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Multiple genes in the 15q13-q14 chromosomal region are associated with schizophrenia

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Abstract

Objective—The chromosomal region, 15q13-q14, including the $\alpha 7$ nicotinic acetylcholine receptor gene, *CHRNA7*, is a replicated region for schizophrenia. This study fine-mapped genes at 15q13-q14 to determine whether the association is unique to *CHRNA7*.

Methods—Family-based and case-control association studies were performed on Caucasian-non-Hispanic and African-American individuals from 120 families as well as 468 individual patients with schizophrenia and 144 well-characterized controls. Single-nucleotide polymorphism (SNP) markers were genotyped, and association analyses carried out for the outcomes of schizophrenia, smoking, and smoking in schizophrenia.

Results—Three genes were associated with schizophrenia in both ethnic populations: *TRPM1*, *KLF13*, and *RYR3*. Two SNPs in *CHRNA7* were associated with schizophrenia in African-Americans, and a second SNP in *CHRNA7* was significant for an association with smoking and smoking in schizophrenia in Caucasians.

Conclusion—Results of these studies support association of the 15q13-q14 region with schizophrenia. The broad positive association suggests that more than one 15q gene may be contributing to the disorder, either in combination or through a regulatory mechanism. *Psychiatr Genet* 22:1–14 © 2012 Wolters Kluwer Health | Lippincott Williams & Wilkins.

Keywords

association; *CHRNA7*; chromosome 15; copy number variation; nicotinic receptor; schizophrenia

Introduction

Schizophrenia is a common mental illness with a large genetic component (Kendler 1988; Tsuang and Faraone, 1994; Freedman *et al.*, 2001b; Owen *et al.*, 2004). Although heritability is estimated at approximately 80% (Sullivan *et al.*, 2003), the disorder is thought

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to be multigenic with a significant environmental component (Tsuang, 2000; Petronis *et al.*, 2003; Howes *et al.*, 2004). Significant or suggestive evidence of linkage to schizophrenia has implicated 18 chromosomal regions (Baron, 2001; Harrison and Owen, 2003). Twelve of these chromosomal regions have replicated linkage, include one or more polymorphisms associated with schizophrenia, and contain biologically plausible candidate genes showing altered expression in the disorder (Harrison and Weinberger, 2005). The 15q13-q14 locus is one such chromosomal region, with linkage replicated in pedigrees from the National Institute of Mental Health (NIMH) Schizophrenia Genetics Initiative (Kaufmann *et al.*, 1998; Leonard *et al.*, 1998; Freedman and Leonard, 2001; Freedman *et al.*, 2001b), and in other sample populations (Coon *et al.*, 1994; Riley *et al.*, 2000; Stöber *et al.*, 2000; Gejman *et al.*, 2001; Liu *et al.*, 2001; Tsuang *et al.*, 2001; Xu *et al.*, 2001a; Stöber *et al.*, 2002; Fallin *et al.*, 2004). Nominal association has also been found for *CHRNA7* in several large studies (Sanders *et al.*, 2008; Stefansson *et al.*, 2008). The locus is, thus, linked to schizophrenia across ethnicities and in multiple independent studies.

