RAPID PUBLICATION

AMERICAN JOURNAL OF medical genetics PART B
Neuropsychiatric Genetics

Genome-Wide Association Study in German Patients With Attention Deficit/Hyperactivity Disorder

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Grant sponsor: German Research Association (DFG); Grant numbers: He1446/9-1, KFO 125, SFB 581, GRK 1156, ME 1923/5-1, ME 1923/5-3, GRK 1389, SCHA 542/10-3; Grant sponsor: Bundesministerium für Bildung und Forschung; Grant number: BMBF 01GV0605; Grant sponsor: US National of Institute of Health; Grant numbers: R13MH059126, R01MH62873, R01MH081803, K23MH066275, MH58277; Grant sponsor: University of Pennsylvania CTR; Grant number: UL1-RR-024134; Grant sponsor: Institutional Development Award to the Center for Applied Genomics from the Children's Hospital of Philadelphia; Grant sponsor: Affymetrix Power Award, 2007; Grant sponsor: NHMRC (Australia); Grant number: 443036; Grant sponsor: Sidney Sax Public Health Fellowship; Grant number: 443036; Grant sponsor: Wellcome Trust, UK for sample; Grant sponsor: UMC Utrecht Genvlag Grant; Grant sponsor: Internal Grant of Radboud University Nijmegen Medical Centre.

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Published online 19 October 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/ajmg.b.31246

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Received 13 July 2011; Accepted 16 September 2011

The heritability of attention deficit hyperactivity disorder (ADHD) is approximately 0.8. Despite several larger scale attempts, genome-wide association studies (GWAS) have not led to the identification of significant results. We performed a GWAS based on 495 German young patients with ADHD (according to DSM-IV criteria; Human660W-Quadv1; Illumina, San Diego, CA) and on 1,300 population-based adult controls (HumanHap550v3; Illumina). Some genes neighboring the single nucleotide polymorphisms (SNPs) with the lowest *P*-values (best *P*-value: 8.38×10^{-7}) have potential relevance for ADHD (e.g., glutamate receptor, metabotropic 5 gene, GRM5). After quality control, the 30 independent SNPs with the lowest *P*-values (*P*-values $\leq 7.57 \times 10^{-5}$) were chosen for confirmation. Genotyping of these SNPs in up to 320 independent German families comprising at least one child with ADHD revealed directionally consistent effect-size point estimates for 19 (10 not consistent) of the SNPs. In silico analyses of the 30 SNPs in the largest meta-analysis so far (2,064 trios, 896 cases, and 2,455 controls) revealed directionally consistent effect-size point estimates for 16 SNPs (11 not consistent). None of the combined analyses revealed a genome-wide significant result. SNPs in previously described autosomal candidate genes did not show significantly lower *P*-values compared to SNPs within random sets of genes of the same size. We did not find genome-wide significant results in a GWAS of German children with ADHD compared to controls. The second best SNP is located in an intron of GRM5, a gene located within a recently described region with an infrequent copy number variation in patients with ADHD. © 2011 Wiley Periodicals, Inc.

Key words: psychiatric; children; early onset; homogeneous

How to Cite this Article:
Hinney A, Scherag A, Jarick I, Albayrak Ö, Pütter C, Pechlivanis S, Dauvermann MR, Beck S, Weber H, Scherag S, Nguyen TT, Volckmar A-L, Knoll N, Faraone SV, Neale BM, Franke B, Cichon S, Hoffmann P, Nöthen MM, Schreiber S, Jöckel K-H, Wichmann H-E, Freitag C, Lempp T, Meyer J, Gilsbach S, Herpertz-Dahlmann B, Sinzig J, Lehmkuhl G, Renner TJ, Warnke A, Romanos M, Lesch K-P, Reif A, Schimmelmann BG, Hebebrand J, Psychiatric GWAS Consortium: ADHD subgroup. 2011. Genome-Wide Association Study in German Patients With Attention Deficit/Hyperactivity Disorder.
Am J Med Genet Part B 156:888–897.

INTRODUCTION

Approximately 5% of children fulfill DSM-IV (Diagnostic and Statistical Manual of Mental Disorders—4th Edition) criteria for attention deficit/hyperactivity disorder (ADHD; American Psychiatric Association, 1994). Family, twin, and adoption studies indicate that ADHD is a highly heritable child and adolescent psychiatric disorder; heritability estimates are consistently around 0.8 [Heiser et al., 2004; Faraone et al., 2005; Franke et al., 2009; Faraone and Mick, 2010; Freitag et al., 2010]. Candidate gene association and linkage studies, which were mostly based on small sample sizes, have provided modest evidence for the involvement of specific genes and chromosomal regions in ADHD. Genetic variants in single, mostly dopaminergic, candidate genes have been implicated in meta-analyses (for review see: [Heiser et al., 2004; Li et al., 2006; Gizer et al., 2009; Banaschewski et al., 2010; Faraone and Mick, 2010; Franke et al., 2010]); however, none of these variants achieved genome-wide significance at a level α of 5×10^{-8} [Speliotes et al., 2010]. A meta-analysis of seven linkage studies identified a single genome-wide significant linkage finding on chromosome 16q22–16q24 [Zhou et al., 2008].

