Genome-Wide Association Study Implicates HLA-C*01:02 as a Risk Factor at the Major Histocompatibility Complex Locus in Schizophrenia

Irish Schizophrenia Genomics Consortium and the Wellcome Trust Case Control Consortium 2

Background: We performed a genome-wide association study (GWAS) to identify common risk variants for schizophrenia.

Methods: The discovery scan included 1606 patients and 1794 controls from Ireland, using 6,212,339 directly genotyped or imputed single nucleotide polymorphisms (SNPs). A subset of this sample (270 cases and 860 controls) was subsequently included in the Psychiatric GWAS Consortium-schizophrenia GWAS meta-analysis.

Results: One hundred eight SNPs were taken forward for replication in an independent sample of 13,195 cases and 31,021 control subjects. The most significant associations in discovery, corrected for genomic inflation, were \( rs204999, p \text{ combined } = 1.34 \times 10^{-9} \) and in combined samples \( rs2523722, p \text{ combined } = 2.88 \times 10^{-16} \) mapped to the major histocompatibility complex (MHC) region. We imputed classical human leukocyte antigen (HLA) alleles at the locus; the most significant finding was with HLA-C*01:02. This association was distinct from the top SNP signal. The HLA alleles DRB1*03:01 and B*08:01 were protective, replicating a previous study.

Conclusions: This study provides further support for involvement of MHC class I molecules in schizophrenia. We found evidence of association with previously reported risk alleles at the TCF4, VRK2, and ZNF804A loci.

Key Words: CACNA1I, genetics, genome-wide association study, HLA-C, major histocompatibility complex, polygene score, schizophrenia

Schizophrenia is a complex psychiatric disorder of substantial heritability \((h^2 \approx 0.8)\) characterized by hallucinations, delusions, disordered thinking, and cognitive deficits (1). The disorder poses a significant public health problem, as it affects approximately 0.8% of the population, causes considerable morbidity, and reduces average life expectancy by 20 to 25 years (2). The onset of illness is typically in early adulthood, but despite current treatments, the evolution of symptoms, severity, and course of the disorder are variable.

Understanding the genetic component of schizophrenia has been a major focus in the field, with the hope that this will aid development of more effective diagnostics and therapeutics. Recently, a number of genome-wide association studies (GWAS) have made encouraging progress in identifying a spectrum of common and rare genetic risk variants that contribute to schizophrenia susceptibility (3–11). They have found evidence for a number of susceptibility loci, including the major histocompatibility complex (MHC) region on chromosome 6p21-6p22. Although the MHC has been implicated in multiple schizophrenia GWAS (3,6,12), the signals reported cover a broad region across the different studies from 27.2 megabase (Mb) to 32.7 Mb.

Here, we report a schizophrenia GWAS conducted as part of the Wellcome Trust Case Control Consortium 2 (WTCCC2) study of 15 complex disorders and traits. The discovery dataset was made up of an Irish cohort of 1606 affected and 1794 unaffected population control subjects. We performed a logistic regression case-control analysis (see Methods and Materials) and selected regions for follow-up with single nucleotide polymorphisms (SNPs) that surpassed a significance level of \( p < 10^{-4} \). Replication data consisted of a large independent set of 13,195 cases and 31,021 control subjects. Imputations based on the 1000 Genomes (13) reference panel yielded information at over six million SNPs after quality control (QC). In addition, we present evidence for association at loci that had met criteria for genome-wide association in previously reported schizophrenia and bipolar disorder GWAS. Finally, we imputed classical human leukocyte antigen (HLA) alleles and performed association analyses with these alleles to further define the MHC association seen in the SNP data.

