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ORIGINAL ARTICLE

Association between genetic variation in a region on chromosome 11 and schizophrenia in large samples from Europe

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Recent molecular studies have implicated common alleles of small to moderate effect and rare alleles with larger effect sizes in the genetic architecture of schizophrenia (SCZ). It is expected that the reliable detection of risk variants with very small effect sizes can only be achieved through the recruitment of very large samples of patients and controls (that is tens of thousands), or large, potentially more homogeneous samples that have been recruited from confined geographical areas using identical diagnostic criteria. Applying the latter strategy, we

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performed a genome-wide association study (GWAS) of 1169 clinically well characterized and ethnically homogeneous SCZ patients from a confined area of Western Europe (464 from Germany, 705 from The Netherlands) and 3714 ethnically matched controls (1272 and 2442, respectively). In a subsequent follow-up study of our top GWAS results, we included an additional 2569 SCZ patients and 4088 controls (from Germany, The Netherlands and Denmark). Genetic variation in a region on chromosome 11 that contains the candidate genes *AMBRA1*, *DGKZ*, *CHRM4* and *MDK* was significantly associated with SCZ in the combined sample ($n = 11\,540$; $P = 3.89 \times 10^{-9}$, odds ratio (OR) = 1.25). This finding was replicated in 23 206 independent samples of European ancestry ($P = 0.0029$, OR = 1.11). In a subsequent imaging genetics study, healthy carriers of the risk allele exhibited altered activation in the cingulate cortex during a cognitive control task. The area of interest is a critical interface between emotion regulation and cognition that is structurally and functionally abnormal in SCZ and bipolar disorder.

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Introduction

Schizophrenia (SCZ) is a severe psychiatric disorder characterized by fundamental and characteristic distortions of thought and perception. It has a lifetime prevalence of around 0.5–1%. Formal genetic studies have shown that genetic factors contribute substantially to the disease risk. Meta-analyses of pooled data from 12 twin studies have estimated that susceptibility to SCZ has a heritability of $\sim$80%.[1] Recent molecular studies have implicated common alleles of small to moderate effect and rare alleles with larger effect sizes in the genetic architecture of SCZ.[2] Genome-wide association studies (GWAS) using single nucleotide polymorphism (SNP) array technologies have been applied to detect common risk alleles, and a total of 12 GWAS of SCZ have been published to date (Supplementary Table S3). Common risk alleles in the major histocompatibility region on chromosome 6 have so far shown the most statistically significant evidence of association.[3–5] Interestingly, the major histocompatibility region, which is involved in the immune response, has long been postulated to harbor variants conferring a risk for SCZ as there is evidence for linkage in this region[6] and research has suggested the involvement of infection in disease development.[7] Genome-wide significance has also been reported for risk alleles at *TCF4*,[3] *NGRN*[3] and *ZNF804A*.[8]

All GWAS of SCZ performed to date have indicated that the strongest common genetic risk factors have odds ratios (OR) that are no greater than 1.15–1.20. In fact, recent molecular genetic evidence points to a substantial polygenic component to the risk of SCZ that involves a large number of common risk alleles of very small effect.[4] It is likely that the reliable detection of risk variants with very small effects can only be achieved through the study of very large samples of patients and controls (that is, tens of thousands) or large, potentially more homogeneous samples that have been recruited from a confined geographical area using the same diagnostic criteria. Using the latter strategy, we performed a GWAS of clinically well characterized and ethnically homogeneous SCZ patients recruited from a confined area of Western Europe (Germany, The Netherlands and Denmark). Our results suggest that genetic variation in a region on chromosome 11, which contains the candidate genes *AMBRA1, DGKZ*, *CHRM4* and *MDK*, is implicated in the etiology of SCZ.

Patients and methods

The following section provides details of sample recruitment and quality control (QC) for the GWAS data set. Information concerning specific aspects of the GWAS data set and data from the follow-up samples are provided in the Supplementary Information Patients and Methods.

Sample ascertainment and selection for the GWAS sample

All participating individuals provided written informed consent. The study protocols were approved by the respective institutional review boards or ethics committees.

German sample (Bonn—Mannheim). The German SCZ patients used in the GWAS step ($n = 487$) were recruited from consecutive hospital admissions and were all of German descent. Lifetime best estimate diagnoses were assigned according to DSM-IV criteria on the basis of multiple sources of information including structured interviews with the SCID[9] or SADS-L,[10] the OPCRIT,[11] medical records and family history. Best estimate diagnoses were assigned by at least two experienced psychiatrists/psychologists. The controls were drawn from three population-based epidemiological studies: (A) PopGen[12] ($n = 490$), (B) KORA[13] ($n = 488$) and (C) the Heinz Nixdorf Recall ($n = 383$) study[14] (risk factors, evaluation of coronary calcification and lifestyle). The recruitment areas for PopGen, KORA and HNR were located in the following: (i) Schleswig-Holstein (Northern Germany); (ii) Essen, Bochum and Mühlheim (Ruhr area); and (iii) Augsburg (Southern Germany), respectively. Ethnicity was assigned to

patients and controls on the basis of self-reported ancestry.