The advent of genome-wide association studies (GWAS) was a paradigm shift in the elucidation of genetic variants underlying complex disorders. For the first time, genome-wide significant findings for multiple categorical and dimensional complex phenotypes are being detected at an unprecedented pace, with frequent confirmation in independent study groups (http://www.genome.gov/26525384). Such GWAS and subsequent replication studies

are usually based on large samples. Small effect sizes of the detected variants (odds ratios mostly <1.3) represent a common underlying theme [Psychiatric GWAS Consortium Steering Committee, 2009]. The first GWAS for some neuropsychiatric disorders (e.g., restless legs syndrome, schizophrenia, and bipolar disorder) resulted in the detection of single SNPs/genes that were confirmed in GWAS performed in parallel and/or in subsequent large scale genotyping studies of promising SNPs [Stefansson et al., 2007, 2009; Winkelmann et al., 2007; Ferreira et al., 2008; Schormair et al., 2008; Sullivan et al., 2008; Cichon et al., 2011]. The functionally relevant variants in the respective candidate genes are still to be identified.

In 2008, the first GWAS for ADHD in adults analyzed 343 in- and outpatients and 250 controls. Hints to novel interesting risk genes were identified; however, results were not significant on a genome-wide level [Lesch et al., 2008]. The first GWAS for ADHD in children and adolescents, based on a total of 909 trios, was performed by the Genetic Association Information Network (GAIN) based on the sample of the International Multicentre ADHD Genetics (IMAGE) project (http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000016.v1.p1). The lowest *P*-values in two chromosomal regions (chr. 6; rs9389835, $P = 6.75 \times 10^{-6}$ in 642 trios and chr. 14; rs2295426, $P = 5.01 \times 10^{-6}$ in 585 trios) were not significant on a genome-wide level, indicating that larger samples may be required to identify risk loci for ADHD [Neale et al., 2008a]. The top SNPs reported in the GWAS by Neale et al. [2008a] were recently analyzed in an independent study group (of 415 trios). None of the SNPs showed a significant association [Lantieri et al., 2010]. Another independent multisite ADHD GWAS was conducted on 732 families from the PUWMa consortium (Pfizer-funded study from the University of California, Los Angeles, Washington University, and Massachusetts General Hospital). Again, none of the SNPs reached genome-wide significance (lowest *P*-value $= 6.7 \times 10^{-7}$). Notably, one of the 20 SNPs with the lowest *P*-values was located in a candidate gene in the GWAS data of interest for ADHD (solute carrier family 9 (sodium/hydrogen exchanger), member 9 gene, *SLC9A9*, rs9810857, $P = 6.4 \times 10^{-6}$). A gene-based test of published candidate genes identified additional evidence of association with *SLC9A9* [Mick et al., 2010]. A further case–control GWAS for ADHD in the IMAGE II consortium sample (896 cases with ADHD and 2,455 repository controls) likewise produced no genome-wide significant result [Neale et al., 2010a].

A recent large-scale meta-analysis analyzed imputed data from four GWAS studies comprising 2,064 trios, 896 cases, and 2,455 controls. Genome-wide significant associations were not identified [Neale et al., 2010b]. Given the high heritability of the disorder, the negative results suggest that the effects of common ADHD risk variants might individually be very small. The missing heritability might also be explained by other types of variants (e.g., rare variants [Neale et al., 2010b]).

We hypothesized that the uniform ascertainment (e.g., only clinically ascertained cases) that has been applied to all German ADHD samples would discover of genetic risk variants that might not have been picked up by the broader recruitment approach of prior ADHD GWAS studies [Kuntsi et al., 2006; Chen et al., 2008; Neale et al., 2008b]. We performed a GWAS on 495 German young ADHD patients (Human660W-Quadv1; Illumina) and on 1,300 population-based adult controls (HumanHap550v3; Illumina).

MATERIALS AND METHODS

Study Groups

GWAS Sample; ADHD Cases. Four hundred ninety-five German young (age range 6–18 years; mean age 11 ± 2.7 years) patients with ADHD were recruited and phenotypically characterized in six psychiatric outpatient units for children and adolescents (Aachen, Cologne, Essen, Marburg, Regensburg, and Würzburg). Patients were included if they were diagnosed with ADHD according to DSM-IV [American Psychiatric Association, 1994], subtypes are given in Table 1. The ascertainment strategy and inclusion criteria have been described previously [Hebebrand et al., 2006; Schimmelmann et al., 2007; Romanos et al., 2008].

