Journal of Psychiatric Research 64 (2015) 67–73

Contents lists available at ScienceDirect

Journal of Psychiatric Research

journal homepage: www.elsevier.com/locate/psychires

Common variants of *IRF3* conferring risk of schizophrenia

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ARTICLE INFO

Article history:
Received 27 November 2014
Received in revised form
12 March 2015
Accepted 13 March 2015

Keywords:
Schizophrenia
Association
eQTL
IRF3
Expression

ABSTRACT

Schizophrenia is a brain disorder with high heritability. Recent studies have implicated genes involved in the immune response pathway in the pathogenesis of schizophrenia. Interferon regulatory factor 3 (*IRF3*), a virus-immune-related gene, activates the transcription of several interferon-induced genes, and functionally interacts with several schizophrenia susceptibility genes. To test whether *IRF3* is a schizophrenia susceptibility gene, we analyzed the associations of its SNPs with schizophrenia in independent population samples as well as reported data from expression quantitative trait loci (eQTL) in healthy individuals. We observed multiple independent SNPs in *IRF3* showing nominally significant associations with schizophrenia ($P < 0.05$); more intriguingly, a SNP (rs11880923), which is significantly correlated with *IRF3* expression in independent samples ($P < 0.05$), is also consistently associated with schizophrenia across different cohorts and in combined samples (odds ratio = 1.075, $P_{meta} = 2.08 \times 10^{-5}$), especially in Caucasians (odds ratio = 1.078, $P_{meta} = 2.46 \times 10^{-5}$). These results suggested that *IRF3* is likely a risk gene for schizophrenia, at least in Caucasians. Although the clinical associations of *IRF3* with diagnosis did not achieve genome-wide level of statistical significance, the observed odds ratio is comparable with other susceptibility loci identified through large-scale genetic association studies on schizophrenia, which could be regarded simply as small but detectable effects.

1. Introduction

Schizophrenia is one of the most severe psychiatric disorders with worldwide lifetime prevalence approaching 1%, and characterized by psychotic features (delusions and hallucinations), disorganization, dysfunction in normal affective responses, and altered cognitive functions (Andreasen, 1995). Previous studies have implicated schizophrenia as an illness involved by interactions of one or more environmental insults with predisposing genetic susceptibility (Cannon et al., 2003; Caspi and Moffitt, 2006; Clarke et al., 2009). Among these environmental hazards, viral infection is one of the most widely accepted factors that could increase risk of future development of schizophrenia.

Viral infections produce considerable gene expression changes as they trigger immune defenses through type I interferons (IFNs) and the mobilization of transcription factors of the signal transducer and activator of transcription (STAT) and interferon regulatory factor (IRF) families (de Veer et al., 2001; Stark et al., 1998).

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http://dx.doi.org/10.1016/j.jpsychires.2015.03.008
0022-3956/

Altered expressions of IRF genes in brains have been implicated to contribute to disrupted brain circuit development, maturation and function and result in behavioral deficits that overlap with those seen in schizophrenia and major depression (Hurlock, 2001; Schaefer et al., 2002a, 2002b). However, sensitivity to environmental stressors like viral infections shows substantial inter-individual variation, and at least part of this variation may be genetically determined and/or involved with gene–environment interactions.

The interferon regulatory factor 3 gene (*IRF3*), located on chromosome 19q13, a genomic region possibly harbors risk genes for psychiatric disorders, i.e., *APOE*, a risk gene for schizophrenia and Alzheimer's disease (Harold et al., 2009; Lambert et al., 2009; Liu et al., 2003; Seshadri et al., 2010). *IRF3* plays an important role in the innate immune system's response to viral infection (Collins et al., 2004), and the protein encoded by *IRF3* is found in an inactive cytoplasmic form that upon serine/threonine phosphorylation forms a complex with *CREBBP*, which can translocate to the nucleus and activate transcription of interferons alpha and beta, as well as other interferon-induced genes (Juang et al., 1998; Lee et al., 2014; Prinarakis et al., 2008).

Thus, *IRF3* is likely a susceptibility gene for schizophrenia based on these lines of epidemiologic and etiologic evidence. Interestingly, a known and predicted protein–protein interactions database (STRING, http://string-db.org/) showed that *IRF3* interacts with many other schizophrenia susceptibility genes (Franceschini et al., 2013), such as *AKT1*, *CREB1*, *ESR1*, and *TP53* (Fig. S1). This observation encouraged our speculation because of recent evidence about protein–protein interactions among schizophrenia risk genes (Luo et al., 2014a; Yu et al., 2014).

Here, we attempt to characterize the genetic contributions of common variants within *IRF3* to schizophrenia susceptibility in independent samples as well as to explore the potential effects on gene expression. The discovery stage involved data from a large screening schizophrenia case–control sample and two independent cohorts containing expression quantitative trait loci (eQTL) data; the replication step includes a variety of independent replications on both clinical and eQTL associations. Our results indicated that *IRF3* is likely a schizophrenia susceptibility gene.

2. Materials and methods

2.1. Case-control and family-based samples

We used the case–control subjects from the Schizophrenia Psychiatric Genomics Consortium (PGC) as our screening sample (Ripke et al., 2013). The schizophrenia PGC performed a large-scale meta-analysis by combining GWAS data in 13,833 schizophrenia cases and 18,310 controls (PGC1). All of the subjects were of European ancestry. Detailed information about sample description, including diagnostic assessments, genotyping, quality control and statistical analysis can be found in the original publication (Ripke et al., 2013).

For replication analysis, we recruited two independent schizophrenia case–control samples and five family-based samples from different populations. Detailed information on each sample, including diagnostic assessment, genotyping, and quality control has been reported previously (Aberg et al., 2013; Alkelai et al., 2011, 2012; Lencz et al., 2013; Zhang et al., 2014). In brief, the seven replication samples are: (1) the Jewish case–control sample: this sample contained 904 schizophrenia cases and 1640 controls (Lencz et al., 2013). (2) The Chinese case–control sample: this sample consisted of 480 schizophrenia cases and 479 controls (Zhang et al., 2014). (3) The Jewish-Israeli sample: this sample comprised 107 schizophrenia families with a total of 331 individuals (Alkelai et al., 2012). (4) The Arab-Israeli sample: this sample contained 58 schizophrenia families with a total of 198 subjects (Alkelai et al., 2011). (5) The European sample: this sample comprised 794 families with 2740 individuals (Aberg et al., 2013). (6) The African sample: this sample consisted of 438 families with 1262 individuals (Aberg et al., 2013). (7) The Asian sample: this sample contained 579 families with 2296 subjects (Aberg et al., 2013). All replication samples showed no overlap with our screening PGC1 samples. In total, 15,217 schizophrenia cases, 20,429 controls and 1976 schizophrenia families were included in the analysis. All studies were conducted under the appropriate ethical approvals, and written informed consent was obtained from all subjects.