Methods and Materials

Discovery Subjects

Discovery Sample Cases. The case sample of 1908 individuals was recruited through community mental services and inpatient units in the Republic of Ireland and Northern Ireland following similar research protocols and with local ethics approval. All participants were interviewed using a structured clinical interview (Structured Clinical Interview for DSM-IV [14]; Schedule for Affective Disorders and Schizophrenia [Lifetime Version] [15]; or Schedule for Clinical Assessment in Neuropsychiatry [16]). Diagnosis of a major psychotic disorder was made by consensus, lifetime best estimate method using DSM-IV criteria with all available information (interview, family or staff report, and chart review). All cases were over 18 years of age, were of Irish origin (being of Irish parents and having all four grandparents born in Ireland or the United Kingdom), and had been screened to exclude substance-induced psychotic disorder or psychosis due to a general medical condition. The final diagnostic determination was made by one of the four lead clinicians on the project (A.C., T.O.N., K.S.K., D.W.). The final analysis included samples from 1606 participants whose SNP data passed quality control filters, detailed in Table S1 in Supplement 1, and met DSM-IV criteria for schizophrenia \((n = 1418)\), schizoaffective disorder \((n = 182)\), or schizophreniform disorder \((n = 6)\).

Discovery Sample Control Subjects. Control subjects \((n = 2048)\) were ascertained with written informed consent from the Irish GeneBank and represented blood donors from the Irish Blood Transfusion Service recruited in the Republic of Ireland. They met the same ethnicity criteria as cases but were not specifically
screened for psychiatric illness. Individuals taking regularly prescribed medication are excluded from blood donation in Ireland and donors are not financially remunerated. As the lifetime prevalence of schizophrenia is relatively low (<1%) and there was no obvious reason for individuals with schizophrenia to be overrepresented in the control subjects, the fraction of putative case individuals in the control collection was expected to be small. Following QC (Table S1 in Supplement 1), the control sample included 1794 participants.

Replication Subjects

The total replication sample included 13,195 cases and 31,021 control subjects. This was composed of nonoverlapping samples from the Psychiatric GWAS Consortium (PGC)-Schizophrenia consortium sample (9393 cases and 12,462 control subjects) and the SGENE+ consortium sample (1053 cases and 12,174 control subjects). It also included a WTCCC2 replication dataset (2749 cases from the United Kingdom and 6385 control subjects from the 1958 Birth Cohort and National Blood Service), genotyped on the Illumina Immunochip, which is referred to as the Immunochip replication sample.

Psychiatric GWAS Consortium Sample. The sample included individuals of European ancestry with a DSM-IV (or equivalent) diagnosis of schizophrenia or schizoaffective disorder. The full sample includes 9394 cases, which were collected with institutional review board approval at multiple centers in 17 individual cohorts representing 11 countries (Australia, Bulgaria, Denmark, Germany, Ireland, Netherlands, Norway, Portugal, Sweden, United Kingdom, and the United States of America). Control subjects consisted of 12,462 samples of European ancestry collected from the same countries. The PGC-Schizophrenia sample is described more fully elsewhere (9). A small number of Irish cases (n = 270) and control subjects (n = 860) overlapped with our discovery sample and were excluded from the replication analysis for this project. Due to the consortium nature of the PGC, the composite populations are genotyped on different platforms and are imputed to give a common set of SNPs. The SNPs for which we attempted replication are therefore not genotyped in all of the individual populations and are either made up of a subset of genotyped populations, with the remaining imputed, or are imputed in all populations.

SGENE+ Sample. Details of the SGENE+ sample have been published elsewhere (12). Part of the SGENE+ sample (identified in their paper as the SGENE-Bonn, Copenhagen, Munich, TOP3, and University of California, Los Angeles sites) had contributed to stage 1 of the PGC analysis and were excluded from the SGENE+ sample for replication purposes.

Immunochip Replication Sample. After quality control, this sample totaled 2749 cases collected in the United Kingdom and 6385 control subjects from 1958 Birth Cohort and National Blood Service cohorts. The control sample did not overlap with WTCCC1 control subjects included in the PGC sample. All cases were diagnosed with schizophrenia and were undergoing monitoring by the United Kingdom Clozaril Monitoring Service for clozapine therapy; full details on the control subjects are provided elsewhere (17). Samples were genotyped on the Illumina Immunochip platform at the Sanger Institute, United Kingdom. The Immunochip SNP chip was designed jointly by a number of groups, including the Wellcome Trust Case Control Consortium 2, and included 2400 SNPs selected from the top hits reported in individual schizophrenia GWAS (including from this study) and from the PGC meta-analysis of schizophrenia GWAS data. For the study presented here, the Immunochip data were used as a shallow replication cohort, where data at less than 100 SNPs were analyzed.