GWAS Sample; Population-Based Controls. One thousand three hundred adult controls were drawn from three population-based epidemiological studies (for details see [Cichon et al., 2011]): (a) the Heinz Nixdorf Recall (Risk Factors, Evaluation of Coronary Calcification, and Lifestyle) study (n = 383; [Schmermund et al., 2002]), (b) PopGen (n = 490; [Krawczak et al., 2006]), (c) KORA (Cooperative Health Research in the Region of Augsburg; n = 488; [Wichmann et al., 2005]). The recruitment areas were Essen, Bochum, and Mülheim (Ruhr area) for (a), Schleswig-Holstein (Northern Germany) for (b), and Augsburg (Southern Germany) for (c), respectively. Ethnicity was assigned to patients and controls according to self-reported ancestry (all German). Written informed consent was given by all individuals or by their parents in case of minors. The study protocols were approved by the respective institutional review board or ethics committees and conducted in accordance with *The Declaration of Helsinki*.

Replication Samples. The "Würzburg sample" consisted of 149 families (510 individuals) with at least one affected child (99 trios) with ADHD (DSM-IV criteria), in 40 families 2, in 8 families 3, and in 2 families 4 or 5 affected children were recruited, respectively. The "Frankfurt/Homburg/Trier sample" consisted of 171 trios

TABLE 1. Clinical Characteristics of the 495 Patients With ADHD Analyzed With the GWAS Approach

	Probands, n (%) or mean (SD)
Sex	
Male	400 (80.8%)
Female	95 (19.2%)
Age	11.0 (SD 2.7)
ADHD subtype[a]	
Combined type	362 (73.1%)
Predominantly inattentive type	108 (21.8%)
Predominantly hyperactive-impulsive type	25 (5.1%)

[a]Current DSM-IV diagnosis according to K-SADS, Kinder-DIPS or PACS.

(513 individuals) with one affected child with ADHD (DSM-IV criteria). All children were recruited from outpatient clinics at the Departments of Child and Adolescent Psychiatry, Psychosomatics and Psychotherapy of the Universities of Würzburg, Frankfurt, and Homburg and the local child psychiatry clinic in Trier. Written informed consent was given by all participants and in case of minors by their parents. The study was approved by the Ethics Committee of the Universities of Würzburg and Frankfurt am Main, and the "Ärztekammer des Saarlandes" and conducted in accordance with the guidelines of *The Declaration of Helsinki*. DSM-IV criteria were used to define caseness (see Renner et al. [2008] and Romanos et al. [2008], for details on the diagnostic procedures). In silico replication was performed in the Psychiatric GWAS Consortium ADHD meta-analysis sample (see [Neale et al., 2010b]).

Genotyping, Quality Control and Statistical Evaluation

Genome-wide association study. The genome-wide genotyping was performed on HumanHap550v3 (Illumina; controls) and Human660W-Quadv1 BeadArrays (Illumina; cases) by (i) Illuminas customer service (all 490 PopGen controls); (ii) the Department of Genomics, Life & Brain Center, University of Bonn, Germany (all 495 ADHD cases and 383 Heinz Nixdorf Recall study controls) and (iii) the Helmholtz Zentrum München, Germany (all 488 KORA controls).

We applied a quality control (QC) protocol to filter genotypes and individuals to the overlapping genotype content of all GWAS data sets. This QC protocol has been previously described in detail [Cichon et al., 2011], in short it accounts for call rates (CR—cut-off 0.98 for SNPs and 0.97 for individuals), heterozygosity, cross-contamination, population stratification, relatedness, deviations from Hardy–Weinberg equilibrium (HWE—cut-offs $P_{\text{exact-cases}} < 1 \times 10^{-6}$, $P_{\text{exact-controls}} < 1 \times 10^{-4}$) and minor allele frequencies (MAF—cut-off <1% in cases or controls). Moreover, we explored the cluster intensity plots of the SNPs that were followed-up manually by two independent raters. After QC and merging both the case and control data sets a total of 487,484 autosomal and 11,917 X-chromosomal SNPs were analyzed in 495 cases and 1,300 controls (see Supplementary Figs. 1 and 2).