Genome-wide nonparametric linkage (NPL) analysis first identified the 15q13-q14 locus through linkage to the P50 deficit, a dominantly inherited sensory gating deficit found in most patients with schizophrenia and half of their first degree relatives (Freedman *et al.*, 1997; Waldo *et al.*, 2000). The P50 deficit was linked (LOD = 5.3) to a microsatellite marker (D15S1360), located in intron 2 of the *CHRNA7* gene. This same study found positive, but less strong linkage to schizophrenia at D15S1360 (LOD = 1.3) (Freedman *et al.*, 1997). Sibpair analysis in the NIMH pedigrees revealed a significant excess of alleles identical by descent ($P < 0.0024$) (Leonard *et al.*, 1998). In addition, a transmission disequilibrium test was used for an association study of the 15q13-q14 locus in parent-child trios in the same cohort. Significant genotype-wise disequilibrium ($P < 0.007$) was found at a microsatellite marker, D15S165, located within one megabase of the *CHRNA7* gene (Freedman *et al.*, 2001a). Biological evidence has firmly implicated the $\alpha 7$ receptor in a pathway regulating the P50 auditory evoked response. Nicotine transiently normalizes the P50 deficit in schizophrenics and their first-degree relatives, consistent with the involvement of a nicotinic receptor in the disease (Adler *et al.*, 1993; Leonard *et al.*, 2001). Animal models of the P50 deficit further support a role for the $\alpha 7$ receptor. Antagonists of the $\alpha 7$ receptor disrupt sensory processing in rats (Luntz-Leybman *et al.*, 1992), and agonists of the $\alpha 7$ receptor normalize aberrant sensory processing in mice (Stevens *et al.*, 1998). The effects of nicotine administration and $\alpha 7$ stimuli on patient and animal models of the P50 deficit supports the hypothesis that smoking may be a form of 'self-medication' for schizophrenics (Adler *et al.*, 1998; Kumari and Postma, 2005; Leonard *et al.*, 2007). This hypothesis is reinforced by the prevalence of smoking in schizophrenics, which is almost three times as high as in the general population (De Leon *et al.*, 1995; Diwan *et al.*, 1998; Leonard *et al.*, 2001; De Leon *et al.*, 2002; De Leon and Diaz, 2005). A significant association of the D15S1360 microsatellite marker in intron 2 of *CHRNA7* to smoking in schizophrenia was also reported (De Luca *et al.*, 2004). Recently, several neuronal nicotinic acetylcholine receptor genes, including *CHRNA2*, *CHRNA5*, *CHRNA3*, and *CHRNA7*, have been associated with nicotine addiction or to heavy smoking (Berrettini *et al.*, 2008; Bierut *et al.*, 2008; Weiss *et al.*, 2008; Furberg *et al.*, 2010; Thorgeirsson *et al.*, 2010).

Ligand binding with an $\alpha 7$ receptor antagonist [125 I]- α -bungarotoxin, showed 50% fewer receptors in the postmortem hippocampus of patients with schizophrenia than in nonmentally ill controls (Freedman *et al.*, 1995). These results were confirmed in cortex (Guan *et al.*, 1999; Marutle *et al.*, 2001) and in the reticular nucleus of the thalamus (Court *et al.*, 1999). Decreased levels of $\alpha 7$ receptors suggest that cognition is likely to be impaired (Rezvani and Levin, 2001; Young *et al.*, 2004; Sacco *et al.*, 2005; Levin *et al.*, 2006). The $\alpha 7$ receptor is the most promising molecular target for development of drugs for cognitive impairments in schizophrenia (Psychiatric News, 2006). Phase I and II trials of an $\alpha 7$

receptor partial agonist, 3-[2,4-dimethoxybenzylidene] anabaseine, resulted in improvement in both the P50 gating deficit and in attention in schizophrenic nonsmokers (Olincy *et al.*, 2006; Freedman *et al.*, 2008). Recently single nucleotide polymorphism (SNP) rs3087454 in the 5' upstream regulatory region of *CHRNA7* was associated with schizophrenia (Stephens *et al.*, 2009) and also with improvement in functional MRI response to 3-[2,4-dimethoxybenzylidene] anabaseine (Tregellas *et al.*, 2010).

The 15q13-q14 region is characterized by large copy number variations (CNV), including both deletions and duplications that include the *CHRNA7* gene. Although the CNVs are rare, they are associated with schizophrenia (Stefansson *et al.*, 2008; Stone *et al.*, 2008). Genetic characterization of *CHRNA7* revealed the gene to be partially duplicated (exons 5–10), with both loci mapping to the 15q13-q14 region (Gault *et al.*, 1998; Riley *et al.*, 2002). The duplicated gene, *CHRFAM7A*, is transcribed in both human brain and in the periphery, and is unique to humans (Locke *et al.*, 2003). The *CHRFAM7A* gene product was recently found to be a dominant negative regulator of $\alpha 7$ receptor function (Araud *et al.*, 2011; De Lucas-Cerrillo *et al.*, 2011).