Dutch sample (Utrecht and Rotterdam). Inpatients and outpatients ($n = 804$) were recruited from various psychiatric hospitals and institutions throughout the Netherlands. Detailed medical and psychiatric histories were collected, and this process included use of the Comprehensive Assessment of Symptoms and History (CASH),[15] an instrument for assessing diagnosis and psychopathology. Only those patients with a DSM-IV diagnosis of SCZ were finally included as cases. The controls from Utrecht ($n = 704$) were volunteers with no history of psychiatric disorder. The Rotterdam control individuals ($n = 2302$) were drawn from a large population-based project on the genetics of complex traits and diseases, which is financed by the Dutch government through the Netherlands Scientific Organization—Large Investments (NWO Groot; 175.010.2005.011). This prospective population-based cohort study of chronic disabling conditions in Dutch individuals aged 55 years and above is described elsewhere.[16,17] All patients and controls had at least three grandparents of Dutch ancestry.

Sample ascertainment and selection for the imaging genetics study sample

Subjects ($n = 122$) were drawn from an ongoing large-scale multicenter imaging genetics study[18] (Esslinger *et al*. plus seven subsequently scanned subjects) that is being conducted at two sites in Mannheim and Bonn, Germany. All participants were healthy German volunteers with parents and grandparents of European origin. None of the participants had any self-reported lifetime or family history of SCZ or affective disorder. All subjects provided written informed consent. The study was approved by the local ethics committees of the Universities of Heidelberg and Bonn.

Genotyping and QC for the GWAS and the imaging genetics study

Ethylenediaminetetraacetic acid anti-coagulated venous blood samples were collected from all participating individuals (GWAS and imaging genetics study). Lymphocyte DNA was isolated by salting-out[19] with saturated sodium chloride solution or by a Chemagic Magnetic Separation Module I (Chemagen, Baesweiler, Germany) used according to the manufacturer's recommendations. The GWAS data set was assembled from seven sub-data sets, which were genotyped by the following: (i) Illumina's customer service, San Diego, CA, USA (all PopGen controls, all German SCZ patients); (ii) the Department of Genomics, Life & Brain Center, University of Bonn (all HNR controls); (iii) the Helmholtz Zentrum München, Germany (all KORA controls); (iv) The Southern California Genotyping Consortium (SCGC) at UCLA, Los Angeles, USA (all Dutch SCZ patients and $n = 704$ Dutch controls); and (v) the genotyping facility of the ErasmusMC Biomics core facility, Rotterdam, The Netherlands ($n = 2302$ Dutch controls, including the controls for the follow-up sample). All genome-wide genotyping for the GWAS was performed on HumanHap550v3 BeadArrays using the Infinium II assay (Illumina). The imaging genetics sample ($n = 122$) was genotyped by the Department of Genomics, Life & Brain Center, University of Bonn. The genome-wide genotyping was performed on Human610-quad beadchips using the Infinium HD assay (Illumina). For a discussion on the possible confounding of case/control status with plate and genotyping procedures please see the Supplementary Information Patients and Methods.

We developed a protocol of filters for the stringent QC of whole-genome and sub-whole genome data sets. This accounted for call rates, heterozygosity, cross-contamination, population stratification, relatedness, non-random-missingness, Hardy–Weinberg equilibrium, minor allele frequency and others. With the exception of the cluster plot investigations for the follow-up SNPs, each QC filter for the GWAS, the imaging genetics study and the follow-up data sets was performed using the PLINK toolset.[20] The samples from the Rotterdam study (controls for the GWAS and the replication step) underwent QC together with the other GWAS samples.

The QC protocol was applied to a total of 1291 patients and 4367 controls for the GWAS step. As a result, 375 individuals including 122 patients and 253 controls (representing 6.70, 9.73 and 5.80%, of the sample) as well as 86 037 SNPs (15.32% of the HumanHap550v3 BeadArray content) were excluded before the association analysis. The final GWAS data set was comprised of 464 030 autosomal (including PAR1 + 2) and 11 397 X-chromosomal SNPs, genotyped in 1169 SCZ patients and 3714 controls. An additional 400 controls were added to the Dutch follow-up data set described above.

The QC protocol was also applied to the 122 healthy German individuals who participated in the imaging genetics study. As a result, one individual and 39 356 SNPs (6.64% of the Human610-quad BeadArray content of evaluable 592 532 SNPs) were excluded before the association analyses. The mean age of the remaining 121 individuals (43.4% males) was 33.1 (s.d. 10.3). For the analysis of the imaging genetics data, only information for rs11819869, which was present in the post QC data set, was extracted and analyzed.

A detailed description of the QC filters is provided in the Supplementary Information Patients and Methods.