DNA Sample Preparation

For all participants in the discovery sample, DNA was extracted from whole blood using standard methods. Genomic DNA for all cases and control subjects was shipped to the Sanger Institute, United Kingdom, or the Broad Institute, United States. Similar procedures for DNA quality control were applied at both sites and are detailed elsewhere (3,17).

Genotype Calling

All discovery samples were genotyped using the Affymetrix 6.0 platform totaling 893,634 autosomal SNPs, either at the Affymetrix (Santa Clara, California) or Broad Institute (Cambridge, Massachusetts) laboratories. A subset of samples (n = 37) were genotyped at both sites and successfully identified as duplicates as part of the QC procedure. The final sample included 1606 cases (1302 were genotyped at Affymetrix) and 1794 control subjects (1001 were genotyped at Affymetrix). For all samples passing laboratory quality control, raw intensities (from the .CEL files) were renormalized within collections using CelQuantileNorm (http://outmodedbonsai.sourceforge.net/). These normalized intensities were used to call genotypes with an updated version of the Chiamo software (https://mathgen.stats.ox.ac.uk/genetics_software/chiamo/chiamo.html), adapted for Affymetrix 6.0 SNP data (17,18).

Quality Control

Full details of the quality control methods employed in the Wellcome Trust Case Control Consortium 2 have been published elsewhere (17,19) and additional details are provided in Supplemental Methods in Supplement 1. After applying all of the quality control criteria, from a total of 3956 samples, 566 samples were excluded, leaving 1606 cases and 1794 control subjects for further analysis (Table S1 in Supplement 1). After quality control of the SNP data (Supplemental Methods in Supplement 1), 696,951 directly assayed SNPs were available for analysis.

The genomic inflation statistic (λ) was 1.10 (Figure S1 in Supplement 1). This is slightly higher than would be ideal for a genome-wide study (20), and several methods were applied to try and understand any underlying structure in the data. Using a label corresponding to the genotyping center as a covariate gave a similar λ to just using the data that was genotyped at Affymetrix (λ = 1.095, λ = 1.09, respectively). Including the first two principal components as covariates also had no effect on reducing the value of λ (λ = 1.095). None of these methods substantially reduced the genomic inflation, suggesting there could be an underlying polygenic component to the inflation (21), as previous studies have found for schizophrenia (3). Therefore, no covariates were used for the following analyses unless explicitly described. Genomic control was not applied to the results of the primary analysis, but for completeness, Table S4 in Supplement 1 shows p values after applying genomic control at SNPs that were taken through to replication. Visual inspection of the Manhattan plot (Figure 1) suggested that the filtered data provide a good basis for association analyses.

Imputation

SNP Imputation. Haplotype phasing and imputation was performed using IMPUTE2 (22). Only samples that had passed the quality control filtering were imputed and before imputation SNPs were subjected to additional quality control to remove any that could adversely affect the quality of the imputation (Supplement 1). Imputation was performed using the HapMap2 and 3 and the 1000 Genomes reference panels; further details are in Supplement 1.

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HA Imputation. Human leukocyte antigen alleles were imputed using an updated version of HLA*IMP (23) (Supplement 1).

Statistical Methods

We report p-values from 1-df Cochran-Armitage tests for trend as implemented in the software SNPTTEST (https://mathgen.stats.ox.ac.uk/genetics_software/snptest/snptest.html). A parallel analysis using the Cochran-Armitage trend test in PLINK (24) gave near identical results (data not shown). We also performed 2-df genotypic tests to verify that none of our associations showed significant deviation from a multiplicative model and two marker logistic regressions to test for interactions between associated markers. Effect size (odds ratio [OR]) estimates represent per-allele increase of risk in a multiplicative model.