Duplicated sequences from the *CHRFAM7A* gene are greater than 99% homologous to sequences from exons 5–10 of the full-length locus and therefore mutation screening of both genes required the use of mRNA (Gault *et al.*, 1998). Results of mutation screening of *CHRNA7* and its partial duplication, *CHRFAM7A*, identified 33 polymorphisms located in the coding region and intron/exon borders of the genes (Gault *et al.*, 2003). A 2bp deletion in exon 6 of the duplicated gene, *CHRFAM7A*, is associated with schizophrenia (Sinkus *et al.*, 2009). The 2 bp deletion is found in an inverted copy of *CHRFAM7A* (Flomen *et al.*, 2008), suggesting that the inversion itself may be pathogenic. In the full-length *CHRNA7* gene, three polymorphisms were nonsynonymous and mapped to the full length *CHRNA7* coding region. These polymorphisms, however, were rare and not associated with schizophrenia or the P50 gating deficit (Gault *et al.*, 2003). Mutation screening also revealed a large number of mutations in the 5' upstream regulatory region of *CHRNA7*, which is not duplicated. Functional analysis of proximal promoter mutations identified polymorphisms that decreased transcription *in vitro*. These functional promoter mutations were statistically more prevalent as a group in schizophrenics than in control participants ($P = 0.007$), and the presence of a promoter polymorphism was associated with the P50 gating deficit ($P < 0.0001$) (Leonard *et al.*, 2002). In addition, we reported that a SNP rs3087454, located at position –1831 bp upstream of exon 1 in *CHRNA7*, is significantly associated with schizophrenia in African-American and Caucasian-non-Hispanic case-control sample populations after multiple testing correction ($P = 0.0009$, African-American; $P = 0.013$, Caucasian-non-Hispanic) (Stephens *et al.*, 2009).

Because the *CHRNA7* and *CHRFAM7A* genes show homology, SNP analysis in other genes in this region might confirm this gene cluster is important for schizophrenia. This study was designed to fine map genes in the 15q13-q14 linkage peak region to determine whether genetic variance in the *CHRNA7* gene is associated with schizophrenia or if variation in flanking genes contributes to the disorder. An informative set of SNPs was selected in each of 16 genes in the region. Ten microsatellite markers were also queried in the region of interest. SNP genotype data were analyzed for association with schizophrenia in Caucasian-non-Hispanic and African-American participants. A portion of this cohort consisted of families from the NIMH Human Genetics Initiative for Schizophrenia which had been previously linked to the 15q13-14 region (Kaufmann *et al.*, 1998; Leonard *et al.*, 1998; Freedman and Leonard, 2001; Freedman *et al.*, 2001b). Smoking history was considered as a secondary outcome.

Results indicate that three genes were associated with schizophrenia in both ethnic populations after correcting for multiple comparisons: *TRPM1*, *KLF13*, and *RYR3*. In addition, two SNPs in *CHRNA7* were associated with schizophrenia in African-Americans. One SNP in *CHRNA7* was significant for an association with smoking and smoking in schizophrenia in Caucasians. A SNP in *RYR3* was associated with smoking in African-Americans. Thus, broad association with schizophrenia in the 15q13-14 region was confirmed.

Materials and methods

Participants

Characteristics of study participants are shown in Tables 1 and 2. DNA samples for case-control association studies included 612 African-American and Caucasian-non-Hispanic participants collected in our laboratory and one randomly chosen individual with schizophrenia per family, from families within the NIMH Human Genetics Initiative for Schizophrenia. SAS version 9 (SAS Institute Inc., Cary, North Carolina, USA) was utilized for random selection from 14 trios, 34 sibling-pairs, and 25 multiplex families in the NIMH Caucasian-non-Hispanic sample and three trios, 15 sibling-pairs, and 28 multiplex families from African-Americans in the collection. Local participants were consented for genetic studies, utilizing a consent form approved by the Colorado Multiple Institutional Review Board. DNA samples contain no identifying information. Ethnicities of case-control participants were recorded from self-report or family interview. The Caucasian-non-Hispanic case-control sample comprised 363 patients with schizophrenia (307 adult onset and 56 childhood onset schizophrenics) and 99 well-characterized controls. The African-American case-control sample included 105 individuals with schizophrenia (103 adult onset and two childhood onset), and 45 controls. Adult case individuals were chosen based on a diagnosis of schizophrenia, utilizing the Structured Clinical Interview for *Diagnostic and Statistical Manual of Disorders*, 4th edition Axis I disorders (First *et al.*, 1996b). Control participants were carefully selected with a screen for evidence of current or past psychosis using a Structured Clinical Interview for *Diagnostic and Statistical Manual of Disorders*, 4th edition Axis I disorders for nonpatients (First *et al.*, 1996a). Childhood onset schizophrenia (COS) cases were diagnosed utilizing the K-SADS-PL (Kaufman *et al.*, 1997). Smoking history was determined on local individuals, utilizing a detailed questionnaire (Breese *et al.*, 1997; Breese *et al.*, 2000).