Statistical analyses for GWAS and follow-up

All association analyses were performed using PLINK[20] (v1.07). In the single-marker analysis, all autosomal (including PAR1 and PAR2) and gonosomal SNPs that passed QC checks were tested for association with SCZ using the Cochran–Mantel–Haenszel test (CMH) with $2 \times 2 \times K$ stratified tables

{disease × SNP disease | cluster} with $K=2$ (assigning German and Dutch individuals to one cluster each) for the GWAS step, $K=4$ (one cluster for the German, Dutch, and Danish samples, respectively) for Replication 1, and $K=6$ for the combined analysis. Following analysis of the initial GWAS, a set of 60 SNPs was chosen for the follow-up step using a top-down approach (Replication 1; more information on the selection of SNPs is provided in the Supplementary Information Patients and Methods). The CMH was also applied to the replication sample as described above. In the replication analysis, the test statistic was one-tailed if the OR was in the same direction as in the GWAS. SNPs that exceeded the threshold for nominal significance ($P=0.05$) were identified. Finally, for all SNPs included in the replication step, a combined analysis of individuals from the GWAS and the replication step was performed using the CMH ($K=6$; test statistic always two-tailed). The Breslow–Day test was used to investigate the homogeneity of the ORs for the replicated SNPs. The meta-analysis for the 15 independent samples of European ancestry (Replication 2) was performed using PLINK[20] (v1.07) and considering a fixed effect model. There was no evidence of heterogeneity (Cochrane's *Q*). In cases where genome-wide data were available, the two-tailed *P*-value of the TREND test was corrected by the genomic inflation factor for the specific sample as calculated from the genome-wide data.

A note on possible stratification

We have assumed that our sample is characterized by a high degree of ethnic (and presumably genetic) homogeneity. Nonetheless, several steps were taken to minimize population stratification. Patients and controls were only included when a minimum of three grandparents originated from the respective country. Outliers were identified using two methods: (1) multidimensional scaling and (2) outlier detection diagnostics as implemented in PLINK.[20] This was performed for the Dutch and German samples individually, and then in the combined sample (Supplementary Figure S6, further information is provided in the Supplementary Information Patients and Methods). Tests were applied to detect differences in allele frequencies and missingness patterns between the single Dutch and German control samples (Rotterdam, Utrecht, Kiel, Munich and Essen). Finally, association analyses were performed using a CMH test and a logistic regression with covariates derived from multidimensional scaling analyses (first six dimensions) to minimize potential stratification effects (Supplementary Figure S5, more information is provided in the Supplementary Information Patients and Methods).

Imaging genetics study

The participants of the imaging genetics study completed a battery of six cognitive tasks that have been used and validated previously in imaging genetics. Specifically, the following tasks were performed: (1) an associative learning task,[21] in which subjects learn and are then tested on face-job associations, (2) an n-back working memory task,[22] in which subjects are presented with a sequence of numbers and press a button corresponding either to the number currently seen (control condition, '0-back') or the number seen two presentations previously ('2-back'), (3) a theory of mind task,[23] in which subjects have to make inferences about the state of mind of a human based on a series of cartoons, (4) a flanker task[24] in which subjects have to perform or withhold a button press depending on a set of either congruent or incongruent stimuli, requiring cognitive control, (5) an implicit emotion recognition task,[25] in which subjects match pictures of angry and fearful faces, (6) a monetary reward task,[26] in which subjects receive or do not receive monetary rewards according to their performance in a reaction time task and a 5 min of rest period. Further information, including details of the imaging parameters, is provided in the Supplementary Information Patients and Methods.

For analysis of the influence of rs11819869, contrast images of incongruent > congruent trials were subjected to a two-sample *t*-test with age, gender and scanning site as covariates of no interest. To avoid small cell sizes, CT heterozygotes and TT homozygotes were combined and compared with the CC homozygous group.

In view of our exploratory approach, we used a significant threshold of $P<0.0083$ (equivalent to $P<0.05$ with Bonferroni correction for six tasks), correcting for multiple comparisons across all voxels of the brain using family-wise error, based on Gaussian random fields theory. Further information is provided in the Supplementary Information Patients and Methods.

Results

We performed a GWAS of 1169 clinically well characterized and ethnically homogeneous SCZ patients who had been recruited from a confined area of Western Europe ($n=464$ from Germany, $n=705$ from The Netherlands) and 3714 ethnically matched controls ($n=1272$ and $n=2442$, respectively; Table 1). A total of 475 427 SNPs (Illumina's HumanHap550v3 BeadArray) were tested using the CMH test (CMH, for $2\times2\times K$ stratified tables, Patients and methods section). No marker exceeded the widely acknowledged genome-wide significance threshold of 5×10^{-8}, or a Bonferroni-corrected significance threshold of 1.1×10^{-7}. The best result ($P_{GWAS}=4.50\times10^{-7}$) was for the SNP rs11154491, which is located in an intron of the *Rho GTPase activating protein 18* gene on chromosome 6 (*ARGHAP18*, Supplementary Figure S2). An overview of the GWAS results is provided in Figure 1a, a quantile–quantile plot of the *P*-values is shown in Supplementary Figure S4.

