No association between DGKH and bipolar disorder in a Scandinavian case–control sample

Martin Tesli, Anna K. Kähler, Bettina Kulle Andreassen, Thomas Werge, Ole Mors, Erling Mellerup, Pernille Koefoed, Ingrid Melle, Gunnar Morken, Katrine V. Wirgenes, Ole A. Andreassen and Srdjan Djurovic

Single nucleotide polymorphisms (SNPs) in diacylglycerol kinase eta (DGKH) have recently been shown to be associated with bipolar disorder (BD). To replicate this finding, we carried out a gene-wide genotyping of 36 tagSNPs in DGKH and performed a population-based association study on two Scandinavian samples, with successful genotyping of 594 BD cases and 1421 healthy controls. We found no significant association after multiple-testing correction between any of these SNPs and BD in our sample. Thus, it is unlikely that these genetic variations confer susceptibility to BD in this large Scandinavian sample. *Psychiatr Genet* 19:269–272 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Introduction

Bipolar disorder (BD) is a spectrum of psychiatric conditions characterized by severe mood fluctuations. It is a common and disabling disorder, without a well-defined etiology (Newberg et al., 2008). Several family, twin and adoption studies implicate a strong genetic component, and the heritability has repeatedly been estimated to be as high as 0.8 (Craddock and Forty, 2006). No single major susceptibility gene has been firmly demonstrated, although several association studies have been performed (Craddock and Forty, 2006).

A recent whole-genome analysis showed nominal significant association between three single nucleotide polymorphisms (SNPs) in diacylglycerol kinase eta (DGKH) and BD (Baum et al., 2008a). The most significant result was obtained for rs1012053, with \( P = 1.5 \times 10^{-8} \), odds ratio = 1.59, and \( P < 0.01 \), after Bonferroni’s correction for the total number of SNPs studied.

DGKH is a gene located in a bipolar linkage region on chromosome 13 (Badner and Gershon, 2002) encoding DGKH, a member of the diacylglycerol enzyme family. DGKH is involved in regulating the intracellular concentrations of diacylglycerol and phosphatidic acid; it has also been shown to be a key protein in the lithium-sensitive phosphatidyl inositol pathway (Berridge, 1989).

Keywords: bipolar disorder, DGKH, single nucleotide polymorphism

*Institute of Psychiatry, *Epi-Gen, Faculty Division Akershus University Hospital, *Department of Biostatistics, University of Oslo, *Departments of Psychiatry, *Medical Genetics, Oslo University Hospital, Ullevål, Oslo, *Research Institute of Biological Psychiatry, Mental Health Center Sc. Hans, Copenhagen University Hospital, *Center for Psychiatric Research, Aarhus University Hospital, Riskøv, *Center of Psychiatry, Righospitalet, Copenhagen, *Laboratory of Neuropharmacology, Department of Neuroscience and Pharmacology, University of Copenhagen, Denmark, *Østmarka Psychiatric Department, St. Olavs Hospital, Trondheim, Norway and *Institute of Neuroscience, Norwegian University of Technology and Science, Trondheim, Norway

Correspondence to Martin Tesli, MD, Section for Psychosis Research, Building 49, Division of Psychiatry, Department for Research and Development, Oslo University Hospital, Ullevål, Kirkeveien 166, N-0407 Oslo, Norway

Tel: +47 22116277; fax: +47 23027332; e-mail: m.tesli@medisin.uio.no

Received 7 January 2009 Revised 23 March 2009 Accepted 20 April 2009

Materials and methods

Patient sample

This study is based on two independent case–control samples from Norway and Denmark, included in the Scandinavian Collaboration of Psychiatric Etiology. A total of 594 cases and 1421 healthy control individuals were successfully genotyped. The Danish sample consisted of 376 cases and 1201 controls, the Norwegian of 218 cases and 220 controls (Table 1). The Norwegian patients had been diagnosed with BD I (\( n = 125 \)), BD II (\( n = 80 \)) or bipolar not otherwise specified (\( n = 13 \)), according to the *Diagnostic and Statistical Manual of Mental Disorders* (fourth edition) DSM-IV using the Structural Clinical Interview for DSM-IV (Spitzer et al., 1992), the Danish patients with bipolar affective disorder F31 according to the International Classification of Diseases (10th revision) (\( n = 296 \)) and BD I (\( n = 80 \)) using the DSM-IV. The Norwegian and Danish healthy controls are described in more detail elsewhere (Hansen et al., 2007; Kahler et al., 2008).