For the family-based study, a total of 329 African-American (47 nuclear families) and Caucasian-non-Hispanic participants (73 nuclear families) from the NIMH Schizophrenia Genetics Initiative were chosen based on a diagnosis of schizophrenia. This cohort has previously shown positive LOD scores and association to markers at the 15q13-q14 locus in both African-American and Caucasian individuals (Kaufmann *et al.*, 1998; Leonard *et al.*, 1998; Freedman *et al.*, 2001b). The structure of the NIMH families included sibpairs (approximately 53% of the sample), as well as trios (approximately 22%), and extended family members (approximately 25%). Detailed information on the NIMH family structure and diagnosis is available from the web site (<http://www.nimhgenetics.org>). Smoking history on the NIMH patients was determined using the Fagerstrom test for nicotine dependence (Fagerstrom and Schneider, 1989) as part of the Diagnostic Interview for Genetic Studies (Nurnberger *et al.*, 1994).

15q13-q14 linkage peak region

To delineate a peak region for schizophrenia at the 15q13-q14 locus, NPL scores from published studies were plotted for microsatellite markers from positive linkage reports (Fig. 1). A region was selected between *CHRFAM7A* and *ACTC*, where markers in the region had

NPL scores greater than 2.0. Genes in the region between *CHRFAM7A* (28,440,735) and *ACTC* (32,869,723) (March 2006 NCBI36/hg18) are listed in Tables 3–5. SNPs in *CHRFAM7A*, the partial duplication of *CHRNA7*, could not be surveyed for this study as the duplication prevents determination of heterozygotic and homozygotic status for polymorphisms (Gault *et al.*, 1998; Riley *et al.*, 2002). Telomeric to *ACTC*, the linkage signal declines. *B2M*, a gene telomeric to the 15q13-q14 linkage peak region was included in the fine-mapping study because its expression is elevated in schizophrenic nonsmokers and normalized in schizophrenic smokers (Mexal *et al.*, 2005).

Single nucleotide polymorphism selection

SNP selection within the 16 genes localized to the 15q13-q14 linkage peak region was performed with the Tagger algorithm as implemented in Haploview (Barrett *et al.*, 2005). TagSNPs were chosen with pairwise analyses, requiring settings $r^2 \geq 0.8$ between each ungenotyped SNP and at least one TagSNP, and a minimum minor allele frequency of 5%. TagSNPs from both the Yoruban African and CEPH (Utah residents with ancestry from northern and western Europe) populations were utilized in an attempt to capture all common genetic variation in both the Caucasian-non-Hispanic and African-American study participants. Exons 5–10 of the *CHRNA7* gene are duplicated (*CHRFAM7A*); therefore, only SNPs from exons 1 to 4 of the full-length gene were identified using HapMap.

Microsatellite selection

Three markers were selected within the region from previous studies. D15S128 and D15S1360 both provided convincing evidence for linkage in replicated reports (Freedman *et al.*, 1997; Kaufmann *et al.*, 1998; Liu *et al.*, 2001; Fallin *et al.*, 2003). Marker D15S659 was included even though it showed evidence of linkage in only one report (Gejman *et al.*, 2001); it is the closest significant microsatellite marker to the *B2M* gene. On the basis of highly significant *P* values in the region centromeric to *CHRNA7*, seven additional microsatellite markers were chosen to expand the region between marker D15S128 and *CHRNA7*. These markers included D15S113 (23,665,494), D15S1002 (25,423,217), D15S1019 (27,350,717), D15S1043 (27,925,207), D15S165 (28,947,842), D15S976 (29,239,627), and D15S1031 (29,775,416).