Polyscore Analyses. We performed a direct replication experiment of the polyscore analysis performed by the International Schizophrenia Consortium (ISC) (3). The principle, described in that paper, is that multiple genetic variants of very small effect (OR < 1.05) may have only a small chance of showing individual significance in a GWAS, but en masse they could contribute a substantial amount of risk. To be as close as possible to the original study, we chose the same 74,062 SNPs as in the original paper and used the same p value thresholds. Because of overlapping samples between this study and the International Schizophrenia Consortium, this analysis was restricted to a sample set of 1310 cases and 1023 control subjects.

Results

The top association in this GWAS was in the MHC region (rs2049999, p = 2.77 × 10^{-8}) (Figures 1 and 2). This SNP is 63 kilobase upstream from, and in weak linkage disequilibrium (1000 Genomes CEU data R^2 = .23) with, the signal reported by Stefansson et al. (12) (rs3131296). After accounting for rs204999, rs3131296 does not add any further signal in our dataset (p = .816). The MHC was the only region in the discovery GWAS with SNPs that had p < 10^{-7}. Association analysis was also carried out on the X chromosome, but following quality control, no SNPs were significant at p < 10^{-4} (Supplement 1).

In total, we selected 79 regions for follow-up, where the most significant SNP had p < 10^{-4}, and in the case of directly genotyped SNPs, the cluster plots met quality control standards. One or more SNPs were chosen for replication in each region, depending on the local pattern of linkage disequilibrium and the availability of SNPs in the replication data (108 SNPs in total). Seven SNPs spanning the association signal from 30.3 Mb to 34.1 Mb in the MHC region were included in the replication phase of the study. Of the other regions, 56 were identified from the genotyped scan (66 SNPs), 10 from the HapMap imputation (11 SNPs), and 12 from the 1000 Genomes imputation (24 SNPs). Replication was carried out in silico using the PGC and SGENE+ samples and SNPs were genotyped directly in the Immunochip replication samples, giving a total of 13,195 cases and 31,021 control subjects. Data were not available for all the SNPs on all samples due to differences in imputation strategies and genotyping platforms (see Methods and Materials).

Of note, a small proportion of the Irish cases in the discovery sample (n = 270) and Irish control subjects (n = 860) were included in the PGC-schizophrenia GWAS meta-analysis. We included these samples in the discovery phase for two reasons. First, this analysis had additional genotype information provided by 1000 Genomes imputation (6,212,339 directly genotyped and imputed SNPs) and analysis of the X chromosome when compared with the PGC analysis (1,252,901 autosomal SNPs). Second, existing data suggest genetic heterogeneity (e.g., at the MHC) may be important in schizophrenia etiology, indicating a value in reporting the results of the complete Irish sample. We performed a sensitivity analysis excluding these overlapping cases and control subjects from our discovery GWAS scan. This reduced the number of regions meeting criteria for follow-up (from 79 to 48) but did not materially affect the study outcome. We provide data on the SNP findings for the full and restricted discovery datasets at all loci selected for follow-up in Table S4 in Supplement 1. Expanding the sample to include additional cases with a broader psychotic disorder phenotype (n = 2049 cases, including mostly bipolar disorder cases) did not significantly alter the results.

Three of the seven SNPs tested in the MHC reached a p < 5 × 10^{-8} in a fixed effects meta-analysis across the four follow-up cohorts (p combined) (Table 1). These three SNPs showed evidence of association in two of the three replication datasets; no replication evidence (p < .05) at the MHC was found in the SGENE+ data (Figure S2 in Supplement 1). These MHC SNPs were the only replication results to meet standard significance criteria (p < .05) when correcting for the number of replication SNPs tested.