Under the assumptions that the most significantly associated SNPs included true SCZ susceptibility

Table 1 Descriptive data for schizophrenia patients and controls following quality control

	GWAS				*Follow-up study (Replication 1)*							
	Germany (Bonn—Mannheim)		*The Netherlands*		*Germany (Munich)*		*The Netherlands*		*Germany (Bonn—Mannheim)*		*Denmark (Aarhus)*	
	Patients	*Controls*	*Patients*	*Controls*	*Patients*	*Controls*	*Patients*	*Controls*	*Patients*	*Controls*	*Patients*	*Controls*
Individuals	464	1272	705	2442	913	1668	178	400	600	1146	878	874
Males (in %)	233 (50.1%)	649 (51.0%)	539 (76.5%)	1224 (50.1%)	572 (62.7%)	810 (48.6%)	124 (69.7%)	165 (41.3%)	372 (62.0%)	566 (49.4%)	479 (54.8%)	475 (54.3%)
Mean age at recruitment in years (s.d.)	34.1 (11.0)	50.4 (11.4)	34.4 (13.1)	59.1 (18.3)	37.7 (11.7)	49.5 (16.0)	37.9 (10.9)	70.0 (9.5)	38.4 (12.3)	46.5 (15.8)	20.1 (2.7)	20.1 (2.7)
Mean age of onset in years (s.d.)	21.9 (6.5)	N/A	N/A	N/A	23.6 (8.7)	N/A	N/A	N/A	29.1 (9.5)	N/A	18.9 (2.8)	N/A

Abbreviations: GWAS, genome-wide association study; N/A, not applicable or not available.

factors and that the failure to reach genome-wide significance in the GWAS step was a result of insufficient power, we performed a subsequent follow-up analysis (Replication 1). A total of 43 SNPs were selected using a top-down *P*-value approach in 2569 additional patients and 4088 controls from Western Europe (Germany/Munich: 913 patients/1668 controls; Bonn/Mannheim: 600/1146; The Netherlands: 178/400; Denmark: 878/874; Table 1). In all 9 of the 43 SNPs (21%) showed nominal significance in the combined replication samples, and all with the same alleles as in the GWAS (Table 2 and Supplementary Figure 1 and Supplementary Table S1a and b). The strongest evidence for association was found for four highly correlated SNPs (rs7112229, rs11819869, rs7130141 and rs12575668). These four SNPs are located in intronic regions of the *activating molecule in beclin-1-regulated* gene (*AMBRA1*) on chromosome 11. The best SNP (rs11819869) showed $P_{\mathrm{REPLI}} = 5.04 \times 10^{-5}$ ($P_{\mathrm{CORR}} = 0.0022$). One further SNP (rs4309482) withstood Bonferroni correction ($P_{\mathrm{CORR}} = 0.013$) for the number of SNPs tested in the replication step. This SNP is located in an intergenic region of chromosome 18 between the *coiled-coil domain-containing 68* gene (*CCDC68*) and the *transcription factor 4* gene (*TCF4*). Our top GWAS SNP (rs11154491), which is located in *ARGHAP18* on chromosome 6q22.33, was not replicated ($P_{\mathrm{REPLI}} = 0.38$).

In an analysis of the combined samples (GWAS + follow-up; $n = 3738$ patients/7802 controls; CMH, $K = 6$), variation in the chromosome 11 region surpassed the threshold for genome-wide significance (rs11819869, $P_{COMB} = 3.89 \times 10^{-9}$, OR = 1.25, Figure 1b). A Breslow–Day test was performed across all of the analyzed samples for all SNPs that had withstood Bonferroni correction in the replication step. This revealed no significant differences in ORs ($P > 0.05$) between the investigated samples. In support of this, subtraction of any one individual replication sample failed to alter the effect sizes to any substantial degree (Supplementary Table S1b).

We then attempted to replicate our genome-wide significant result for rs11819869 in 15 independent samples of European ancestry (Replication 2; 4734 patients and 18 472 controls). We observed significant association for the T risk allele of rs11819869, as had been observed in the GWAS study ($P_{\mathrm{META}} = 0.0029$, OR = 1.11; Table 3). We then applied a functional magnetic resonance imaging test battery for imaging genetics in a total of 121 healthy subjects (53 males, 68 females) of German descent with no family history of affective disorder or SCZ. This demonstrated a significant effect of the rs11819869 genotype on medial prefrontal activation during a flanker task, which contrasted incongruent and congruent stimuli configurations ($P < 0.05$, family wise error corrected for multiple testing, cluster size: $K = 9$, maximum activation found at (−3, 36, −18), $T(116) = 5.522$, $P_{\mathrm{CORR}} < 0.008$ (see Patients and methods for details of the task and the statistics). Carriers of the