2.2. Healthy subjects for expression quantitative loci (eQTL) analysis

To identify the potential eQTL single-nucleotide polymorphisms (SNPs) for *IRF3*, we used two well-characterized gene expression databases. The first expression database is BrainCloud (Colantuoni et al., 2011) (http://braincloud.jhmi.edu/). The BrainCloud is comprised of 261 postmortem dorsolateral prefrontal cortex (DLPFC) of normal subjects, including 113 Caucasian subjects and 148 African American individuals across the lifespan. The raw genotype data was extracted from BrainCloud, and the expression data and demographic information such as RIN, race, sex, and age were also obtained. The samples were initially divided into two groups (prenatal and postnatal), and the postnatal samples were further divided according to their ethnicities (Caucasian and African American). The statistical analysis was conducted using linear regression, with RIN, sex, and age as covariates, and race was also included when analyzing prenatal samples.

The second database is from Genevar (Yang et al., 2010) (http://www.sanger.ac.uk/resources/software/genevar/). Among Genevar (there are several datasets in Genevar), we used the European data set (CEU, Caucasians living in Utah USA, a total of 109 subjects) from Stranger et al.'s study (2012), which correlated genome-wide gene expression in lymphoblastoid cell lines with SNPs located in the region *cis* to the genes. The mRNA quantification and correlation between expression level and genotype can be found in the original study (Stranger et al., 2012).

2.3. SNP selection and genotyping

SNP selection was based on three criteria. First, tagging SNPs. The whole SNPs within *IRF3* genomic region (chr19: 50152826-50179132) were downloaded from Caucasians in 1000-Human-Genome and the Haploview program (version 4.1, Broad Institute of MIT and Harvard, Cambridge, MA) was applied to test the linkage disequilibrium (LD) between paired SNPs, to define the haplotype blocks and to select the tagging SNPs using the r^2 confidence interval (CI) algorithm (Barrett et al., 2005). Second, eQTL SNPs. By utilizing mRNA expression data from BrainCloud, we screened potential *cis* SNPs (a total of 11 SNPs in this region were available in BrainCloud) within 50 kb to *IRF3* and analyzed their associations with *IRF3* expression, and significant eQTL SNPs ($P < 0.05$) in both Caucasians and African Americans samples were chosen (Colantuoni et al., 2011). Third, potential functional SNPs. These SNPs might affect protein structure, mRNA expression and alternative splicing of the gene, such as non-synonymous SNPs and 5′-UTR SNPs. In total, eleven SNPs were selected for screening in the PGC1 sample, and top significant SNPs were further analyzed in additional samples. The LD map of the eleven SNPs in Caucasians is shown in Fig. S2 and the SNP information is shown in Table 1. For the genotyping in replication samples, we used the SNaPshot

Table 1
Associations of *IRF3* tagging, functional and eQTL SNPs with schizophrenia in the PGC1 GWAS data.

CHR	SNP	POS	Allele 1	Frequency of allele 1	*P*-value	OR	95% CI	SNP Annotation
19	rs11880923	50122078	T	0.6162	0.00188	1.063	1.023–1.105	eQTL
19	rs7259683	50153332	T	0.6125	0.00242	1.065	1.023–1.109	eQTL
19	rs10415600	50158913	A	0.3710	0.00217	0.939	0.902–0.978	tagging
19	rs61743199	50161091	A	0.9703	0.924	1.004	0.923–1.093	tagging
19	rs35272206	50162221	T	0.0540	0.00342	1.092	1.030–1.158	tagging
19	rs7251	50162909	C	0.5549	0.00380	1.058	1.018–1.099	missense
19	rs10415576	50164390	T	0.4808	0.00457	1.055	1.017–1.095	tagging
19	rs2230666	50166463	A	0.0114	0.796	1.046	0.743–1.473	splicing
19	rs2304207	50167726	C	0.8884	0.0313	1.056	1.005–1.110	tagging
19	rs3204440	50168940	A	0.9575	0.643	1.026	0.921–1.143	5′-UTR
19	rs881785	50173291	C	0.7283	0.0531	1.059	1.000–1.122	tagging

CHR - chromosome; POS – position in genomic region; OR – odds ratio; 95% CI – 95% confidence interval.

method in our Han Chinese case–control sample, and in others samples Illumina based assays were used.

2.4. Statistical analysis

Association *p*-values and allele-specific odds ratios (ORs) for each individual sample were calculated by a logistic regression model with an additive effect (cases-control samples) or family-based analysis (family-based samples). Meta-analyses were then conducted based on Z-scores by combining data from both case–control and family-based samples in the R package (Meta module) using the Mantel-Haenszel method under the fixed effects model, and the combined *p*-values and ORs were generated. Before pooling, we performed Cochran's (Q) λ^2 test of heterogeneity to ensure that each group of studies was suitable for meta-analysis. We used a forest plot to graphically present the individual ORs and their 95% confidence intervals, i.e., each sample was represented by a square in the forest plot. All protocols and methods used in this study were approved by the institutional review board of Kunming Institute of Zoology, Chinese Academy of Sciences and adhere to all relevant national and international regulations.

3. Results

3.1. Identification of eQTL SNPs for IRF3

In BrainCloud of DLPFC samples, we screened the potential *cis* SNPs within 50 kb to *IRF3* and analyzed their associations with mRNA expression. Among the 11 SNPs available in BrainCloud, we identified two SNPs (rs11880923 and rs7259683) significantly associated with *IRF3* expression in both Caucasian and African American postnatal samples ($P < 0.05$, Fig. 1), but they were not significant in prenatal samples ($P > 0.5$, Fig. S3), which is not unexpected given its small sample size (N = 36). No other *cis* SNPs were associated with *IRF3* expression in both populations ($P > 0.05$). For replication analysis using the data of Stranger et al.'s (2012) study from Genevar, the significant associations between these two SNPs and *IRF3* expression were successfully validated in healthy Caucasians ($P = 0.0177$ for rs11880923 and $p = 0.0093$ for rs7259683, Fig. S4), and the direction of effects was the same. We also assessed the impacts of these two SNPs on other adjacent genes expression in BrainCloud samples, but none of them showed significance in both Caucasians and African Americans (Table S1).

Genomic coordination and linkage structure analyses showed that rs7259683 and rs11880923 are located about 9.5 kb and 40.7 kb downstream of *IRF3*, respectively. These two SNPs were in moderate LD in BrainCloud Caucasian samples ($r^2 = 0.68$) and 1000-Human-Genome Caucasians ($r^2 = 0.45$), but the LD was quite low in Africans ($r^2 = 0.05$ in BrainCloud and $r^2 = 0.00$ in 1000-Human-Genome) and in Han Chinese ($r^2 = 0.30$, Fig. S5). Of note, the allele frequencies of both SNPs are apparently different between Caucasians (CAUC) and African Americans (AA) (CAUC/AA: 0.294/0.647 for rs11880923 [C], and 0.252/0.714 for rs7259683 [C]).

Taken together, the eQTL analyses suggested that these two SNPs may be associated with *IRF3* expression, but whether they have functional effects themselves or are because of shared LD with causal variants need to be further investigated.

3.2. IRF3 SNPs are associated with schizophrenia in screening PGC1 sample

We screened the above eleven SNPs, including 2 eQTL, 6 tagging and 3 functional SNPs (Table 1), in the PGC1 sample. Among these SNPs, seven SNPs showed nominally significant associations and one SNP had marginal significance. The LD analysis indicated that most of these SNPs were in low to moderate LD in Caucasians (Fig. S2), implying that they unlikely shared the same association signal.