Six other regions showed some evidence for association in the replication dataset (Table 1). In each case, the direction of effect was consistent in the majority of replication datasets, although the combined p values were >5 × 10^{-6} (Table 1, Figure 2; Figure S2 in Supplement 1). These included two variants on chromosome 22, rs9611198 (p combined = 7.56 × 10^{-6}) and rs9607658 (p combined = 1.11 × 10^{-5}), at the calcium channel gene CACNA1I. This gene is of potential interest, as its protein product interacts with the
Figure 2. Regional association plots of each of the loci described in Table 2. The $-\log_{10}(p)$ values for the multiplicative model single nucleotide polymorphism (SNP) association in SNPTEST are shown on the upper part of the plot. Single nucleotide polymorphisms are colored based on their $R^2$ calculated in the 58C control data. Where the SNP was chosen from the 1000 Genome imputation results, the plot includes the imputed SNPs. Circles represent the directly genotyped SNPs, and triangles represent the imputed SNPs. The bottom part of each plot shows the fine scale recombination rate estimated from individuals in the HapMap panel, and genes are marked by horizontal blue lines. Chr, chromosome; cM, centimorgan; Mb, megabase.
Table 1. Loci with Evidence for Association with Schizophrenia Identified in this Study

<table>
<thead>
<tr>
<th>Candidate Locus</th>
<th>Risk Allele</th>
<th>Case</th>
<th>Control</th>
<th>P-Value Discovery*</th>
<th>P-Value Replication*</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1p21 rs987913</td>
<td>G</td>
<td>4</td>
<td>10</td>
<td>0.011</td>
<td>0.078 (1.10–1.32)</td>
<td>1.20 (1.10–1.32)</td>
</tr>
<tr>
<td>3p14 rs38285819</td>
<td>G</td>
<td>5</td>
<td>10</td>
<td>0.011</td>
<td>0.078 (1.10–1.32)</td>
<td>1.20 (1.10–1.32)</td>
</tr>
<tr>
<td>3q13 rs7618341</td>
<td>A</td>
<td>10</td>
<td>10</td>
<td>0.033</td>
<td>0.028 (1.10–1.32)</td>
<td>1.20 (1.10–1.32)</td>
</tr>
<tr>
<td>5p15 rs12595425</td>
<td>G</td>
<td>10</td>
<td>10</td>
<td>0.011</td>
<td>0.078 (1.10–1.32)</td>
<td>1.20 (1.10–1.32)</td>
</tr>
</tbody>
</table>

*All values shown are two-sided, and (−) denotes that the direction of effect is opposite to that in the discovery analysis.
HLA-DQB1, and HLA-DRB1 (see Supplemental Methods in Supplement 1).

An association test for each allele was performed in the Irish sample (Table S5 in Supplement 1), and HLA-C*01:02 showed the strongest evidence of association with schizophrenia (p = 3.83 × 10^{-4}), where presence of HLA-C*01:02 was associated with increased risk of schizophrenia [OR (95%CI) = 1.65 [1.25–2.18]]. HLA-C*01:02 was found at a frequency of 4.1% in cases and 2.5% in control subjects and was imputed with high specificity and sensitivity (see Supplemental Methods in Supplement 1). This allele was also associated with risk of schizophrenia in the Immunochip replication data, where HLA imputation was available for 2690 cases and 6279 control subjects (p = .045, OR [95% CI] = 1.19 [1.01–1.41]).

When conditioning on HLA-C*01:02, the top SNP from the discovery scan, rs204999, was still the most significant SNP (p = 2.51 × 10^{-7}), and similarly, when conditioning on rs204999, HLA-C*01:02 was still significant (p = 2.82 × 10^{-3}). This suggests that both signals may be important in this dataset.

Stefansson et al. (12) found that SNP haplotypes tagging DRB1*03 and HLA-B*08 were protective for schizophrenia. In this study, we replicated these findings at the imputed HLA alleles directly rather than at tagging SNP haplotypes: HLA-B*08:01 p = .024 and DRB1*03:01 p = .068 (one-sided p values shown). Both of these HLA alleles were imputed with high specificity and sensitivity (see Supplemental Methods in Supplement 1). DRB1*03:01 and B*08:01 were in high linkage disequilibrium (LD) (R^2 = .61) and from these data it appears that these two HLA alleles were tagging the same signal.