Using PLINK v1.07 [Purcell et al., 2007] we applied Cochran–Armitage Trend tests to assess all autosomal SNPs for association with the disorder as recommended [Sasieni, 1997; Guedj et al., 2008] while X chromosomal SNPs were tested by the allelic test (2 × 2 Table) which basically weights X alleles of women and men equally. In addition, we performed analyses stratified by sex and compared the allele frequencies in cases and controls to those of the parents in the replication samples for the SNPs displayed in Table 2 (results not shown). These sensitivity analyses indicated no strong discrepancies by sex or age group that could either mask true effects or lead to spurious effects. All reported nominal *P*-values are two-sided; *P*-values adjusted for genomic inflation ($\lambda = 1.042$) and effect sizes estimators (odds ratios) are also provided (see Table 2). The ratio of the median of the empirically observed distribution of the test statistic to the expected median is defined as the genomic inflation factor λ which can be used to address possibly population stratification effects [Devlin and Roeder, 1999].

Validation of GWAS genotypes. After QC we validated the three (rs4862110, rs2556378, and rs5016282) initially best GWAS SNPs (according to *P*-value) by genotyping the GWAS cases by independent genotyping methods; (a) rs4862110: TaqMan assay C_27905256_10 was used according to the manufacturer; (b) rs2556378: polymerase chain reaction (PCR) with subsequent diagnostic restriction fragment length polymorphism (RFLP) analyses was performed; forward primer "TAG TCA AGC CAA AGG GGC TA"; reverse primer "ACA ACT TGA GGG GGA AAA GG," digestion with *Bsm*AI. (c) rs5016282: amplification refractory mutation system (ARMS)-PCR; forward outer primer "ACT ATT CCT GCA AAA TTG TTT TAC C"; reverse outer primer "ACA ACA TGA TTG TGT TCT TTG AGT T"; forward inner primer "TAT TCT TCA TGG CTA TGT TTC TCT G," reverse inner primer "TCA AAT TTA GAT AAC CTT GAT GGC T." For validity of the genotypes, allele assignments were made by at least two experienced individuals independently. Discrepancies were solved unambiguously either by reaching consensus or by repeating of the genotyping. Comparison with the GWAS chip data revealed a high concordance (<1% discrepancies) for SNPs rs2556378 (4 differences in 495 samples) and rs5016282 (3 differences in 495 samples). However, for the initially best SNP rs4862110 (initial *P*-value 1.02×10^{-9}), the discrepancy rate was high (18.4%; 91 differences in 495 samples). Re-genotyping of rs4862110 by TaqMan confirmed the initial TaqMan genotypes. Re-sequencing (SeqLab, Göttingen) also confirmed the TaqMan genotypes, but not the chip data. Hence we decided to use the Taqman data for the association analysis. The new analysis revealed a *P*-value of 0.742 for rs4862110, so that the SNP was excluded from further replication attempts.

Replication. The 30 best, independent (distance between SNPs at least 1 Mb, in case of more SNPs in linkage disequilibrium (LD) the SNP with the lowest *P*-value was chosen) SNPs (ranked by *P*-value; see Table 2) which passed the QC filter were genotyped in the independent "Würzburg sample" (see above). SNP genotyping of 28 of these SNPs 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 MassArray® iPlex® standard operation procedure. Primer sequences are available from the authors. SNP rs5016282 was genotyped as described above ("validation of GWAS genotypes"). Genotyping of the two best SNPs in the "Frankfurt/Homburg/Trier sample": rs2556378 and rs5016282 were genotyped by ARMS-PCR as described above ("validation of GWAS genotypes"). As the replication was performed in nuclear families we checked all samples for Mendelian inconsistencies and determined deviations from HWE in the parents (all $P_{\text{exact}} > 0.01$ except for rs1430961). We used the family-based association test (FBAT) [Laird and Lange, 2006] for the association analysis to address the impact of one or more offspring affected with ADHD for each family.

We also report the findings from the meta-analyses of both the GWAS and the replication data sets applying Fisher's combined probability test which weights each data set equally. Applying inverse variance weightings did not alter our conclusions.

Gene-set enrichment analyses. For 64 previously described autosomal ADHD candidate genes [see Banaschewski et al.,

TABLE 2. Thirty Best ADHD SNPs From a Genome-Wide Association Study in 495 German Children and Adolescents With ADHD and 1,300 Population-Based Controls; Replication in ADHD Nuclear Families ($n_{max} = 1{,}023$[a])