Notably, the eQTL SNP rs11880923 showed the strongest association with schizophrenia ($P = 0.00188$); another eQTL SNP rs7259683 also showed significant association ($P = 0.00242$). Additionally, a non-synonymous SNP rs7251 in *IRF3*, which was in moderate LD with rs11880923 ($r^2 = 0.52$) and rs7259683 ($r^2 = 0.63$) in Caucasians (Fig. S2), was also associated with schizophrenia ($P = 0.00380$). However, if we performed a stringent Bonferroni correction according to the number of tested SNPs (N = 11), some SNPs cannot remain significant after the correction for multiple testing ($P > 0.05/11$). None of these SNPs showed genome-wide significant associations, suggesting that they may show small but detectable effects on schizophrenia.

3.3. Replication and meta-analysis of IRF3 SNPs with schizophrenia

To further confirm the associations, we chose SNPs rs11880923 and rs7259683, which showed associations with both *IRF3* expression and schizophrenia, and conducted a series of replication studies in independent case–control and family-based samples.

For rs11880923, although the association with schizophrenia did not achieve significance level ($P = 0.05$) in any case–control replication cohort, it did show a trend of association in both samples, and the effect size (shown as OR) in replication samples was even higher than in our discovery sample (1.089 and 1.170 versus 1.063). We speculated that the non-significant result was likely due to the limited statistical power caused by small sample size. In the family-based samples, rs11880923 was significantly associated with schizophrenia in the Caucasian sample ($P = 0.0064$) and showed marginal significance in Jewish family-based sample ($P = 0.0544$). Meta-analysis of all replication samples yielded a

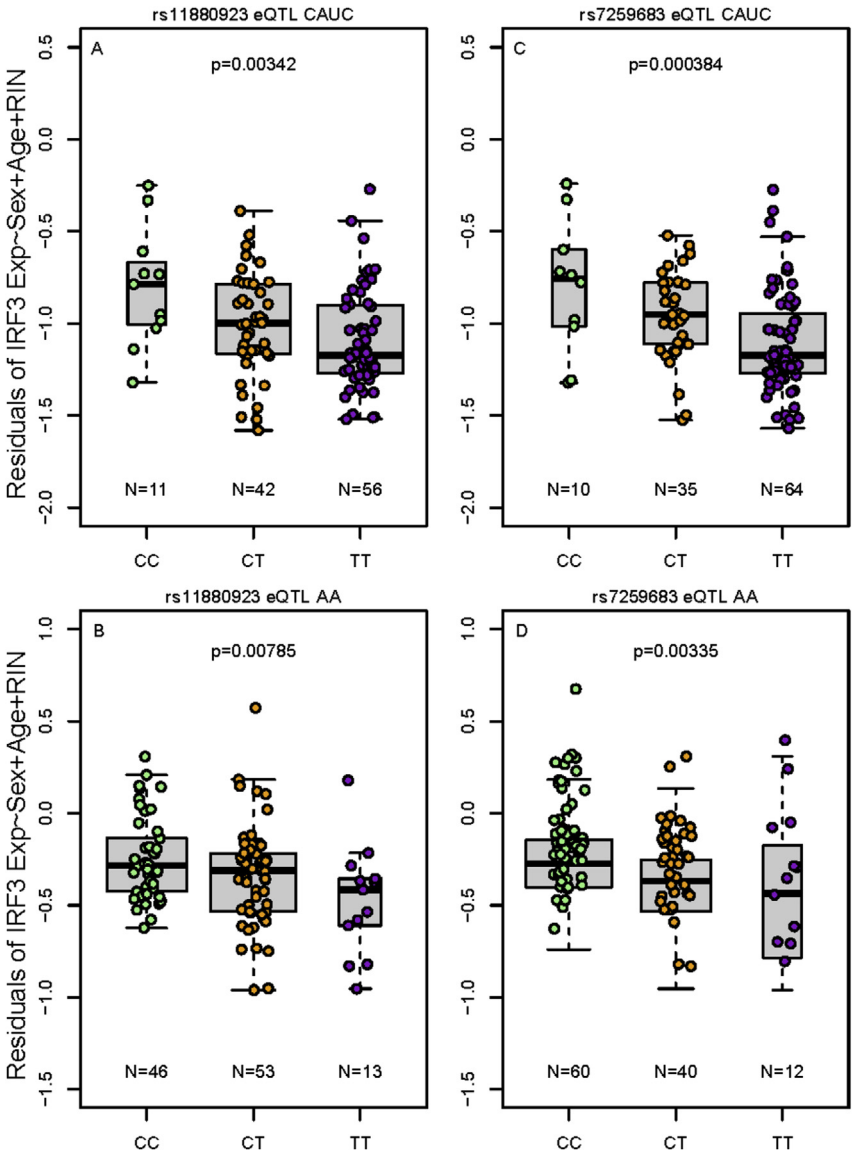

Fig. 1. The associations of rs11880923 and rs7259683 with *IRF3* expression in adult brains of the BrainCloud dataset. A, rs11880923 in Caucasians (CAUC). B, rs11880923 in African Americans (AA). C, rs7259683 in Caucasians. D, rs7259683 in African Americans.

significant association of rs11880923 with schizophrenia ($P = 0.0019$, OR = 1.109, Table 2). When the discovery and replication samples were combined together, the associations were further strengthened ($P = 0.000021$, OR = 1.075, Table 2), and even remained significant after Bonferroni correction according to the number (N = 11) of tested SNPs (corrected $P = 0.00023$). We used the fixed effect model for meta-analysis because there was no significant heterogeneity among either replication samples

Table 2
Associations of *IRF3* rs11880923 [T] with schizophrenia in multiple samples.

Sample	Sample size (case/control)	*P*-value	OR	95% CI	Data source
Discovery sample					
PGC1	13,833/18,310	0.00188	1.063	1.023–1.105	(Ripke et al., 2013)
Case-Control replication samples					
Jewish 01	904/1640	0.125	1.089	0.977–1.214	(Lencz et al., 2013)
Chinese	480/479	0.166	1.170	0.936–1.462	this study
Family-based replication samples[a]					
Jewish 02	107	0.0544	1.515	0.979–2.344	(Alkelai et al., 2012)
Arab	58	0.232	1.600	0.920–2.783	(Alkelai et al., 2011)
Caucasians	794	0.0064	1.212	1.056–1.392	(Aberg et al., 2013)
Asian	579	0.842	0.985	0.852–1.139	(Aberg et al., 2013)
African	438	0.761	1.035	0.829–1.292	(Aberg et al., 2013)
All replication samples	/	**0.0019**	**1.109**	**1.039–1.184**	/
All samples	/	**0.000021**	**1.075**	**1.040–1.111**	/

[a] The sample size for family-based replication samples were shown as number of nuclear families.

