, BPD (Ferreira et al, 2008). Thus, both hypothesis-free and hypothesis-driven genetic data as well as animal models argue for a role of NPAS3 in psychoses. Indeed, one (rs17455703) of the two NPAS3 SNPs tested in the present study again was associated with BPD. A nominally significant association for the same SNP with aADHD was also found, arguing that the connection between NPAS3 and psychiatric disorders crosses diagnostic boundaries. In line with this, three out of 282 NPAS3 SNPs tested in our aADHD GWAS (Lesch et al, 2008) were associated with disease, also following correction for multiple testing on a gene-based level (rs4503707, rs10483437, rs12100538). Further studies have to reveal whether NPAS3 is involved in cognitive functioning or rather emotional regulation, as both domains are affected in all three disorders (schizophrenia, BPD, and aADHD).

DGKH

The most prominent finding of our study however related to DGKH, suggested to be associated with BPD in the GWAS by Baum et al (2008a,b). However, replication failed in studies on BPD and lithium response (Manchia et al, 2009; Tesli et al, 2009), while two other studies were ambiguous (Ollila et al, 2009; Squassina et al, 2009). We attempted to replicate previously associated SNPs (rs9315885 and rs1170191), and by calculating a meta-analysis a role for rs9315885 was confirmed (Figure 3a), which however was not due to a signal in our sample. Our most significant SNPs were tagging SNPs, which have not been previously reported. While our data thus are again arguing for a role of DGKH in BPD, they cannot be considered a replication in a strict statistical sense and thus follow-up studies have to further test the top SNPs described here. Nevertheless, a recent report demonstrated increased expression of DGKH in BPD (Moya et al, 2010), lending further support to the notion for an involvement of this molecule in BPD. The role of DGKH is to metabolize diacylglycerol (DAG), which is produced upon cleavage of PIP2 into IP3 and DAG by phospholipase C. DAG, in turn, activates protein kinase C.

Figure 3 Forest plots displaying meta-analyses of minor vs major allele of rs9315885 (a) and rs1170191 (b).
(PKC), which phosphorylates a variety of proteins including Disheveled, an inhibitor of GSK3β. Thus, although the precise role of DGKH is not known yet, it clearly is involved in crucial pathways for psychiatric disorders and especially the mechanism of action of lithium. Intriguingly, DGKH knockdown in HeLa cells impaired the MEK/ERK pathway activated by EGF, while overexpression of the gene activated the pathway (Yasuda et al, 2009). Thus, DGKH is also linked to EGFR/CMTM8 mentioned above.

In our study, 6 out of 21 tested SNPs in DGKH were associated with BPD, and two SNPs withstood correction for multiple testing. Re-analysis of our aADHD GWAS (Lesch et al, 2008) revealed that 8 out of 52 SNPs were nominally associated with disease, one of which also survived correction on the gene level (rs10492444; nominal p = 0.0004, corrected p = 0.0212) and was located 505 bp away from rs9525584 being at the 3' end of the risk hapllocpblock delineated below. Furthermore, two frequent haplotypes were significantly associated with disease, especially rs994856/rs9525580/rs9525584 GAT. Apart from rs347405, all associated SNPs were also nominally associated with UPD (with three SNPs surviving correction for multiple testing) and again the hapllocpblock rs994856/rs9525580/rs9525584 was associated. In aADHD, four of the eight SNPs replicated, one of which withstanding correction and again, rs994856/rs9525580/rs9525584 GAT was associated with disease. When looking at absolute haplotype frequencies, it becomes apparent that the GAT haplotype is always more frequent in cases (controls, 23%; BPD, 31%; UPD, 29%; aADHD, 30%), while the AGC haplotype is always less frequent in cases (controls, 48%; BPD, 42%; UPD, 39%; aADHD, 45%). While our data as well as HapMap CEU suggest that AGT are the major alleles, the sub-Saharan HapMap subset suggest that the AGC alleles are evolutionary older, that is the AGC haplotype can be considered ancient. The GAT haplotype appears to be evolutionary younger and thus it seems to convey the risk variant. The hapllocpblock spans a genomic region of 27 kb covering exons two to six of the gene, that is amino acids 65–243. Three non-synonymous variants (no frequency data available) are deposited in databases, rs59790803 causing a G > A transition resulting in a possibly damaging Asp > Asn exchange (Polyphen-2. Adzhubei et al, 2010) score: 0.736, sensitivity: 0.81, specificity: 0.90) in exon 6 at position 107; rs9566925, causing a stop mutation at position 90; and rs1344826, causing a A > C transition, resulting in a Thr > Pro exchange at position 65. These regions contain a pleckstrin homology (PH) domain (AA 65–158) and a phorbol-ester/DAG-type zinc finger (AA 175–225). While the latter is the DAG sensor, the PH binds phosphatidylinositol and proteins such as PKC. This domain is, therefore, involved in intracellular targeting and enables DGKH to interact with other signal transduction pathways. Both variants, therefore, might well alter the function of the protein by either impairing catalysis or changing protein–protein interactions, thereby disturbing intraneuronal second- and third-messenger pathways.

**Outlook and Conclusions**

By applying a two-stage design, we here demonstrated an association of DGKH with BPD, UPD, and aADHD. These disorders share the feature of mood instability with varying amplitude and frequency. Thus, genetic variation at the DGKH locus might be associated with this psychopathological phenotype. This is yet another example that a common genetic variant is associated with more than one psychiatric phenotype, which was also the case with other risk genes picked up by GWAS, for example, CACNA1C and ANK3. The integration of such findings might pinpoint distinct molecular pathways whose identification might enhance psychiatric diagnostics and research on neurobiological underpinnings.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)