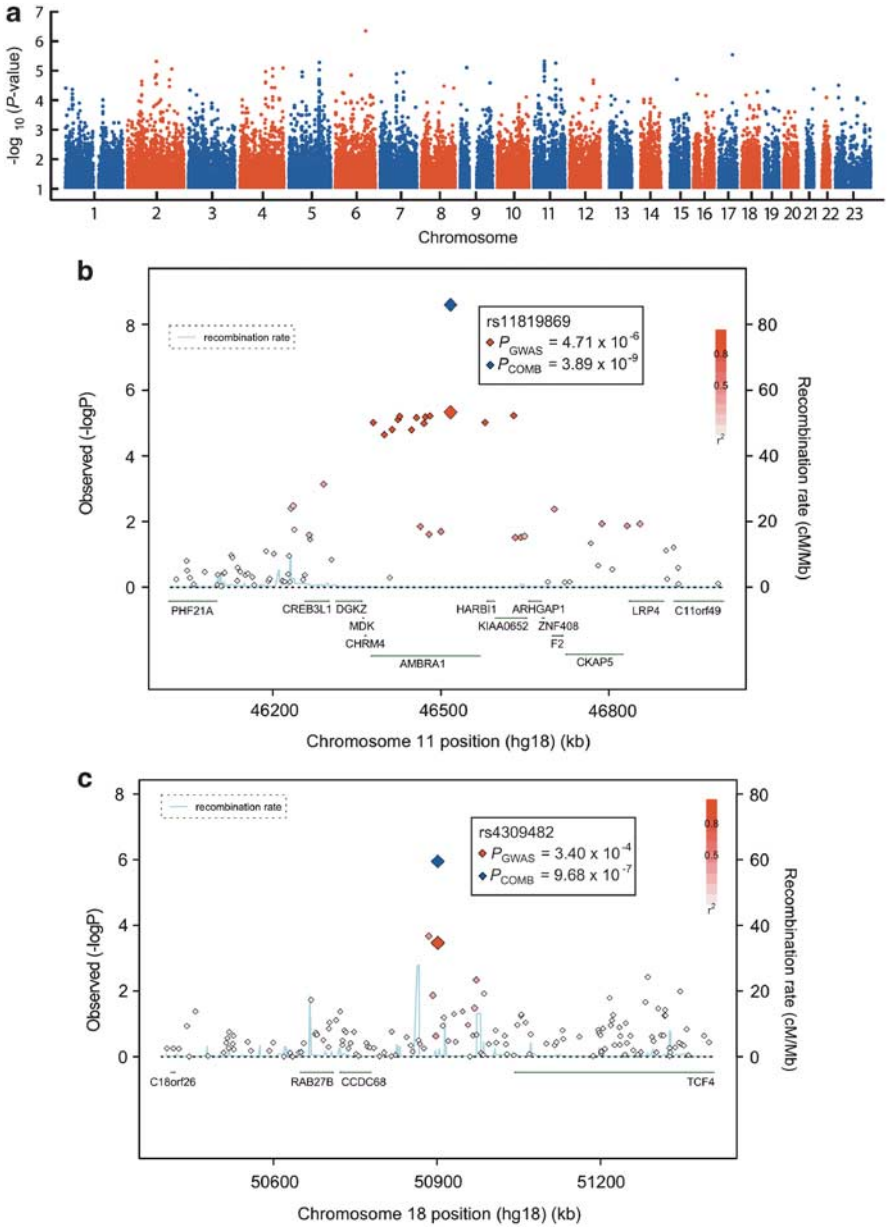

Figure 1 Association results for the genome-wide association study (GWAS) and the two best-supported genes from the follow-up study (Replication 1). (**a**) Manhattan plot. Regional plots depicting (**b**) *AMBRA1*/*CHRM4*/*DGKZ*/*MDK* region and (**c**) *CCDC68*/*TCF4* region. The best-associated marker from the GWAS (enlarged red diamond) is centered in a genomic window of 1 Mb (hg18, RefSeq genes); its *P*-value from the combined analysis (P_{COMB}) is shown (enlarged blue diamond). The linkage disequilibrium (LD) strength (r^2) between the sentinel single nucleotide polymorphism from the GWAS and its flanking markers is demonstrated by the red (high) to white (low) colored bar. The recombination rate (cM/Mb; second *y* axis) is plotted in blue, according to HapMap[27] CEU.

risk allele showed increased activation during incongruent conditions, whereas homozygote carriers of the C-allele showed decreased activation (Figure 2b).

Discussion

The SNP rs11819869 is located in the gene *AMBRA1*, in a region of strong linkage disequilibrium (LD) that spans ~360 kb ($r^2 > 0.8$ based on HapMap Phase 2 CEU data,[27] Supplementary Figure S3). Other RefSeq genes in the LD region are *KIAA0652/ATG13*, *CHRM4*, *DGKZ*, *MDK*, *HARBI1*, *ARGAPH1* and *ZNF408*. In our initial GWAS, 11 of the 25 most significant SNPs were located in this region. Although this region also showed evidence for association in the GWAS study of the SGENE consortium,[3] it was not among the top findings selected for follow-up.[3]

AMBRA1 has a major role in the development of the nervous system and has been reported to be a member of the Autophagy Interaction Network.[28]

Table 2 Nine SNPs replicated in the follow-up study (Replication 1) with the same alleles as in the GWAS