($P = 0.20$) or all combined samples ($P = 0.21$), as shown by the forest plot of this meta-analysis (Fig. 2). Further, we also conducted the analysis by dividing the samples according to ethnicities (Caucasians, Asians, and Africans). In Caucasians, rs11880923 was significantly associated with schizophrenia with similar effect size compared to the total samples ($P = 0.0000246$, OR = 1.078, Table S2). However, in Asians and Africans, the association between rs11880923 and schizophrenia was not significant ($P > 0.5$, Table S2), although the direction of effect was consistent with that in Caucasians. Therefore, we conclude rs11880923 is a risk SNP for schizophrenia in Caucasians, while in Asians and African Americans, it may not be associated with schizophrenia in the present sample and need more validation in a larger sample.

For rs7259683, the replication analysis showed marginal significance in a Jewish case–control sample ($P = 0.07$), but it did not show any evidence of association/trend in other replication samples, and the results in all replication samples were also not significant ($P = 0.09$, Table S3). Therefore, we were unable to validate that rs7059683 is a risk SNP for schizophrenia.

4. Discussions

Growing evidence has suggested that the interactions between immune and nervous systems may play important roles in the pathogenesis of schizophrenia (Potvin et al., 2008). Dysregulation of immune system on downstream cellular and molecular pathways have been reported in the pathogenesis of schizophrenia (Drexhage et al., 2010; Potvin et al., 2008; Sainz et al., 2013), and recent GWA studies have also confirmed the involvement of immune related genes, such as major histocompatibility complex (MHC) region in schizophrenia across independent samples (International Schizophrenia Consortium et al., 2009; Irish Schizophrenia Genomics Consortium and the Wellcome Trust Case Control Consortium 2, 2012; Jia et al., 2012; Shi et al., 2009; Stefansson et al., 2009). By taking full advantage of the available large-scale data and an aggregate/comprehensive data-mining analysis as well as replication study in newly recruited samples, we showed that SNPs of *IRF3* were associated with schizophrenia. Our data further support the conclusion of "immune hypothesis" of schizophrenia, as *IRF3* is a member of interferon regulatory factor families, which have diverse roles in immune system via the target "interferon". Additionally, interferon is released by host cells in response to the presence of pathogens such as viruses, bacteria or parasites, therefore our study further confirmed the likelihood between viral infection and schizophrenia (Kneeland and Fatemi, 2013).

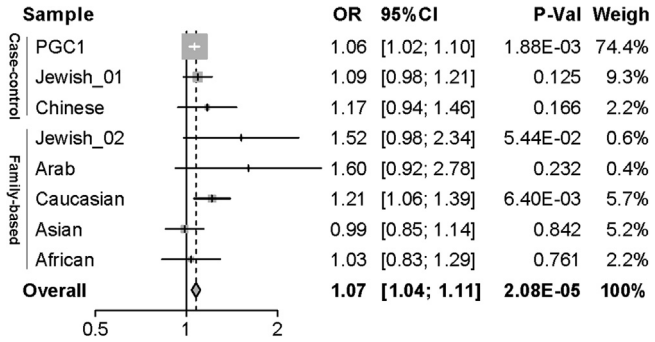

Fig. 2. Forest plot of odds ratio with 95% confidence interval for all schizophrenia samples included in the meta-analysis of rs11880923. The T allele is overrepresented in all the tested cohorts except for the Asian family based samples. The data source was listed in Table 2.

We employed a strategy to achieve a higher coverage of the *IRF3* gene. For the SNP selection criterion, we not only employed the commonly used "tagging SNP" method, but also considered the *cis* eQTL SNPs of *IRF3*, even they are not located within this gene region, as emerging evidence has suggested that schizophrenia susceptibility alleles are enriched for eQTL in human brain, and vice versa (Bacanu et al., 2014; Richards et al., 2012). Intriguingly, through this approach, we identified an *IRF3* eQTL SNP rs11880923 showed a significant association with schizophrenia, although it did not achieve the genome-wide level of statistical significance. Previous aggregated analyses have indicated that there may be true findings among those markers passing nominal significance (International Schizophrenia Consortium et al., 2009), and the herein observed OR for rs11880923 is 1.075, which is comparable with those observed ORs in other large-scale association studies (Chen et al., 2011; Li et al., 2014; Luo et al., 2014b).

More importantly, the risk SNP rs11880923 is associated with schizophrenia not only in PGC1 case–control sample, but also showed strong and marginal significant associations in two family-based samples (Caucasians and Jewish 02), respectively. It is well known that family-based samples could reduce population stratification and reflect the unbiased authentic signal more easily, thus we were confident with the association of this SNP. In addition, although with no nominal significance, the SNP effect sizes in populations outside European, e.g. Chinese and Arab, are even higher than that of PGC1 data (1.170 for Chinese and 1.600 for Arab compared with 1.063 for PGC1 sample). We speculate that the non-significant results are likely due to the limited power of small sample size. It should be mentioned that in the latest large-scale PGC2 GWAS, rs11880923 is also significantly associated with schizophrenia ($P = 0.000044483$) (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). This observation further supported our results.

There are, however, limitations to the interpretation of our results. First, although we identified a risk eQTL SNP, we cannot rule out the possibility that it is co-inherited with other functional variants in *IRF3*. It should also be noted that there is the possibility of underlying rare variants that may create synthetic associations (Dickson et al., 2010) because rare missense mutations and copy number variations could increase risk for schizophrenia (Girard et al., 2011; Kirov, 2010; St Clair, 2009; Xu et al., 2011). Therefore, we could not identify the causative risk variant in this region unless further fine-grained analyses and functional assays are warranted. Second, given the fact that most of the significant SNPs in *IRF3* (Table 1) seem to be in moderate LD with rs11880923 in Caucasians, we cannot rule out the possibility that these risk associations represents one association within a haplotype block that may harbor one or more causal variants, and thus further fine scale mapping in future studies are needed. Third, although we identified an association of risk SNP rs11880923 with *IRF3* expression, the exact regulative mechanism is still unclear, given the long genomic distance between rs11880923 and the *IRF3* gene, we cannot exclude the possibility that rs11880923 is located within a long non-coding RNA (lncRNA) near *IRF3*, since previous studies have also shown the effects of *in cis* lncRNAs on adjacent genes' expression level (Bao et al., 2013). Finally, we have identified the risk genotype showing association with *IRF3* expression, but recent genome-wide expression analyses did not find evidence for the alterations of this gene in schizophrenia patients (Hakak et al., 2001; Kuzman et al., 2009; Mistry et al., 2013), thus we are cautious to interpret that expression change of *IRF3* is a risk factor for schizophrenia. But this is not unexpected given the fact that *IRF3* is vulnerable by virus infection, a major environmental risk factor causing schizophrenia. To the best of our knowledge, virus infection has not been well considered as a major covariate

in expression analyses of schizophrenia patients, which may be achievable in future studies.

In summary, using a hypothesis-driven method combining with large-scale data and expression quantitative techniques, we were able to identify genetic evidence of associations between *IRF3* and schizophrenia, further supporting the role of immune system in such illness.

Role of funding source

This study was supported by the Strategic Priority Research Program (B) of the Chinese Academy of Sciences (XDB02020000 to Y.-G. Y.).

Contributors

Authors XL, ML and YGY designed the study. Authors XL, WZ, TL, AD, AA, BL, HYJ, DFZ, LY and XFX generated the experimental data. Authors ML and YGY analyzed all data and wrote the paper. Authors HYJ and XFX provided the patient samples. All authors contributed to and have approved the final manuscript.