The Norwegian Scientific–Ethics Committees, the Norwegian Data Protection Agency, the Danish Scientific

To further investigate the potential involvement of the DGKH in BD susceptibility, we genotyped 36 tagSNPs in DGKH and tested them for association with BD in two independent case–control samples of Scandinavian origin.
Committees, and the Danish Data Protection Agency, approved the study. All patients have given written informed consent before inclusion to the project.

TagSNP selection
To cover most of the common variants with tagSNPs, we used a structured gene-wide approach, based on the HapMap CEU population. TagSNP selection was performed at the HapMap website using pair-wise tagging, with \( r^2 \geq 0.8 \) (De Bakker et al., 2005; hapmap.org; HapMap Data Rel 22/phaseII Apr07) and minor allele frequency \( \geq 0.05 \). The actual tagging efficiency of successfully genotyped tagSNPs was calculated at the Tagger website (www.broad.mit.edu/mpg/tagger/server.html). Our tagSNPs covered 58\% of the common variants with \( r^2 \geq 0.8 \) and 94\% with \( r^2 \geq 0.5 \). The mean \( r^2 \) was 0.81 and minimum \( r^2 \) was 0.31.

Genotyping
Genomic DNA was extracted from whole blood. All tagSNPs were genotyped using the GoldenGate 1536plex assay (Illumina Inc., San Diego, California, USA) on Illumina BeadStation 500GX at the SNP Technology Platform, Uppsala University, Sweden (www.genotyping.se), accredited by the Swedish accreditation agency SWE-DAC, and approved according to a quality system based on the international SS-EN ISO/IEC 17025 standard.

SNP conversion rate was 92.6\%, reproductivity was 99.996\% (there were five duplicate errors in 124 684 duplicate genotype calls), and the average sample call rate per SNP assay was 96.9\%. The SNP rs1012053 was genotyped separately, using the TaqMan SNP Genotyping Assay ID: C___1931998_10 (Applied Biosystems, Foster City, California, USA). The rate per SNP assay was 96.9\%. The SNP rs1012053 was genotyped separately, using the TaqMan SNP Genotyping Assay ID: C___1931998_10 (Applied Biosystems, Foster City, California, USA). The SNP rs1012053 was genotyped separately, using the TaqMan SNP Genotyping Assay ID: C___1931998_10 (Applied Biosystems, Foster City, California, USA).

Statistics
All SNPs in the controls were tested for deviation from Hardy–Weinberg equilibrium by the corresponding exact test implemented in the genetics package in R (www.sourceforge.net). Genotype-based association tests between the SNPs and the phenotype BD were calculated by the MAX-test (Freidlin et al., 2002). This test uses the maximal test statistic for the recessive, dominant, and additive inheritance pattern implemented by different weights in the Cochrane–Armitage trend test. For each SNP, 10 000 permutations are made to evaluate the underlying null distribution, and thus leading to corresponding \( P \) values. All calculations were performed with R.

Results and discussion
Thirty-six tagSNPs in \( DGKH \) were successfully genotyped in 594 cases and 1421 controls of Scandinavian origin. The results are presented in Table 2. None of the SNPs showed significant association with the bipolar phenotype after correcting the MAX \( P \) value with 10 000 permutations.

Table 2 Single marker association results for \( DGKH \) in the bipolar disorder Scandinavian case–control samples