SNP	Chromosome	Base pairs[b]	Nearest gene[c]	GWAS *P*-value[d]	GWAS risk allele/ frequency[e]	GWAS odds ratio[f]	Replication FBAT[a] *P*-value	Direction of effect[g]	Combination *P*-value[h]
rs2556378	2	60762502	BCL11A	8.38×10^{-7}	T/0.14	1.61	0.4751	+	1.70×10^{-6}
rs5016282	11	88741660	GRM5	1.78×10^{-6}	A/0.86	1.85	0.3428	+	2.55×10^{-6}
rs2532274	17	44247164	KIAA1267	5.82×10^{-6}	C/0.20	1.49	0.1585	+	3.76×10^{-6}
rs7105122	11	47449544	PSMC3	9.10×10^{-6}	C/0.71	1.47	0.5279	+	1.76×10^{-5}
rs9844608	3	33774574	CLASP2	9.31×10^{-6}	A/0.67	1.45	0.5347	+	1.82×10^{-5}
rs11757000	6	28484869	GPX6	1.17×10^{-5}	T/0.85	1.72	0.9177	−	7.63×10^{-5}
rs4804149	19	11284028	ANKRD25	1.20×10^{-5}	C/0.27	1.42	0.1096	−	7.81×10^{-5}
rs6458351	6	43791080	VEGF	1.39×10^{-5}	T/0.07	1.72	0.1967	−	8.95×10^{-5}
rs7984422	13	92808689	GPC5	1.42×10^{-5}	A/0.02	2.41	0.0896	+	5.08×10^{-6}
rs1430961	4	90552920	SNCA	1.49×10^{-5}	C/0.07	1.76	0.6698[i,j]	+	3.47×10^{-5}
rs17031719	12	102022264	MYBPC1	1.62×10^{-5}	G/0.15	1.53	0.2489	−	1.03×10^{-4}
rs13119057	4	46807501	COX7B2	1.86×10^{-5}	T/0.24	1.43	0.0265[h]	−	1.17×10^{-4}
rs11142062	9	90658749	CCRK	2.05×10^{-5}	T/0.10	1.59	0.9013	+	6.14×10^{-5}
rs3760201	17	21193618	MAP2K3	2.05×10^{-5}	T/0.67	1.44	0.2466	+	1.84×10^{-5}
rs10838881	11	48387275	OR4C3	2.24×10^{-5}	T/0.85	1.66	0.1083	+	9.29×10^{-6}
rs2114961	5	134574232	H2AFY	2.70×10^{-5}	T/0.72	1.46	1.0000	−	1.65×10^{-4}
rs2509689	11	102368719	MMP7	2.84×10^{-5}	C/0.78	1.52	0.0499	+	5.62×10^{-6}
rs4549394	4	129431298	PGRMC2	3.58×10^{-5}	T/0.13	1.52	0.4250	+	5.13×10^{-5}
rs3741410	11	48168664	PTPRJ	3.84×10^{-5}	A/0.84	1.62	0.0792	+	1.15×10^{-5}
rs655824	11	120834480	GRIK4	3.98×10^{-5}	C/0.04	1.85	0.6473	+	8.34×10^{-5}
rs7694947	4	22576409	GPR125	4.22×10^{-5}	T/0.51	1.36	0.5637[j]	−	2.48×10^{-4}
rs6732434	2	182901257	SSFA2	4.68×10^{-5}	A/0.24	1.41	0.0042	−	2.73×10^{-4}
rs2773822	9	135861033	GFI1B	5.04×10^{-5}	C/0.86	1.66	0.7357	+	1.17×10^{-4}
rs9929758	16	84430781	KIAA0703	5.47×10^{-5}	T/0.83	1.57	0.5485	+	9.60×10^{-5}
rs13270024	8	142201477	DENND3	5.75×10^{-5}	C/0.16	1.46	NA[k]	NA[k]	NA[k]
rs1859063	2	130363731	LOC151121	5.83×10^{-5}	G/0.02	2.35	0.3173	−	3.34×10^{-4}
rs2580817	2	232798723	NPPC	6.15×10^{-5}	A/0.10	1.54	0.2763	−	3.50×10^{-4}
rs10515549	5	144697489	MGC21644	6.55×10^{-5}	G/0.43	1.34	0.8175	+	1.64×10^{-4}
rs650957	1	191429227	RGS18	7.38×10^{-5}	T/0.42	1.33	0.5858	+	1.34×10^{-4}
rs1623319	12	76270756	PHLDA1	7.57×10^{-5}	A/0.23	1.38	0.4862	+	1.16×10^{-4}