Conflict of interest

The authors have no conflicts of interest to declare.

Acknowledgement

We are grateful to all the voluntary donors of DNA samples in this study. We thank members of schizophrenia Psychiatric Genomic Consortium, who shared the PGC1 GWAS data.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jpsychires.2015.03.008.

References

Aberg KA, Liu Y, Bukszar J, McClay JL, Khachane AN, Andreassen OA, et al. A comprehensive family-based replication study of schizophrenia genes. JAMA Psychiatry 2013;70:573–81.
Alkelai A, Lupoli S, Greenbaum L, Giegling I, Kohn Y, Sarner-Kanyas K, et al. Identification of new schizophrenia susceptibility loci in an ethnically homogeneous, family-based, Arab-Israeli sample. FASEB J 2011;25:4011–23.
Alkelai A, Lupoli S, Greenbaum L, Kohn Y, Kanyas-Sarner K, Ben-Asher E, et al. DOCK4 and CEACAM21 as novel schizophrenia candidate genes in the Jewish population. Int J Neuropsychopharmacol 2012;15:459–69.
Andreasen NC. Symptoms, signs, and diagnosis of schizophrenia. Lancet 1995;346:477–81.
Bacanu SA, Chen J, Sun J, Richardson K, Lai CQ, Zhao Z, et al. Functional SNPs are enriched for schizophrenia association signals. Mol Psychiatry 2014;19:276–7.
Bao J, Wu J, Schuster AS, Hennig GW, Yan W. Expression profiling reveals developmentally regulated lncRNA repertoire in the mouse male germline. Biol Reprod 2013;89:107.
Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005;21:263–5.
Cannon TD, van Erp TG, Bearden CE, Loewy R, Thompson P, Toga AW, et al. Early and late neurodevelopmental influences in the prodrome to schizophrenia: contributions of genes, environment, and their interactions. Schizophr Bull 2003;29:653–69.
Caspi A, Moffitt TE. Gene-environment interactions in psychiatry: joining forces with neuroscience. Nat Rev Neurosci 2006;7:583–90.
Chen X, Lee G, Maher BS, Fanous AH, Chen J, Zhao Z, et al. GWA study data mining and independent replication identify cardiomyopathy-associated 5 (CMYA5) as a risk gene for schizophrenia. Mol Psychiatry 2011;16:1117–29.
Clarke MC, Tanskanen A, Huttunen M, Whittaker JC, Cannon M. Evidence for an interaction between familial liability and prenatal exposure to infection in the causation of schizophrenia. Am J Psychiatry 2009;166:1025–30.
Colantuoni C, Lipska BK, Ye T, Hyde TM, Tao R, Leek JT, et al. Temporal dynamics and genetic control of transcription in the human prefrontal cortex. Nature 2011;478:519–23.
Collins SE, Noyce RS, Mossman KL. Innate cellular response to virus particle entry requires IRF3 but not virus replication. J Virol 2004;78:1706–17.
de Veer MJ, Holko M, Frevel M, Walker E, Der S, Paranjape JM, et al. Functional classification of interferon-stimulated genes identified using microarrays. J Leukoc Biol 2001;69:912–20.
Dickson SP, Wang K, Krantz I, Hakonarson H, Goldstein DB. Rare variants create synthetic genome-wide associations. PLoS Biol 2010;8:e1000294.
Drexhage RC, Knijff EM, Padmos RC, Heul-Nieuwenhuijzen L, Beumer W, Versnel MA, et al. The mononuclear phagocyte system and its cytokine inflammatory networks in schizophrenia and bipolar disorder. Expert Rev Neurother 2010;10:59–76.
Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, Roth A, et al. STRING v9.1: protein-protein interaction networks, with increased coverage and integration. Nucleic Acids Res 2013;41:D808–15.
Girard SL, Gauthier J, Noreau A, Xiong L, Zhou S, Jouan L, et al. Increased exonic de novo mutation rate in individuals with schizophrenia. Nat Genet 2011;43:860–3.
Hakak Y, Walker JR, Li C, Wong WH, Davis KL, Buxbaum JD, et al. Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia. Proc Natl Acad Sci U. S. A 2001;98:4746–51.
Harold D, Abraham R, Hollingworth P, Sims R, Gerrish A, Hamshere ML, et al. Genome-wide association study identifies variants at CLU and PICALM associated with Alzheimer's disease. Nat Genet 2009;41:1088–93.
Hurlock ECt. Interferons: potential roles in affect. Med Hypotheses 2001;56:558–66.
International Schizophrenia Consortium, Purcell SM, Wray NR, Stone JL, Visscher PM, O'Donovan MC, et al. Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. Nature 2009;460:748–52.
Irish Schizophrenia Genomics Consortium, the Wellcome Trust Case Control Consortium 2. Genome-wide association study implicates HLA-C*01:02 as a risk factor at the major histocompatibility complex locus in schizophrenia. Biol Psychiatry 2012;72:620–8.
Jia P, Wang L, Fanous AH, Chen X, Kendler KS, International Schizophrenia Consortium, et al. A bias-reducing pathway enrichment analysis of genome-wide association data confirmed association of the MHC region with schizophrenia. J Med Genet 2012;49:96–103.
Juang YT, Lowther W, Kellum M, Au WC, Lin R, Hiscott J, et al. Primary activation of interferon A and interferon B gene transcription by interferon regulatory factor 3. Proc Natl Acad Sci U. S. A 1998;95:9837–42.
Kirov G. The role of copy number variation in schizophrenia. Expert Rev Neurother 2010;10:25–32.
Kneeland RE, Fatemi SH. Viral infection, inflammation and schizophrenia. Prog Neuropsychopharmacol Biol Psychiatry 2013;42:35–48.
Kuzman MR, Medved V, Terzic J, Krainc D. Genome-wide expression analysis of peripheral blood identifies candidate biomarkers for schizophrenia. J Psychiatr Res 2009;43:1073–7.
Lambert JC, Heath S, Even G, Campion D, Sleegers K, Hiltunen M, et al. Genome-wide association study identifies variants at CLU and CR1 associated with Alzheimer's disease. Nat Genet 2009;41:1094–9.
Lee HC, Narayanan S, Park SJ, Seong SY, Hahn YS. Transcriptional regulation of IFN-lambda genes in hepatitis C virus-infected hepatocytes via IRF-3.IRF-7.NF-kappaB complex. J Biol Chem 2014;289:5310–9.
Lencz T, Guha S, Liu C, Rosenfeld J, Mukherjee S, DeRosse P, et al. Genome-wide association study implicates NDST3 in schizophrenia and bipolar disorder. Nat Commun 2013;4:2739.
Li M, Luo XJ, Rietschel M, Lewis CM, Mattheisen M, Muller-Myhsok B, et al. Allelic differences between Europeans and Chinese for CREB1 SNPs and their implications in gene expression regulation, hippocampal structure and function, and bipolar disorder susceptibility. Mol Psychiatry 2014;19:452–61.
Liu W, Breen G, Zhang J, Li S, Gu N, Feng G, et al. Association of APOE gene with schizophrenia in Chinese: a possible risk factor in times of malnutrition. Schizophr Res 2003;62:225–30.
Luo X, Huang L, Jia P, Li M, Su B, Zhao Z, et al. Protein-protein interaction and pathway analyses of top schizophrenia genes reveal schizophrenia susceptibility genes converge on common molecular networks and enrichment of nucleosome (chromatin) assembly genes in schizophrenia susceptibility loci. Schizophr Bull 2014a;40:39–49.
Luo XJ, Li M, Huang L, Steinberg S, Mattheisen M, Liang G, et al. Convergent lines of evidence support CAMKK2 as a schizophrenia susceptibility gene. Mol Psychiatry 2014b;19:774–83.
Mistry M, Gillis J, Pavlidis P. Genome-wide expression profiling of schizophrenia using a large combined cohort. Mol Psychiatry 2013;18:215–25.
Potvin S, Stip E, Sepehry AA, Gendron A, Bah R, Kouassi E. Inflammatory cytokine alterations in schizophrenia: a systematic quantitative review. Biol Psychiatry 2008;63:801–8.
Prinarakis E, Chantzoura E, Thanos D, Spyrou G. S-glutathionylation of IRF3 regulates IRF3-CBP interaction and activation of the IFN beta pathway. EMBO J 2008;27:865–75.
Richards AL, Jones L, Moskvina V, Kirov G, Gejman PV, Levinson DF, et al. Schizophrenia susceptibility alleles are enriched for alleles that affect gene expression in adult human brain. Mol Psychiatry 2012;17:193–201.
Ripke S, O'Dushlaine C, Chambert K, Moran JL, Kahler AK, Akterin S, et al. Genome-wide association analysis identifies 13 new risk loci for schizophrenia. Nat Genet 2013;45:1150–9.
Sainz J, Mata I, Barrera J, Perez-Iglesias R, Varela I, Arranz MJ, et al. Inflammatory and immune response genes have significantly altered expression in schizophrenia. Mol Psychiatry 2013;18:1056–7.