SNP data			*Association data*												*Gene data*
			GWAS				*Replication 1*				*Combined analysis (GWAS + Replication 1)*				
			CMH (K = *2*)		*MAF*		*CMH* (K = *4*)		*MAF*		*CMH* (K = *6*)		*MAF*		
SNP	*Chromosome*	*Alleles*	P_{GWAS}	*OR*	*Patients* n = *1169*	*Controls* n = *3714*	P_{REPLI}	*OR*	*Patients* n = *2569*	*Controls* n = *4088*	P_{COMB}	*OR*	*Patients* n = *3738*	*Controls* n = *7802*	*Nearest gene or transcript*
rs11819869	11p11.2	T/C	4.71×10^{-6}	1.32	0.19	0.15	5.04×10^{-5}	1.20	0.18	0.16	3.89×10^{-9}	1.25	0.19	0.16	*AMBRA1*, intronic
rs7112229	11p11.2	T/C	1.03×10^{-5}	1.32	0.18	0.15	5.32×10^{-5}	1.21	0.17	0.14	7.38×10^{-9}	1.25	0.17	0.14	*AMBRA1*, intronic
rs7130141	11p11.2	T/C	6.87×10^{-6}	1.32	0.19	0.16	6.47×10^{-5}	1.20	0.18	0.16	6.96×10^{-9}	1.24	0.19	0.16	*AMBRA1*, intronic
rs12574668	11p11.2	A/C	9.71×10^{-6}	1.31	0.19	0.15	7.17×10^{-5}	1.20	0.18	0.16	1.02×10^{-8}	1.24	0.19	0.16	*AMBRA1*, intronic
rs4309482	18q21.2	G/A	3.40×10^{-4}	0.84	0.38	0.43	2.93×10^{-4}	0.88	0.39	0.41	9.68×10^{-7}	0.87	0.39	0.42	*CCDC68/TCF4*, intergenic
rs6465845	7q22.1	C/T	1.14×10^{-5}	1.28	0.25	0.21	0.0064	1.11	0.23	0.21	4.31×10^{-6}	1.17	0.24	0.21	*CUX1*, intronic
rs370760	7q22.1	G/A	9.31×10^{-5}	1.26	0.22	0.18	0.0084	1.12	0.19	0.18	2.65×10^{-5}	1.17	0.20	0.18	*CUX1*, intronic
rs404523	7q22.1	A/G	8.19×10^{-5}	1.26	0.22	0.18	0.0119	1.11	0.19	0.18	3.84×10^{-5}	1.17	0.20	0.18	*CUX1*, intronic
rs2717001	2p16.1	C/T	3.20×10^{-4}	0.84	0.38	0.42	0.0174	0.93	0.39	0.41	1.00×10^{-4}	0.89	0.39	0.42	*VRK2*, intronic

Abbreviations: Alleles, major/minor allele where minor allele refers to dbSNP build129 and was determined in patients and controls in each analysis; CMH, Cochran–Mantel–Haenszel test; MAF, minor allele frequency; GWAS, genome-wide association study; K, CMH's cluster variable; OR, odds ratio referring to minor allele; SNP, single nucleotide polymorphism.
CMH was one tailed for the replication analysis if OR was in the same direction as in the GWAS and two tailed for the meta-analysis; the table is sorted according to the results of the follow-up analysis (Replication 1).

Table 3 Association results for rs11819869 at all stages of the analysis: GWAS, Replication 1 and Replication 2

SNP	*Sample (Patients/Controls)*	*% Males*		*Test, P*	*OR*	*MA*	*MAF*	
		Pat	*Con*				*Pat*	*Con*
rs11819869	**GWAS (1169/3714)**			**CMH ($K=2$), 4.71×10^{-6}**	**1.32**			
	Germany	50.1	51.0	TREND, 0.008	1.31	T	0.19	0.15
	The Netherlands	76.5	50.1	TREND, 1.85×10^{-4}	1.34	T	0.20	0.16
	Replication 1 (2569/4088)			**CMH ($K=4$), 5.04×10^{-5}**	**1.20**			
	Germany (Bonn—Mannheim)	62.0	49.4	TREND, 0.012	1.24	T	0.18	0.15
	The Netherlands	69.7	41.3	TREND, 0.033	1.36	T	0.19	0.15
	Germany (Munich)	62.7	48.6	TREND, 0.038	1.15	T	0.17	0.15
	Denmark (Aarhus)	54.8	54.3	TREND, 0.016	1.20	T	0.20	0.17
	GWAS + Replication 1 (3738, 7802)			**CMH ($K=6$), 3.89×10^{-9}**	**1.25**			
	Replication 2 (4734/18472)			**META ($n=15$), 0.0029**	**1.11**			
	Denmark (Copenhagen)	58.6	58.1	LOGISTIC, 0.133	1.12	T	0.18	0.17
	England	76.3	54.5	LOGISTIC, 0.172	1.29	T	0.20	0.16
	Finland (Helsinki)	59.3	65.3	LOGISTIC, 0.256	1.26	T	0.13	0.11
	Finland (Kuusamo)	62.6	52.0	LOGISTIC, 0.423	*0.73*	T	0.08	0.11
	Iceland	64.5	49.9	LOGISTIC, 0.101	1.11	T	0.19	0.17
	Wales/UK (Cardiff)	67.8	49.2	LOGISTIC, 0.052	1.16	T	0.19	0.17
	Italy	57.1	56.2	LOGISTIC, 0.784	*0.91*	T	0.18	0.19
	Scotland	72.2	58.1	LOGISTIC, 0.211	1.09	T	0.18	0.16
	Denmark (Aarhus)	53.7	35.7	LOGISTIC, 0.698	*0.94*	T	0.16	0.17
	Poland	46.9	51.6	LOGISTIC, 0.098	1.19	T	0.18	0.16
	Belgium	68.3	43.7	LOGISTIC, 0.317	1.10	T	0.17	0.16
	Hungary	43.6	41.6	LOGISTIC, 0.354	1.07	T	0.20	0.19
	Russia	27.8	38.0	LOGISTIC, 0.172	1.13	T	0.17	0.16
	Sweden	62.7	62.0	LOGISTIC, 0.461	1.02	T	0.14	0.14
	Norway	59.2	50.4	LOGISTIC, 0.112	1.24	T	0.18	0.15