Taking the large set of autosomal score alleles (n = 74,062) from the ISC dataset, we investigated the aggregate risk scores for individuals in the WTCCC2 schizophrenia sample. We examined increasingly liberal p value thresholds (PT) from PT < .01 to PT < .7. We identified higher mean score in cases compared with control subjects in the WTCCC2 dataset. The ISC-derived score was highly associated with disease at PT < .5, as has previously been reported in other schizophrenia datasets (PGC) (36). In our sample, the most significant PT finding was at PT < .01 to PT < .15 (Figure S4 in Supplement 1).

Discussion

We report a schizophrenia GWAS in a sample of 1606 cases and 1794 control subjects. We found strong support for association at three SNPs in the MHC, including the lead SNP in the discovery data rs204999. Multiple SNPs in the MHC region have been identified in previous schizophrenia GWAS (3,6,12). Conditional analysis of the associated SNPs suggests that in this data the signal is being driven by association with rs204999, although there is evidence of association with other SNPs at the locus. Previous work to pinpoint HLA alleles that could be responsible for the signal in the MHC have had varied success. Comparison of association with HLA alleles across studies is complicated by differences in the methodology used to call alleles, as well as differences in the populations being studied. In this study, the strongest HLA allele association was found with HLA-C*01:02, and this was separate from the top SNP signal. We also replicated association with two protective HLA alleles, HLA-B*08:01 and DRB1*03:01, which had previously been identified by Stefansson et al. (5). Our analysis suggests that these alleles tag the same association signal.

There is growing awareness of the important role that MHC genes play in nervous system development and function (37). Major histocompatibility complex class I molecules are expressed in the brain and function both presynaptically and postsynaptically in forming and regulating cortical connectivity across brain circuitry (38). Our study identifies risk and protective HLA alleles for schizo...

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phenia, which may be particularly relevant because of autoimmune or infectious risk factors in this population.

Because of extensive linkage disequilibrium, localizing signals within the MHC is extremely challenging, as evident from Figure 2. The SNP rs204999 is in strong LD with seven other SNPs ($R^2 = 0.66$–0.68; rs3130284, rs3134608, rs3130347, rs3134945, rs3096967, rs3139497, rs3132965), which span a region from 32.22 Mb to 32.26 Mb containing PPT2, PRT1, EGF18, AGPAT1, and RNFS. None of these genes feature in the pre-GWAS schizophrenia genetics literature and there is no strong in silico information available to suggest a functional role for rs204999 in the regulation of these genes or others. Single nucleotide polymorphism rs2523722 is at 30.27 Mb on chromosome 6 and is located in an intron of TRIM26. This variant is in complete LD ($R^2 = 1$) with 11 other SNPs within a 10 kilobase region, all of which map to TRIM26. Nine of these SNPs are intronic, one (rs2523721) is a coding silent SNP, and one (rs971570) is located in the 5' untranslated region. There is no prior evidence of association between TRIM26 and schizophrenia and the gene is a member of a tripartite motif family of unknown function.

We performed a polygene score analysis using the large set of autosomal score alleles identified in the previous ISC schizophrenia study. The proportion of variance explained (estimated by Nagelkerke’s $R^2$) in the original analysis was 3% to 4% and was 6% in this sample, adding support to the hypothesis that a substantial fraction of schizophrenia heritability is accounted for by a polygenic component. In this study, we attempted replication at 79 regions and identified nominal significant evidence of association ($p < 0.05$) with the same risk allele at 17 regions; this is significantly more than expected ($p = 3.09 \times 10^{-17}$). Larger studies are required to confirm which of these regions harbor schizophrenia susceptibility variants. We identified suggestive evidence of association at loci that could implicate three novel genes in schizophrenia susceptibility (IMMP2L, PAK6, and CACNA1I2). Two of these loci were identified from imputation based on reference panels from the 1000 Genomes data, suggesting the inclusion of the imputation data was a useful addition to this study. Based on the experience for other common disorders, further investigation of these loci in a larger meta-analysis of GWAS datasets may be warranted.

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