DNA isolation

Nucleated cells were obtained from anticoagulated blood (EDTA) by means of lysis with a high sucrose solution (0.3 mol/l of sucrose, 0.01 mol/l of Tris-HCl, pH 7.5, 0.005 mol/l of MgCl₂, 1% Triton X-100) and subsequent centrifugation. DNA was then isolated from these cells as previously described (Miller *et al.*, 1988). DNA samples for the NIMH Genetics Initiative participants were obtained from the NIMH collection at Rutgers (<http://www.rucdr.org/ASHG/Schizophrenia.htm>).

Genotyping assays

TagSNP genotyping was done using the Applied Biosystems SNPLEX assay on an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, California, USA). This method assays genotypes in up to 48 SNP PCR multiplex reactions using an oligonucleotide ligation procedure (Tobler *et al.*, 2005). Data analyses were carried out with the Applied Biosystems Genemapper software v4.0.

Twenty-seven probe pools were designed for this study, each containing approximately 47–48 SNP primers. All genotypes called by the Genemapper software were manually reviewed and rescored when necessary. If less than 95% of genotypes were called on any given pool, the entire assay was repeated only for samples in that pool missing at least 10 genotype

calls. For a probe/pool exceeding 95% of genotypes called, the assay was repeated only on samples for which no genotypes were obtained. SNPs for which the allele clusters were indistinguishable were excluded from analysis, as were SNPs not conforming to Hardy–Weinberg Equilibrium (HWE) in the control sample ($P < 0.01$).

Microsatellite marker loci were amplified by PCR using fluorescently (6-FAM) labeled primers and AmpliTaq Gold. The GeneAmp PCR System 9600 (Perkin-Elmer, Foster City, California, USA) was utilized for fragment amplification; thermal cyclers conditions were unique to each amplicon (Supplemental Table 1 <http://links.lww.com/PG/A24>). Fragments were characterized on an Applied Biosystems 3100 Avant Genetic Analyzer (Applied Biosystems, Foster City, California, USA). Genotypes were determined using the Genemapper 3.5 software (Applied Biosystems).

Statistical analysis

Case–control sample—The case group of individuals with schizophrenia included one randomly chosen schizophrenic individual per family with SAS version 9.1 software (SAS Institute Inc.). For each marker, allele frequencies were estimated and conformance of genotype frequencies with HWE expected proportions was tested in the control participants by means of a 1 *d.f.* χ^2 goodness-of-fit test using Haploview (Barrett *et al.*, 2005). For microsatellite loci, the exact test for deviations from HWE was calculated using PEDSTATS (Wigginton and Abecasis, 2005). Linkage disequilibrium parameters D' and r^2 were calculated for each pair of SNP markers, and tests for differences in allele frequencies between cases and controls were computed using a χ^2 test in Haploview. Chi-squared tests of association for each SNP and microsatellite marker under a genotypic model were calculated using the UNPHASED software version 3.0.13 (Frank Dudbridge, MRC Biostatistics Unit, Cambridge, UK) (Dudbridge, 2003). In addition, tests for differences in allele frequencies between cases and controls for each microsatellite marker were conducted in UNPHASED. To avoid spurious genetic associations because of multiple testing, a Bonferroni correction for the number of markers tested was applied, which is the most stringent correction for regional significance. We report nominal and corrected P values; corrected P values are indicated with an (*).

Odds ratio (OR) estimates and 95% confidence intervals (CI) for comparing relevant genotype or allele classes were computed with SPSS version 16.0 software (SPSS Inc., Chicago, Illinois, USA).

Power estimates, for case–control analyses at a significance level of 5%, were obtained with the Quanto software (Irvine, California, USA) (Gauderman, 2002a, 2002b). The Caucasian–non-Hispanic schizophrenic/control sample size had power between 0.74 and 0.99 to detect a genotype relative risk (GRR) greater than or equal to 2.0 over a range of marker allele frequencies between 0.10 and 0.40. At GRRs less than 2.0, power was substantially lower (0.12–0.88) for most allele frequencies. The African-American schizophrenic/control sample size had less power to detect a GRR greater than or equal to 2.0 (0.38–0.87) over a range of marker allele frequencies between 0.10 and 0.40. Similarly, at GRRs less than 2.0, power was considerably reduced (0.07–0.49) for most allele frequencies.