Schaefer M, Engelbrecht MA, Gut O, Fiebich BL, Bauer J, Schmidt F, et al. Interferon alpha (IFNalpha) and psychiatric syndromes: a review. Prog Neuropsychopharmacol Biol Psychiatry 2002a;26:731–46.
Schaefer M, Schmidt F, Neumer R, Scholler G, Schwarz M. Interferon-alpha, cytokines and possible implications for mood disorders. Bipolar Disord 2002b;4(Suppl. 1):111–3.
Schizophrenia Working Group of the Psychiatric Genomics Consortium. Biological insights from 108 schizophrenia-associated genetic loci. Nature 2014;511:421–7.
Seshadri S, Fitzpatrick AL, Ikram MA, DeStefano AL, Gudnason V, Boada M, et al. Genome-wide analysis of genetic loci associated with Alzheimer disease. JAMA 2010;303:1832–40.
Shi J, Levinson DF, Duan J, Sanders AR, Zheng Y, Pe'er I, et al. Common variants on chromosome 6p22.1 are associated with schizophrenia. Nature 2009;460:753–7.
St Clair D. Copy number variation and schizophrenia. Schizophr Bull 2009;35:9–12.
Stark GR, Kerr IM, Williams BR, Silverman RH, Schreiber RD. How cells respond to interferons. Annu Rev Biochem 1998;67:227–64.
Stefansson H, Ophoff RA, Steinberg S, Andreassen OA, Cichon S, Rujescu D, et al. Common variants conferring risk of schizophrenia. Nature 2009;460:744–7.
Stranger BE, Montgomery SB, Dimas AS, Parts L, Stegle O, Ingle CE, et al. Patterns of cis regulatory variation in diverse human populations. PLoS Genet 2012;8:e1002639.
Xu B, Roos JL, Dexheimer P, Boone B, Plummer B, Levy S, et al. Exome sequencing supports a de novo mutational paradigm for schizophrenia. Nat Genet 2011;43:864–8.
Yang TP, Beazley C, Montgomery SB, Dimas AS, Gutierrez-Arcelus M, Stranger BE, et al. Genevar: a database and Java application for the analysis and visualization of SNP-gene associations in eQTL studies. Bioinformatics 2010;26:2474–6.
Yu H, Bi W, Liu C, Zhao Y, Zhang JF, Zhang D, et al. Protein-interaction-network-based analysis for genome-wide association analysis of schizophrenia in Han Chinese population. J Psychiatr Res 2014;50:73–8.
Zhang W, Xiao MS, Ji S, Tang J, Xu L, Li X, et al. Promoter variant rs2301228 on the neural cell adhesion molecule 1 gene confers risk of schizophrenia in Han Chinese. Schizophr Res 2014;160:88–96.

Supporting Online Material for

Common variants of *IRF3* conferring risk of schizophrenia

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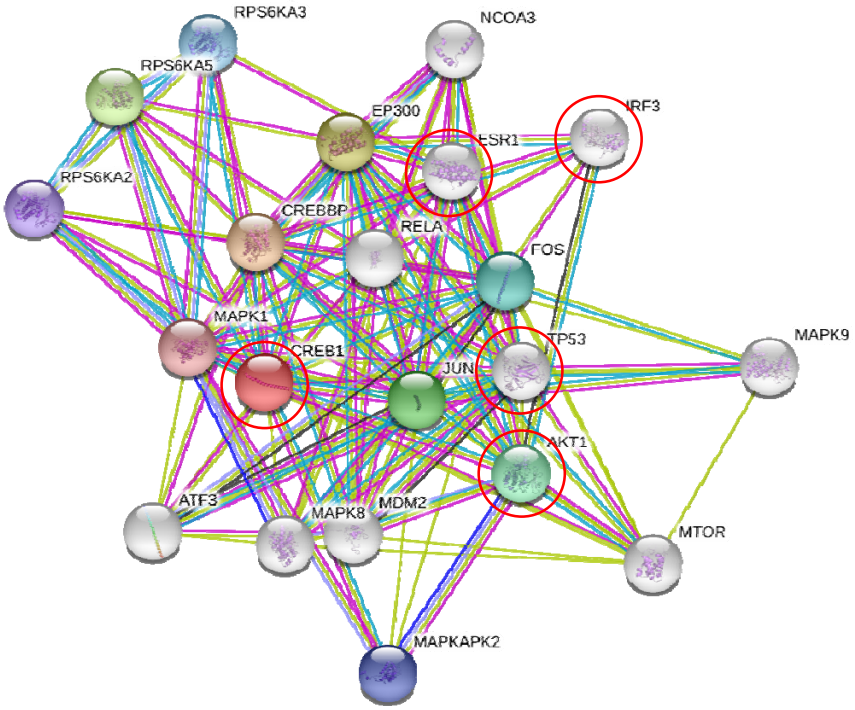


Figure S1. Protein-protein interactions network involved IRF3 in STRING (http://string-db.org/) (Franceschini et al., 2013). The reported schizophrenia risk genes and the *IRF3* gene are marked in red circle.