| SNP         | MAF | HWE | MAX |
|-------------|-----|-----|-----|
| rs17062754  | 0.15| 0.61| 0.28|
| rs6561029   | 0.48| 0.71| 0.25|
| rs9594670   | 0.20| 0.31| 0.19|
| rs8561030   | 0.15| 0.21| 0.67|
| rs2148656   | 0.32| 0.81| 0.86|
| rs12584909  | 0.18| 0.79| 0.66|
| rs1170183   | 0.26| 0.27| 0.14|
| rs1033951   | 0.06| 0.48| 0.56|
| rs816567    | 0.16| 0.84| 0.70|
| rs2767390   | 0.48| 0.67| 0.84|
| rs1170192   | 0.20| 0.44| 0.27|
| rs7984523   | 0.39| 0.24| 0.12|
| rs8562377   | 0.17| 0.65| 0.08|
| rs858810    | 0.34| 0.59| 0.18|
| rs9533002   | 0.29| 0.65| 0.64|
| rs7981733   | 0.46| 0.71| 0.19|
| rs2767388   | 0.20| 0.39| 0.25|
| rs17519698  | 0.32| 0.16| 0.24|
| rs8530007   | 0.46| 0.49| 0.22|
| rs12876965  | 0.11| 0.31| 0.35|
| rs716934    | 0.26| 0.53| 0.05|
| rs11616202  | 0.05| 0.81| 0.78|
| rs4598803   | 0.11| 0.33| 0.77|
| rs11617256  | 0.24| 0.92| 0.67|
| rs2324890   | 0.07| 0.55| 0.15|
| rs17598799  | 0.41| 0.47| 0.15|
| rs1170098   | 0.30| 0.80| 0.27|
| rs1170101   | 0.29| 1.00| 0.76|
| rs9562396   | 0.41| 0.74| 0.83|
| rs347416    | 0.47| 0.63| 0.83|
| rs347405    | 0.46| 0.71| 0.19|
| rs180870    | 0.34| 0.55| 0.38|
| rs347382    | 0.15| 0.64| 0.94|
| rs347383    | 0.19| 0.03| 0.90|
| rs9533040   | 0.11| 0.49| 0.28|
| rs545602    | 0.22| 0.89| 0.51|

\( DGKH \), diacylglycerol kinase eta; HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency; SNP, single nucleotide polymorphism.

* \( P \) value of MAX test, 10 000 permutations for each SNP.
Of the 36 tagSNPs in our sample, one (rs681657) can, according to www.hapmap.org, be used as a proxy for the SNP (rs1012053) strongly associated with BD in the sample of Baum et al. (2008a) ($r^2 = 1$). This SNP (rs681657) attained a corrected $P$ value of 0.70 in this study. To confirm this finding, we genotyped rs1012053 separately using TaqMan SNP Genotyping Assay. This SNP attained an unadjusted $P$ value of 0.76. The frequency of the minor allele C was 0.16 for the affected and 0.15 for the unaffected individuals, a finding that was in accordance with the frequencies for the minor allele G in rs681657 (0.16 for affected and 0.15 for unaffected individuals).

Our sample size is comparable with the size of the above-mentioned whole-genome study of BD by Baum et al. (2008a). In this study, they used two independent samples, both of European origin, and a test-replication design. First sample consisted of 461 cases and 563 controls, the second of 772 cases and 876 controls. The initial screen was performed using pooled DNA; then, selected SNPs that showed significant association in both samples were confirmed by individual genotyping.

Our findings are in accordance with three recent whole-genome studies of BD. Neither Sklar et al. (2008), the WTCCC (2007), nor a recent large collaborative study by Ferreira et al. (2008) did find evidence of association between variants in DGKH and BD. The first study included 1461 cases and 2008 controls, all of North American and British origin (Sklar et al., 2008); the second consisted of approximately 2000 cases and 3000 controls from Great Britain (WTCCC, 2007); whereas the third sample of 4387 cases and 6209 controls comprised the samples from WTCCC (2007), Sklar et al. (2008), and a third sample from Great Britain (Ferreira et al., 2008).

It was shown in a recent study that there is little population stratification between the Northern European countries (Lao et al., 2008). Thus, it is unlikely that our sample differs significantly genetically from these other studies. Furthermore, the diagnostic reliability of our two samples has been ascertained thoroughly and is unlikely to be the cause of the observed discordance.

Three of four studies (including our own) have shown no significant association between DGKH and BD. This may implicate that this gene is not involved in the etiology of BD, or reflect allelic heterogeneity at the same risk locus (Hennah et al., 2008). As commented upon in a letter to the editor (Baum et al., 2008b), at least six SNPs within 2 kb of DGKH showed nominal association with $P$ values less than 0.006 in the WTCCC (2007) study.

These diverging findings may also indicate a polygenic mechanism, in which many genes, each with a small effect, together, and in interaction with environmental factors, confer susceptibility to BD (Craddock et al., 1995). Therefore, gene–gene and gene–environment interaction studies may be needed to further investigate the role of DGKH in BD.