As a secondary analysis, a permutation procedure implemented with set-based tests in PLINK (Purcell *et al.*, 2007) was utilized to obtain empirical P values for each gene (corrected for all SNPs in a gene with 10000 permutations) with the a priori outcome of schizophrenia. Genotype and phenotype information for SNP rs3087454 was included in this analysis as this SNP is located in the upstream regulatory region of *CHRNA7*, and is significantly associated with schizophrenia in African-American and Caucasian–non-Hispanic case–control sample populations (Stephens *et al.*, 2009). Case–control samples of

both ethnicities, including with schizophrenia patients from the NIMH collection, were evaluated for the presence of population stratification in a previous study from our laboratory (Stephens *et al.*, 2009). These analyses indicated no significant differences in average admixture proportions between cases and controls within each ethnic group.

Family-based data—Before data analysis, the PEDCHECK software (Pittsburgh, Pennsylvania, USA) was utilized to detect errors in Mendelian inheritance (O'Connell and Weeks, 1999). Pedigree disequilibrium tests of association at SNP and microsatellite markers were implemented in the UN-PHASED software version 3.0.13 (Dudbridge, 2003). Similar to the case-control analyses, a Bonferroni correction was used to generate *P* values corrected for the number of markers tested.

Results

Genotyping assays and Hardy–Weinberg equilibrium conformance

Approximately 98.4% of the genotypes were collected for each of the 27 SNP probe pools. Of the 1264 SNPs surveyed in 16 genes (Table 3), 36 (2.8%) were eliminated from statistical analysis because their allele clusters were indistinguishable from one another. Of the remaining 1228 SNPs, all but 31 (2.5%) were consistent with HWE in one or more ethnic populations. Figure 2 shows the proportion of SNPs in Hardy–Weinberg disequilibrium, in each gene, across the region of interest. Several of these nonconforming SNPs lie in regions with CNVs associated with schizophrenia (Stefansson *et al.*, 2008; Stone *et al.*, 2008).

Association analyses

Case-control analyses with single nucleotide polymorphism markers—Results for single marker analyses under a genotypic model of association at a significance level of 5%, with the primary *a priori* outcome of schizophrenia as well as smoking and smoking in schizophrenia are shown in Table 6, and Fig. 3a and b. *P*-values were corrected for multiple testing utilizing a Bonferroni correction within ethnic group. A correction of 560 tests was applied to SNPs in the Caucasian-non-Hispanic sample as only 560 of the 1228 SNPs were polymorphic in Caucasians. A correction of 1043 tests was applied in the African-American sample. Figure 3a and b, displays $-\log P$ -value scatter plots for the outcome of schizophrenia. All positive associations were for SNPs in the intronic regions of the genes.

In the Caucasian sample population, five SNPs were significantly associated with schizophrenia under a genotypic model of association after a Bonferroni adjustment of the *P* values, designated with an (*): rs7171212, *MTMR15*; rs1035706, *TRPM1*; rs10162727, *TRPM1*; rs12439853, *KLF13*; and rs16957135, *RYR3*.

In the African-American sample population, nine SNPs were significantly associated with schizophrenia, under a genotypic model after Bonferroni correction: rs10162727, *TRPM1*; rs16956707, *KLF13*; rs11071532, *CHRNA7*; rs2651415, *CHRNA7*; rs11071897, *SGNE1*; rs16973259, *GREM1*; rs7173813, *RYR3*; rs12917360, *RYR3*; and rs527834, *AVEN*. Of the 15 SNPs associated with schizophrenia in one or more ethnic populations, six are located in genes centromeric to *CHRNA7* (*MTMR15*, *TRPM1* and *KLF13*), two reside in the *CHRNA7* gene, and seven are located in genes telomeric to *CHRNA7* (*SGNE1*, *GREM1*, *RYR3* and *AVEN*). Results of allelic tests of association supported results obtained under a genotypic model and are shown in Supplementary Tables 2(A) <http://links.lww.com/PG/A25>, and 2(B) <http://links.lww.com/PG/A26>. Additional exploratory analyses were included under the assumption that COS schizophrenics may be biologically distinct from non-COS schizophrenics. To explore this hypothesis, the *a priori* analysis was repeated with the COS

cases excluded to determine whether results changed considerably. *P* values were very similar to those obtained with the combined groups.