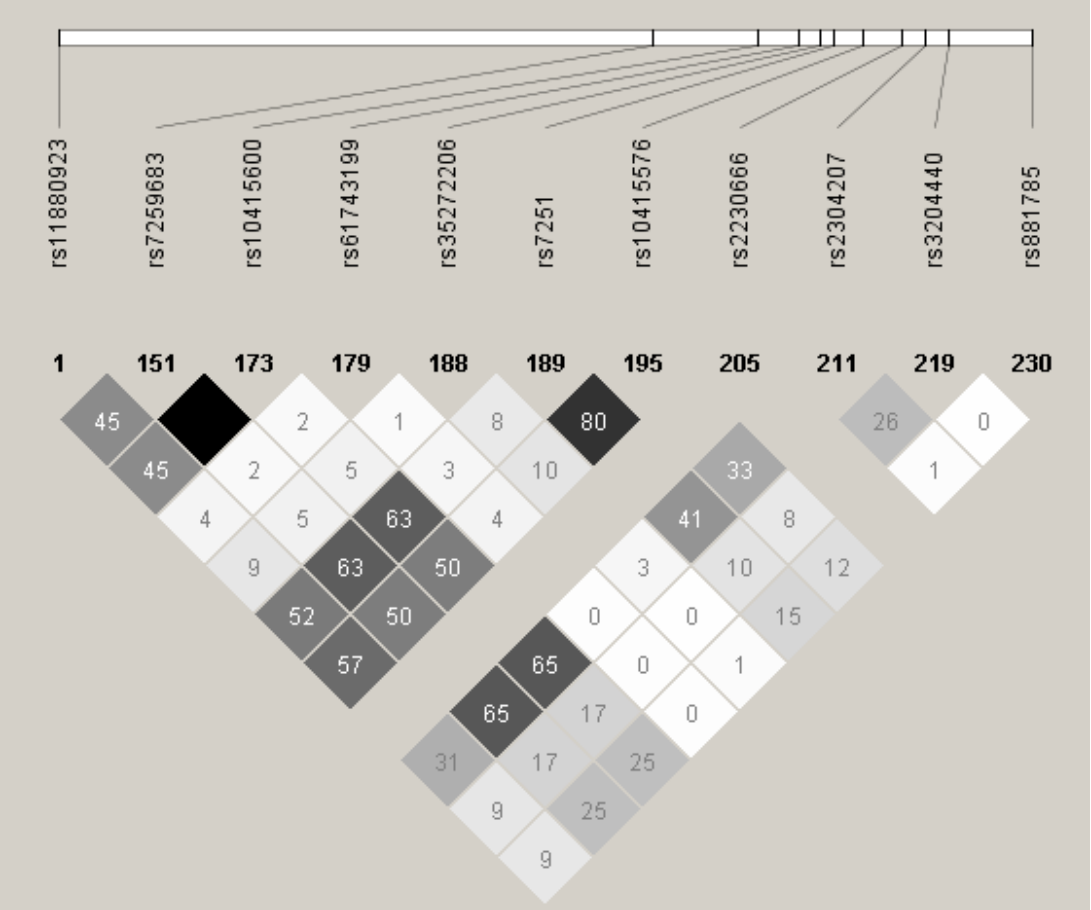


Figure S2. Linkage disequilibrium map of the 11 SNPs in Caucasians from 1000-Human-Genome. The LD mapped was constructed using Haploview 4.1 (Barrett et al., 2005), the numeral under each SNP name was their number among all IRF3 SNPs, as we extracted all SNPs from this region to perform the LD analysis.

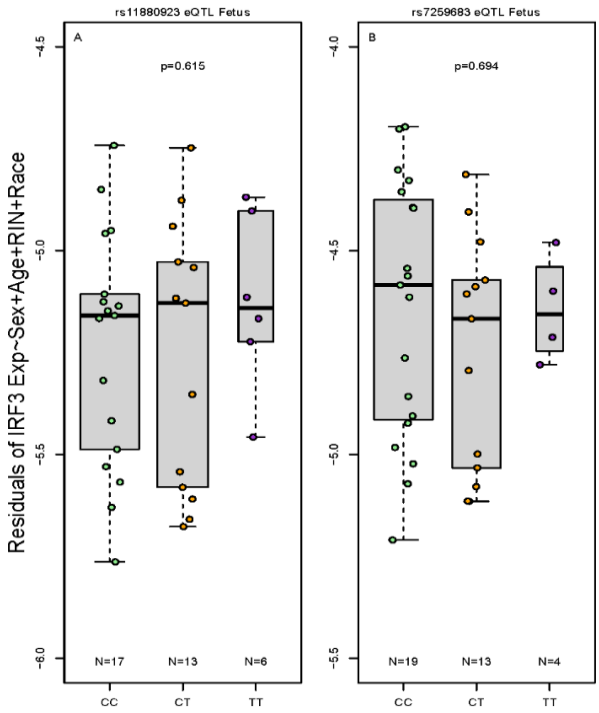


Figure S3. Associations of SNPs with *IRF3* expression in the BrainCloud fetal samples (Colantuoni et al., 2011).

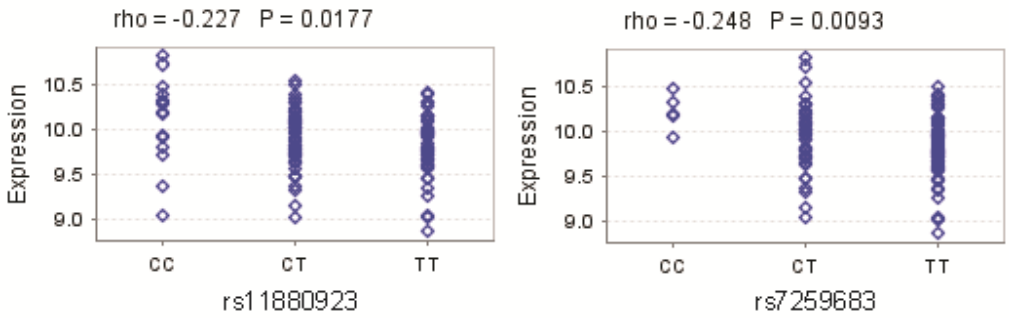
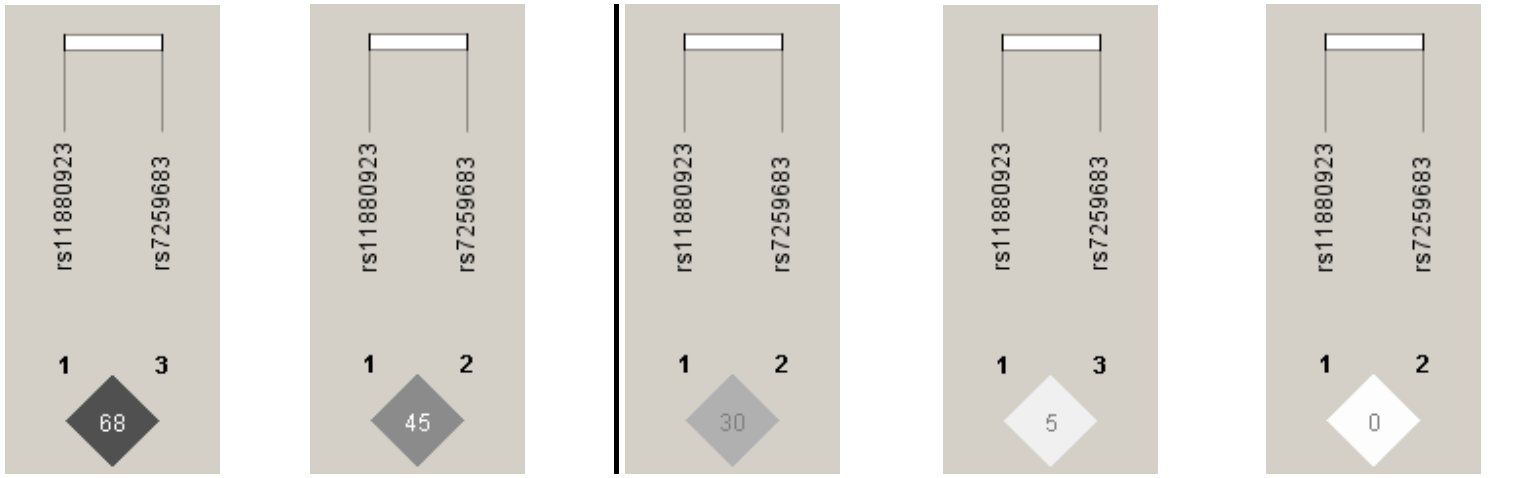