Abbreviations: Con, controls; CMH, Cochran–Mantel–Haenszel test; GWAS, genome-wide association study; LOGISTIC, Logistic regression; MAF, minor allele frequency; META, meta-analysis; SNP, single nucleotide polymorphism; TREND, Cochran–Armitage test; Pat, patients;
CMH was one tailed for Replication 1, and two tailed for GWAS and the combined analysis of GWAS plus Replication 1; TREND was one tailed when OR was in the same direction as in the GWAS for Replication 1 subsamples and two tailed for GWAS subsamples; LOGISTIC (additive effect) was one tailed when OR was in the same direction as in the GWAS for Replication 2 subsamples; META (random effects model) was two tailed; OR, odds ratio referring to the MA, *italic* when OR was in the opposite direction to GWAS.

In mouse models, AMBRA1 functional deficiency results in severe neural tube defects that are associated with impaired autophagy, accumulation of ubiquitinated proteins, unbalanced cell proliferation and excessive apoptotic cell death.[29,30] The gene is highly expressed in the most ventral part of the undifferentiated neural tube during embryogenesis.[30] Another gene in the chromosome 11 region of strong LD (*KIAA0652*/*ATG13*) is also a member of the Autophagy Interaction Network[28] and acts as a regulator of autophagy.[28] Together with the association findings for genetic variation in *TCF4* and *NRGN*,[3] these reports and our findings may be regarded as supportive evidence for the role of brain-development genes in the etiology of SCZ.

Although *AMBRA1* is an interesting functional candidate gene and includes the top associated SNP, (Figure 1b, Supplementary Figure S3) it is difficult to pinpoint the potential susceptibility gene on the basis of the genetic data alone due to the presence of strong LD in the associated region. Another very interesting gene that is covered by LD in this region is the *muscarinic acetylcholine receptor M4* gene (*CHRM4*). Neuropsychopharmacological and neuroimaging studies have produced strong evidence that muscarinic cholinergic receptors are involved in SCZ.[31] Studies of muscarinic receptor knockout mice have suggested that *CHRM4* has an impact on the homeostatic control of cholinergic activity and dopaminergic neurotransmission in mesolimbic and hippocampal brain regions.[32] Interestingly, the muscarinic agonist Xanomeline, which is selective for the CHRM1 and CHRM4 subtypes, exhibited functional dopamine antagonism and resulted in antipsychotic-like effects in rodent models predictive of antipsychotic behaviors.[32] Furthermore, a recent study proposed allosteric modulation of the muscarinic M_4 receptor as a potential approach to the treatment of SCZ.[33] Further information concerning the impact of *CHRM4* on the pharmaceutical treatment of SCZ is provided in the Supplementary Information.

Another gene of interest in the LD region of chromosome 11 (Figure 1b, Supplementary Figure S3) is the *diacylglycerol kinase zeta* gene (*DGKZ).*

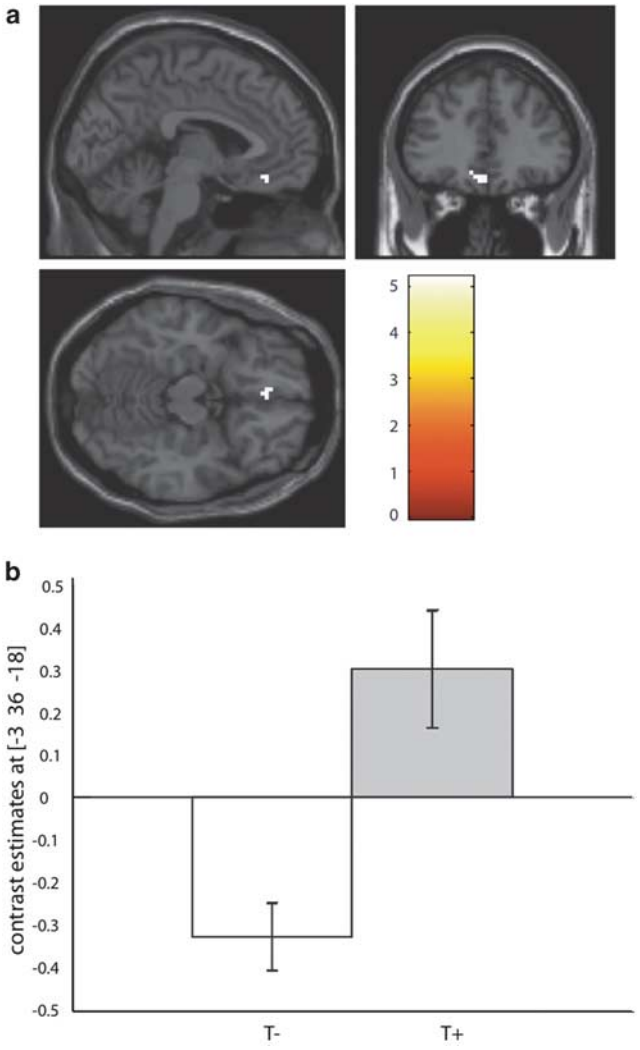

Figure 2 Effect of the rs11819869 genotype on brain activation during a flanker task (contrast incongruent trials > congruent trials). (**a**) The only cluster showing a significant group difference between carriers of at least one T allele and homozygote C allele carriers after correction for multiple testing ($P<0.05$, family wise error corrected for the entire brain and the number of different tasks tested). (**b**) Mean contrast estimates (+/− standard errors) for the significant cluster, reflecting an increased activation during incongruent trials for the T-allele carriers and during congruent trials for participants homozygous for the C allele.