Utilizing smoking as an outcome with the Caucasian-non-Hispanic sample, one SNP located in *CHRNA7* (rs8028396) had a significant *P* value ($P = 0.006^*$) under an allelic model of association and a nominally significant *P* value ($P = 0.0002, 0.112^*$) with a genotypic model. This same SNP was associated with smoking in schizophrenia ($P = 0.0045^*$) with an allelic model, and suggestive ($P = 0.056^*$) with a genotypic model. In the African-American sample, with the outcome of smoking, a SNP located in *RYR3* (rs8035184) had a significant *P* value ($P = 0.041^*$) under a genotypic model of association. This SNP was nominally significant ($P = 0.034$) for an association with smoking in schizophrenia.

Table 6 shows the ORs for a primary outcome of schizophrenia for SNPs significant in the case-control analysis. In the Caucasian-non-Hispanic sample, ORs range from 1.72 (95% CI = 1.31–2.81) for SNP rs7171212 (*MTMR15*), to 3.97 (95% CI = 2.38–5.88) for rs10162727 (*TRPM1*). In the African-American sample population, ORs range from 1.66 (95% CI = 1.09–2.53) for rs11071532 (*CHRNA7*) to 11.89 (95% CI = 5.2–27.16) for rs16973259 (*GREM1*). 95% CI are much narrower for SNPs significant in the Caucasian-non-Hispanic population because of the larger sample size.

Results of a secondary analysis, utilizing a gene (set)-based permutation procedure (PLINK) (Purcell *et al.*, 2007) are shown in Tables 7 and 8. The gene-based empirical *P* value (column 5) was calculated from the mean of the single SNP statistics, permuted 10000 times. In the Caucasian-non-Hispanic sample, gene-based empirical *P* values were significant for *TRPM1*, *KLF13*, *RYR3*, and suggestive for *CHRNA7* (empirical $P = 0.05$). In the African-American sample, empirical *P* values were significant for *TRPM1*, *KLF13*, *CHRNA7*, *SGNE1*, *GREM*, and *RYR3*.

Family-based association analyses with single nucleotide polymorphisms—In Caucasian-non-Hispanic families, three SNPs were associated with schizophrenia (rs2911855 $P = 0.01^*$, rs2288242 $P = 0.039^*$, rs4396507 $P = 0.002^*$). Two of these SNPs are in *TRPM1* (rs2911855 and rs2288242) and one in *SGNE1* (rs4396507). In African-American family members there were no SNPs significant for an association with schizophrenia, however, the *P* value for rs2911855 (*TRPM1*) was nominally significant ($P = 0.0009, P = 0.94^*$). No significant *P* values were obtained for the outcomes of smoking, smoking in schizophrenia. Tables 9 and 10 show the results of family-based association studies with single SNPs.

Association analyses with microsatellite markers—In the case-control sample, there were no significant associations with the nine microsatellite markers included in the statistical analysis after adjusting for multiple comparisons. Results are shown in Supplementary Tables 3(A) <http://links.lww.com/PG/A27>, 3(B) <http://links.lww.com/PG/A28>, and 3(C) <http://links.lww.com/PG/A29>.

Family-based-association tests with microsatellite markers found one significant association after adjusting for multiple comparisons. D15S1043 was associated with smoking ($P = 0.032^*$) and nominally associated with smoking in schizophrenia ($P = 0.01$) in Caucasian-non-Hispanic participants. This microsatellite marker, which lies upstream of the *CHRNA7* gene, has been implicated in schizophrenia in two previous linkage studies (Riley *et al.*, 2000; Liu *et al.*, 2001).

Discussion

In this study, genetic variation was surveyed in genes mapping to a region on chromosome 15 with consistent evidence for linkage to schizophrenia. A portion of this sample consisted of families from the NIMH Human Genetics Initiative for Schizophrenia which had been previously linked to the 15q13-14 region (Kaufmann *et al.*, 1998; Leonard *et al.*, 1998; and Leonard, 2001; Freedman *et al.*, 2001b). Fine mapping of genes in the region and association analyses of case-control participants continue to support the *CHRNA7* gene as a candidate for schizophrenia. These results are consistent with a recent large association study by Sanders *et al.* (2008) in which investigators found two SNPs in *CHRNA7* nominally positive for schizophrenia. Although the same SNPs were not examined in this study, we found other positive SNPs within 50kb of those reported. Stefansson *et al.* (2008) also found nominally significant association with SNPs in the same region.