[a]For all SNPs 171 families comprising at least one child with ADHD and both parents were analyzed; only for SNPs rs2556378 and rs5016282 a total of 320 families (at least one child with ADHD and both parents) was included in the analyses (see "Materials and Methods" section).
[b]Base pairs according to hg19—NCBI Build 37.1 (GRCh37).
[c]Gene abbreviations: *BCL11A*: B-cell CLL/lymphoma 11A (zinc finger protein); *GRM5*: glutamate receptor, metabotropic 5; *PSMC3*: proteasome (prosome, macropain) 26S subunit, ATPase, 3; *KIAA1267*: KIAA1267; *CLASP2*: cytoplasmic linker associated protein 2; *ANKRD25*: KN motif and ankyrin repeat domains 2; *GPC5*: glypican 5; *VEGF*: vascular endothelial growth factor A; *GPX6*: glutathione peroxidase 6 (olfactory); *CCRK*: cyclin-dependent kinase 20; *SNCA*: synuclein, alpha (non A4 component of amyloid precursor); *COX7B2*: cytochrome c oxidase subunit VIIb2; *OR4C3*: olfactory receptor, family 4, subfamily C, member 3; *MYBPC1*: myosin binding protein C, slow type; *MMP7*: matrix metallopeptidase 7 (matrilysin, uterine); *MAP2K3*: mitogen-activated protein kinase kinase 3; *H2AFY*: H2A histone family, member Y; *GRIK4*: glutamate receptor, ionotropic, kainate 4; *PTPRJ*: protein tyrosine phosphatase, receptor type, J; *GPR125*: G protein-coupled receptor 125; *RGS18*: regulator of G-protein signaling 18; *PGRMC2*: progesterone receptor membrane component 2; *NPPC*: natriuretic peptide C; *LOC151121*: Homo sapiens cDNA FLJ32036 fis, clone NTONG2000492; *MGC21644/PRELID2*: PRELI domain containing 2; *KIAA0703*: KIAA1267; *PPP1R1C*: Homo sapiens protein phosphatase 1 regulatory subunit 1A mRNA; *DENND3*: DENN/MADD domain containing 3; *GFI1B*: growth factor independent 1B transcription repressor; *PHLDA1*: pleckstrin homology-like domain, family A, member 1; *FOLH1*: folate hydrolase (prostate-specific membrane antigen) 1.
[d]*P*-values deflated for a genomic control inflation factor $\lambda = 1.042$ ranged between 1.39×10^{-6} and 1.06×10^{-4}.
[e]Risk allele frequency as derived in the controls.
[f]Odds ratio for the risk allele derived for logistic regression model with additive allele coding.
[g]+ Risk allele is the same for GWAS and replication; − risk allele is not the same for GWAS and replication.
[h]By Fisher's combined probability test.
[i]Test for deviations from HWE in parents: $P_{exact} < 0.01$.
[j]Call rate <0.8.
[k]Not genotyped in replication sample.

2010; Won et al., 2011] we performed a gene-set enrichment analysis (GSEA) by testing in the style of the Meta-Analysis Gene-set Enrichment of variaNT Associations (MAGENTA) algorithm; [Segrè et al., 2010]. For a total of 17,680 unique autosomal genes (full gene list downloaded from the University of California Santa Cruz (UCSC) Genome Browser [http://genome.ucsc.edu/]; cleansed as described in [Segrè et al., 2010]), PLINK v1.07 was used to calculate gene-wise empirical *P*-values based on 1,000,000 permutations of the trait status. As proposed by Segrè et al. [2010], any SNP that lies within 110 kb upstream the gene's most extreme

transcript start site and around 40 kb downstream to the genes most extreme transcript end site is assigned to the corresponding gene. For gene-set enrichment testing, the null hypothesis that the 64 candidate gene-wise *P*-values are randomly distributed is compared to the alternative hypothesis that there is an over-representation of gene-wise *P*-values below the 95th percentile of all autosomal genes ($P_{\text{cutoff},95} = 0.0436$) in the candidate gene set compared to 10,000 randomly sampled autosomal gene sets of identical size. A GSEA *P*-value P_{GSEA} was calculated as the fraction of randomly sampled gene sets with more than four gene-wise *P*-values below $P_{\text{cutoff},95}$ as observed in the candidate gene set. Alternative GSEA statistical tests, such as the Mann–Whitney rank-sum test and the *t*-test are also provided.

RESULTS

The GWAS in 495 German children and adolescents with ADHD and 1,300 population-based adult controls revealed 30 independent SNPs with *P*-value below 7×10^{-5} (best *P*-value: 8.38×10^{-7}; Table 2). These were chosen for independent confirmation.

We performed two confirmation attempts: (1) We hypothesized that German children with ADHD should represent a relatively homogeneous replication group. Hence we performed de novo genotyping of 30 SNPs in up to 320 German families comprising at least 1 child with ADHD and both parents (Table 2). This approach revealed directionally consistent effect-size point estimates for 19 of the SNPs (of the 29 SNPs that were available for this analysis; $P_{\text{one-sided binomial sign test}} = 0.07$), whereas 10 were directionally not consistent. However, only one of the 19 SNPs showed an unadjusted *P*-value below 0.05, and none of the combined *P*-values reached the genome-wide significant *P*-value below $\alpha = 5 \times 10^{-8}$. Of course, this observation may not be too surprising given the limited power of both our GWAS data set and the German replication set to detect common variants of effect sizes usually reported for GWAS. For both, the GWAS (at $\alpha = 5 \times 10^{-8}$) and the German replication data set of up to 320 trios (at $\alpha = 0.001 < 0.05/30$, i.e., correcting for 30 SNPs) we had a comparison-wise power >80% to detect genetic risk alleles with a MAF >0.1 and a (log-) additive genetic effect ≥2.0 (odds ratio).