There are a total of 10 diacylglycerol kinase enzymes (DGKs) (DGKalpha, DGKbeta, DGKgamma, DGKdelta, DGKepsilon, DGKzeta, DGKeta, DGKtheta, DGKiota and DGKkappa) and these metabolize 1,2,diacylglycerol to phosphatidic acid. Diacylglycerol kinases are central to a wide range of signal transduction pathways of potential relevance to neuropsychiatric disorders.[34] The diacylglycerol kinase eta (DGKH), a key protein in the lithium-sensitive phosphatidyl inositol pathway, has recently been implicated in the etiology of bipolar disorder.[35] A further member of this protein family, DGKbeta, has been reported to promote dendritic outgrowth and spine maturation in developing hippocampal neurons.[36] In addition, gene-wide evidence for association with SCZ has been reported for the gene that encodes for DGKiota (*DGKI*).[33] One independent study[3] found evidence for association between SCZ and a *DGK1* variant. In view of these findings, *DGKZ* may represent a further promising candidate gene for SCZ in the LD region of chromosome 11.

Last but not least, *Midkine* (*MDK*) is an interesting candidate gene in the region of our top finding. It has been shown that Mdk(−/−) mice exhibited a delayed hippocampal development with impaired working memory and increased anxiety.[37] In addition, midkine was found to accumulate in senile plaques in the hippocampus of patients with Alzheimer's disease.[38] Ohgake *et al*.[39] reported that Mdk(−/−) mice showed a significantly disrupted Prepulse inhibition (PPI) test when compared with Mdk(+/+) mice. This is interesting, because PPI has previously been shown to be a valuable tool for evaluating models or model organisms relevant to SCZ.[40] The PPI reduction was compensated when Mdk(−/−) mice were pre-treated with either haloperidol or clozapine (both known to reverse PPI deficits).[39] In view of their findings, Ohgake *et al*.[39] hypnotized that Mdk(−/−) mice might act as a putative animal model of SCZ.

As none of the 11 top SNPs, which are all located in introns of *AMBRA1,* have any known disease causing function, the SNAP tool[41] was used to test whether our top SNP (rs11819869) was in strong LD ($r^2>0.8$, based on HapMap[27] CEU Phase 2 and 3 data) with SNPs that are functional variants. We found that a *DGKZ* splice-site variant (rs2046768) was in strong LD with our top SNP ($r^2=0.935$ for HapMap[10] CEU data of Phase 2 release 22, Supplementary Figure S3). As this SNP was not present in our post QC data set, it was necessary to impute the information for the GWAS data set using the strategy described in the Supplementary Information Patients and Methods (rs2046768, $P_{IMPUT}=8.79\times10^{-5}$). Although the result for the functional variant itself lags behind the GWAS result for the top SNP (rs11819869, $P_{GWAS}=4.71\times10^{-6}$), we cannot excluded the possibility that this is the functionally relevant effect that was picked up by our top SNP in AMBRA1.

Irrespective of which specific gene in the region is tagged by the identified SNPs, the impact of the risk allele on brain function and SCZ can be tested using imaging genetics. This approach has high specificity when applied to risk genes for SCZ.[42] The stringent statistical approach, which combines Bonferroni correction over tasks with family-wise error correction over brain regions tested, provides strong protection against false positive findings.[42] The present results therefore indicate a regionally specific impact on the function of the subgenual cingulate during a cognitive control task. This represents a critical interface between emotion regulation and cognition that is structurally[43] and functionally[44] abnormal in SCZ and bipolar disorder. The present

findings therefore provide evidence that the identified risk allele is functional in a neural system of relevance to the disorder.

Our second best result was for rs4309482 ($P_{COMB} = 9.68 \times 10^{-7}$, OR = 0.87, Figure 1c), which is located near *CCDC68* and *TCF4* on chromosome 18. This SNP, unlike the SNPs in the chromosome 11 region, was included in the follow-up of the SGENE study and achieved a combined *P*-value of 7.1×10^{-5} (Supplementary Table S2 in Stefansson *et al.*[3]). However, as a substantial proportion of our combined case sample (and a smaller proportion of our control sample) was included in that study, our result for this SNP cannot be regarded as an independent replication.

In summary, the present study has identified a susceptibility region for SCZ on chromosome 11, which contains four excellent functional candidate genes: *AMBRA1, DGKZ*, *CHRM4* and *MDK*. In addition, we found evidence that the identified risk allele is functional in a neural system that is of relevance to the disorder. The aim of future studies will be to determine which of the genes in the region contribute to the risk for SCZ. *CHRM4* is of particular interest as it may lead to novel therapeutic interventions for SCZ.

Conflict of interest

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Molecular Psychiatry website (http://www.nature.com/mp)

Appendix

Genetic Risk and Outcome in Psychosis (GROUP Investigators)

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