Acknowledgements
The authors thank patients and controls for their participation in the study, and the health professionals who facilitated our work. They also thank Thomas D. Bjella for assistance with the database, Bente Bennike, Knut-Erik Gylder, Trude Lien, and Elin Underhaug for molecular genetic technical assistance, and Kristina Larsson, Per Lundmark, Tomas Axelsson, and Ann-Christine Syvänen at the SNP Technology Platform in Uppsala for performing the genotyping. The SNP Technology Platform is supported by Uppsala University, Uppsala University Hospital and by the Knut and Alice Wallenberg Foundation. This study was supported by grants to the TOP study group from the University of Oslo, the Research Council of Norway (#167153/V50, #163070/V50), the SouthEast Norway Health Authority (#2004123), the Danish National Psychiatric Research Foundation, the Lundbeck Foundation, and The Stanley Medical Research Institute.

References
Badner JA, Gershon ES (2002). Meta-analysis of whole-genome linkage scans of bipolar disorder and schizophrenia. Mol Psychiatry 7:405–411.
Baum AE, Akula N, Cabanero M, Cardona I, Corona W, Klemens B, et al. (2008a). A genome-wide association study implicates diacylglycerol kinase eta (DGKH) and several other genes in the etiology of bipolar disorder. Mol Psychiatry 13:197–207.
Baum AE, Harshmere M, Green E, Cichon S, Rietschel M, Nothen MM, et al. (2008b). Meta-analysis of two genome-wide association studies of bipolar disorder reveals important points of agreement. Mol Psychiatry 13:466–467.
Berridge MJ (1989). The Albert Lasker medical awards. Inositol trisphosphate, calcium, lithium, and cell signaling. JAMA 262:1834–1841.
Craddock N, Forty L (2006). Genetics of affective (mood) disorders. Eur J Hum Genet 14:660–668.
Craddock N, Khodel V, Van EP, Reich T (1996). Mathematical limits of multilocus models: the genetic transmission of bipolar disorder. Am J Hum Genet 57:690–702.
De Bakker PI, Yelensky R, Pe’er I, Gabriel SB, Daly MJ, Altshuler D (2005). Efficiency and power in genetic association studies. Nat Genet 37:1217–1223.
Excoffier L, Laval G, Schneider S (2005). Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinformatics Online 1: 47–50.
Ferreira MA, O’Donovan MC, Meng YA, Jones IR, Ruderfer DM, Jones L, et al. (2008). Collaborative genome-wide association analysis supports a role for ANK3 and CACNA1C in bipolar disorder. Nat Genet 40:1056–1058.
Freidlin B, Zheng G, Li Z, Gastwirth JL (2002). Trend tests for case-control studies of genetic markers: power, sample size and robustness. Hum Hered 53:146–152.
Hansen T, Olsen L, Lindow M, Jakobsen KD, Ullum H, Jonsson E, et al. (2007). Brain expressed microRNAs implicated in schizophrenia etiology. PLoS ONE 2:e873.
Hennah W, Thomson P, McQuilin A, Bass N, Loukola A, Anjorin A, et al. (2008). DISC1 association, heterogeneity and interplay in schizophrenia and bipolar disorder. Mol Psychiatry. doi: 10.1038/mp.2008.22.
Kahler AK, Djurovic S, Kulle B, Jonsson EG, Agartz I, Hall H, et al. (2008). Association analysis of schizophrenia on 18 genes involved in neuronal migration: MDGA1 as a new susceptibility gene. Am J Med Genet B Neuropsychiatr Genet 147B:1089–1100.
Lao O, Lu TT, Nothnagel M, Junge O, Freitag-Wolf S, Calebi A, et al. (2008). Correlation between genetic and geographic structure in Europe. Curr Biol 18:1241–1248.
Moskvina V, Schmidt KM (2008). On multiple-testing correction in genome-wide association studies. *Genet Epidemiol* 32:567–573.

Newberg AR, Catapano LA, Zarate CA, Manji HK (2008). Neurobiology of bipolar disorder. *Expert Rev Neurother* 8:93–110.

Sklar P, Smoller JW, Fan J, Ferreira MA, Perlis RH, Chambert K, et al. (2008). Whole-genome association study of bipolar disorder. *Mol Psychiatry* 13:558–569.

Spitzer RL, Williams JB, Gibbon M, First MB (1992). The structured clinical interview for DSM-III-R (SCID). I: history, rationale, and description. *Arch Gen Psychiatry* 49:624–629.

WTCCC (2007). Genome-wide association study of 14,000 cases of seven common diseases and 3000 shared controls. *Nature* 447:661–678.