(2) We attempted replication by in silico analyses of imputed GWAS data from the currently largest meta-analysis of the international ADHD GWAS data available in the Psychiatric GWAS Consortium (2,064 trios, 896 cases, and 2,455 controls) [Neale et al., 2010b]. Of note most of the German index patients used for replication (see 1) were also included in this large-scale meta-analysis [Neale et al., 2010b]. We detected directionally consistent effect-size point estimates for 16 SNPs (out of 27 that were available for this analysis; $P_{\text{one-sided binomial sign test}} = 0.44$; 11 were directionally not consistent). None of the *P*-values was below 0.05 (Supplementary Table A); combined analyses did not reveal a genome-wide significant result at $\alpha = 5 \times 10^{-8}$.

Additionally, we analyzed 64 previously published autosomal ADHD candidate genes which were either derived from candidate gene studies or from GWAS regions (see Supplementary Table B) for ADHD (for 63 see recent review by Banaschewski et al. [2010] plus the recently described ADHD candidate gene G protein-coupled receptor kinase-interacting protein-1 gene (*GIT1*) [Won et al., 2011]; for gene names and positions see Supplementary Table B). To analyze if the GWAS derived gene-wise empirical *P*-values in these genes were significantly lower than expected by chance when picking 64 such *P*-values out of all being attributable to the 17,680 autosomal genes, we used gene set enrichment analyses (GSEA) [Segrè et al., 2010; http://www.broadinstitute.org/mpg/magenta/]. GSEA just failed to reveal a difference between gene-wise empirical *P*-values according to the "ADHD candidate genes" in comparison to randomly sampled autosomal gene sets of the same size ($P_{\text{GSEA}} = 0.4045$; $P_{\text{Mann–Whithney}} = 0.07518$; $P_{t\text{-test}}$ 0.06058; complete results for all analyzed genes are shown in Supplementary Table B).

DISCUSSION

This GWAS in a small group of clinically ascertained young German patients with ADHD and population-based controls did not reveal genome-wide significant findings. Replication attempts in further German and international samples did also not lead to a *P*-value(s) below 5×10^{-8}.

We subsequently analyzed all previously published candidate genes for ADHD (including the best hits of the previous ADHD GWAS) by GSEA methodology. Again, we detected no evidence for an involvement of this set of ADHD genes in our patients with ADHD. The power of our GWAS and subsequent analyses was sufficient to detect risk variants with an OR of 2.0. For another complex phenotype (obesity), we were able to derive a (study specific) genome-wide significant result in study groups of a similar size (487 obese cases vs. 442 healthy lean controls) [Hinney et al., 2007] using a similar approach of homogeneous diagnostic assessments in combination with high population homogeneity. Apart from the sample size limitations, the cases and controls do also differ with regard to sex—the case sample is enriched for males—and age—the cases were children and adolescents whereas the controls were adults. At the genome-wide level we cannot exclude that effects of sex and age may have masked some of the true variants. However, the sensitivity analyses for the 30 best SNPs (Table 2) indicated no strong sex- or age-related discrepancies. Another limitation of our study is the use of different genotyping chips for the cases and controls (Human660W-Quadv1 BeadArrays for the cases and HumanHap550v3 for the controls; both from Illumina), while the same quality control (QC) measures were applied to the raw genotyping data for all chips [see also Cichon et al., 2011]. As a word of caution regarding chip based genotyping results, we like to point out that we have used all commonly recommended QC checks (e.g., check of intensity plots, see [Pluzhnikov et al., 2010]) for the 31 best hits. Surprisingly, the formerly best SNP (rs4862110) did pass all QC checks, but showed massive problems in the re-genotyping (TaqMan) approach; so that eventually the SNP had to be removed from further analysis (see "Materials and Methods" section). We recommend to re-genotype the best chip-based GWAS SNPs with an independent method prior to large-scale confirmation attempts.