Figure S4. Associations of SNPs with *IRF3* expression in Caucasian samples reported in Stranger et al.'s study (Stranger et al., 2012).



CAUC BrainCloud | CAUC 1000-Human-Genome | Chinese 1000-Human-Genome | AA BrainCloud | AA 1000-Human-Genome

Figure S5. Linkage disequilibrium between *IRF3* eQTL SNPs in the Caucasian (CAUC) and African American (AA) samples from the BrainCloud data set (Colantuoni et al., 2011) and 1000-Human-Genome dataset (www.1000genomes.org).

Table S1. Association of rs11880923 and rs7259683 with nearby gene expression in BrainCloud.

Gene	rs11880923						rs7259683					
	Caucasians			African American			Caucasians			African American		
	Beta	SE	P-value	Beta	SE	P-value	Beta	SE	P-value	Beta	SE	P-value
CPT1C	-.038	.040	0.346	-.0048	.038	0.899	-.053	.041	0.196	-.028	.036	0.432
FCGRT	-.114	.054	0.037	-.0086	.052	0.868	-.055	.056	0.322	-.028	.049	0.564
NOSIP	-.031	.038	0.425	.0096	.029	0.741	-.0009	.039	0.981	-.021	.028	0.457
PRMT1	.011	.025	0.670	-.049	.032	0.130	.0043	.025	0.864	-.061	.030	0.046
PRR12	-.018	.080	0.823	.039	.068	0.571	.0097	.081	0.905	.0094	.065	0.885
PRRG2	-.076	.045	0.094	-.0031	.040	0.938	-.084	.046	0.069	.021	.038	0.581
RCN3	-.040	.035	0.255	.048	.032	0.142	-.030	.036	0.402	.083	.030	0.007
RRAS	-.050	.057	0.379	.018	.050	0.727	.017	.058	0.770	-.0032	.048	0.946
SCAF1	-.069	.056	0.221	.016	.043	0.713	-.017	.057	0.767	-.012	.041	0.771

Table S2. Associations of *IRF3* rs11880923 [T] with schizophrenia in different populations

Sample	Sample size	P-value	OR	95%CI	Data Source
Caucasian samples		***0.0000246***	***1.078***	***1.041-1.117***	
PGC1	13,833/18,310	0.00188	1.063	1.023-1.105	(Ripke et al., 2013)
Jewish_01	904/1,640	0.125	1.089	0.977-1.214	(Lencz et al., 2013)
Jewish_02	107	0.0544	1.515	0.979-2.344	(Alkelai et al., 2012)
Arab	58	0.232	1.600	0.920-2.783	(Alkelai et al., 2011)
Caucasians	794	0.0064	1.212	1.056-1.392	(Aberg et al., 2013)
Asian samples		***0.545***	***1.039***	***0.918-1.175***	
Asian	579	0.842	0.985	0.852-1.139	(Aberg et al., 2013)
Chinese	480/479	0.166	1.170	0.936-1.462	this study
African American samples		***0.761***	***1.035***	***0.829-1.292***	
African	438	0.761	1.035	0.829-1.292	(Aberg et al., 2013)

Table S3. Associations of *IRF3* rs7259683 [T] with schizophrenia in multiple samples.

Sample	Sample size	P-value	OR	95%CI	Data Source
Discovery sample					
PGC1	13,833/18,310	0.00242	1.065	1.023-1.109	(Ripke et al., 2013)
Case-Control replication samples					
Jewish_01	904/1,640	0.070	1.126	0.990-1.280	(Lencz et al., 2013)
Chinese	480/479	0.656	1.048	0.854-1.285	this study
Family-based replication samples					
Jewish_02	107	0.116	1.400	0.907-2.160	(Alkelai et al., 2012)
Arab	58	0.734	0.967	0.580-1.610	(Alkelai et al., 2011)
All replication samples	**/**	**0.091**	**1.083**	**0.986-1.188**	**/**
All samples	**/**	**0.0005**	**1.068**	**1.029-1.109**	**/**

Supplementary References

Aberg KA, Liu Y, Bukszar J, McClay JL, Khachane AN, Andreassen OA, et al. A comprehensive family-based replication study of schizophrenia genes. JAMA Psychiatry. 2013;70:573-581.

Alkelai A, Lupoli S, Greenbaum L, Giegling I, Kohn Y, Sarner-Kanyas K, et al. Identification of new schizophrenia susceptibility loci in an ethnically homogeneous, family-based, Arab-Israeli sample. FASEB J. 2011;25:4011-4023.

Alkelai A, Lupoli S, Greenbaum L, Kohn Y, Kanyas-Sarner K, Ben-Asher E, et al. DOCK4 and CEACAM21 as novel schizophrenia candidate genes in the Jewish population. Int J Neuropsychopharmacol. 2012;15:459-469.

Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005;21:263-265.

Colantuoni C, Lipska BK, Ye T, Hyde TM, Tao R, Leek JT, et al. Temporal dynamics and genetic control of transcription in the human prefrontal cortex. Nature. 2011;478:519-523.

Franceschini A, Szklarczyk D, Frankild S, Kuhn M, Simonovic M, Roth A, et al. STRING v9.1: protein-protein interaction networks, with increased coverage and integration. Nucleic Acids Res. 2013;41:D808-815.

Lencz T, Guha S, Liu C, Rosenfeld J, Mukherjee S, DeRosse P, et al. Genome-wide association study implicates NDST3 in schizophrenia and bipolar disorder. Nat Commun. 2013;4:2739.

Ripke S, O'Dushlaine C, Chambert K, Moran JL, Kahler AK, Akterin S, et al. Genome-wide association analysis identifies 13 new risk loci for schizophrenia. Nat Genet. 2013;45:1150-1159.

Stranger BE, Montgomery SB, Dimas AS, Parts L, Stegle O, Ingle CE, et al. Patterns of cis regulatory variation in diverse human populations. PLoS Genet. 2012;8:e1002639.