Although we did not detect genome-wide significant results, some of the genes in the vicinity of the 30 best SNPs of our GWAS merit a closer look. The second best hit, a SNP within an intron of *GRM5* seems to be especially interesting. In our GWAS data set,

none of the ADHD cases were homozygous carriers of the (protective) GG genotype. This absence of GG carriers was similarly observable in both the parents and the ADHD offspring of the replication sample which might indicate a recessive mode of inheritance at this locus. In the literature, there is some evidence that the loss of one *GRM5* copy is associated with a severe form of ADHD [Elia et al., 2010]. Rare structural variants were recently described for ADHD [Elia et al., 2010; Williams et al., 2010; Lesch et al., 2011]. Analysis of 335 patients with ADHD revealed one parent with ADHD and three affected offspring who harbored a deletion within *GRM5* [Elia et al., 2010]. However, Williams et al. [2010] did not detect this deletion in an independent sample of 410 children with ADHD and 1,156 controls. Based on the association observed in our GWAS (discovery sample), mutations in *GRM5* might be relevant for ADHD, though confirmation attempts failed (see Table 2 and Supplementary Table A).

A second gene of interest is glypican 5 (*GPC5*). The gene product belongs to the glypican family; another member of this family—*GPC6*—has already been described as a candidate gene for ADHD in adults [Lesch et al., 2008; summarized in Banaschewski et al., 2010]. Glypicans comprise a family (six members in vertebrates) of glycosyl-phosphatidylinositol-anchored heparan sulfate proteoglycans. Control of cell growth and division seems to be influenced by glypicans [Banaschewski et al., 2010]. SNPs in close vicinity to the *GPC6* ADHD-SNP [Lesch et al., 2008] showed trends for association in a GWAS for bipolar disorder [Sklar et al., 2008]. Interestingly, *GPC5* is located in a linkage region for bipolar disorder. However, a mutation screen in individuals with bipolar disorder did not reveal relevant mutations [Maheshwari et al., 2002].

GWAS have been very successful for detection of risk alleles of SNPs for a variety of different complex disorders and phenotypes [Hindorff et al., 2009] (www.genome.gov/gwastudies). In contrast, the success for psychiatric disorders has proven to be rather limited. Smaller sample sizes seemingly only partly account for this difference between complex psychiatric and other disorders. Although heritability estimates for several psychiatric disorders are high [Hebebrand et al., 2010] currently, only nine genetic variants/regions have been detected for five psychiatric disorders for which GWAS have been published so far (autism—2 genes, ADHD—none, bipolar disorder—4 genes, unipolar depression—none, schizophrenia—3 genes, anorexia nervosa, none; see www.genome.gov/gwastudies). Meta-analyses of GWAS for psychiatric disorders comprised up to several thousands of patients and controls; these numbers are still much lower than those for common traits like body mass index (BMI). A recent large-scale meta-analysis and replication for BMI loci comprised a total of 249,796 individuals from the general population and led to the detection of 18 novel genomic regions and redetection of 14 regions [Speliotes et al., 2010]. Inclusion of similar numbers of patients will be very difficult to achieve for psychiatric disorders. However, as genome-wide significance for some loci is nearly achieved for some of the analyzed psychiatric disorders, large-scale international approaches (like in the Psychiatric GWAS Consortium see above) might well lead to significant findings in the near future.

ACKNOWLEDGMENTS

We thank the children and their families for their participation. We thank the German Research Association (DFG) who funded the GWAS analyses and confirmatory studies (He1446/9-1 to J. Hebebrand, K.-P. Lesch, A. Hinney and T. Renner, KFO 125, SFB 581, GRK 1156 to K.P. Lesch; ME 1923/5-1, ME 1923/5-3 to J. Meyer and C. M. Freitag, GRK 1389 to J. Meyer, SCHA 542/10-3 to H. Schäfer) and the Bundesministerium für Bildung und Forschung (BMBF 01GV0605 to K.P. Lesch). We thank the START-Program EK 119/05 of the Medical Faculty, RWTH Aachen, Germany. We thank the Cardiff group of IMAGE2 for providing summary statistics for the 30 best SNPs; the group comprises Anita Thapar, Michael C O'Donovan, Michael J. Owen, Kate Langley, Nigel Williams, Peter Holmans at the MRC Centre for Neuropsychiatric Genetics and Genomics, Cardiff University, UK. The ADHD meta-analysis was supported by the following grants: US National of Institute of Health Grants R13MH059126, R01MH62873 and R01MH081803 to S.V.Faraone and K23MH066275 to J. Elia, The University of Pennsylvania CTR grant UL1-RR-024134 to J. Elia and H. Hakonarson and Institutional Development Award to the Center for Applied Genomics from the Children's Hospital of Philadelphia to H. Hakonarson; Affymetrix Power Award, 2007 to B. Franke; NHMRC (Australia) and Sidney Sax Public Health Fellowship (443036) to S.E. Medland; Wellcome Trust, UK for sample collection to L Kent. MH58277 to S. Smalley. UMC Utrecht Genvlag Grant, an Internal Grant of Radboud University Nijmegen Medical Centre to J. Buitelaar.

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