A limitation in this study, and other genome-wide-association studies, is the incomplete interrogation of *CHRNA7*. Exons 5–10 and intervening introns in *CHRNA7* are duplicated, forming the chimeric gene, *CHRFAM7A*. The homology of the duplicated *CHRNA7* sequence is nearly 100% (Gault *et al.*, 1998). Thus, not all SNPs in *CHRNA7* can be evaluated, possibly resulting in an underestimate of association. Although next generation sequencing of the *CHRNA7* gene cluster may be possible in the future, current sequencing technologies would require cloning of the duplicated regions from each participant. There is still considerable evidence for *CHRNA7* as a candidate gene for schizophrenia, including biological studies in humans and animals, as well as linkage and association studies. We have mapped polymorphisms unique to the coding region of the full length gene and its duplication (Gault *et al.*, 2003); however, most of these SNPs are rare and as a consequence have limited power to detect association with schizophrenia.

Two SNPs were associated with smoking and smoking in schizophrenia. One SNP in *CHRNA7* (rs8028396) was significant for association with smoking and smoking in schizophrenia in the Caucasian-non-Hispanic population, under an allelic model. Overall, SNPs associated with smoking and smoking in schizophrenia were not associated with an outcome of schizophrenia. These results indicate that significant associations found in these studies are to schizophrenia and not to smoking in schizophrenia.

Results of secondary analysis with a gene-based set test supported results of single SNP analysis of genes in this region. This analysis included SNP rs3087454 (*CHRNA7* 5' UTR) in the derivation of the gene-based empirical *P* value. This SNP, in a nonduplicated region, has previously been associated with schizophrenia (Stephens *et al.*, 2009), and with improvement in a functional MRI measure in the disorder after administration of an $\alpha 7$ receptor agonist (Tregellas *et al.*, 2010). Although single SNP analysis in the Caucasian-non-Hispanic sample found no significant associations, inclusion of rs3087454 resulted in a suggestive empirical *P* value for *CHRNA7* in this same sample.

The number of informative families in the family-based sample limited positive findings in this part of the study. Although there were 73 Caucasian-non-Hispanic families, the number available for family-based association tests in UNPHASED averaged 15. Similarly, there was an average of only 10 informative African-American families out of 47. Significant *P* values were obtained in Caucasian-non-Hispanic family members at two SNPs in the *TRPM1* gene (rs2911855 and rs2288242). There were more informative families for SNPs rs2911855 and rs2288242 (16 and 18, respectively) compared with SNP rs10162727 (12 informative families in Caucasian-non-Hispanics). However, SNP rs10162727 was still significant in both Caucasian-non-Hispanic and African-American case-controls. Although no SNPs in African-American family members passed the threshold for significance, a

nominally significant *P* value at rs2911855 (*TRPM1*) with schizophrenia lent further support to this gene. Thus, family-based association studies supported some results obtained in the case– control samples.

Evidence of association for three additional genes was seen in both ethnicities. *KLF13* is a zinc–finger protein belonging to a large superfamily of transcription factors (Berg, 1990; Klug and Schwabe, 1995). The SP/XKLF family is a subgroup of this superfamily and is composed of factors that bind to GC-rich and related GT or CACCC boxes (Hagen *et al.*, 1992; Sogawa *et al.*, 1993; Cook *et al.*, 1998). *KLF13* is conserved across species and is expressed mainly in the heart and skeletal muscle, but also in brain, liver, and the epithelial layer of the dermis (Schohy *et al.*, 2000; Lavalley *et al.*, 2006). The *CHRNA7* promoter is GC rich with multiple Sp-family binding sites (Leonard *et al.*, 2002). Thus, it is possible that both *CHRNA7* and *KLF13* may confer susceptibility to schizophrenia.

TRPM1 functions to mediate Ca^{++} entry into the cell (Xu *et al.*, 2001b). When *TRPM1* is expressed in HEK293 cells, it increases the cytoplasmic-free Ca^{++} concentration (Miller *et al.*, 2004). The gene is expressed in brain at low levels (Massullo *et al.*, 2006). Although the expression profile of *TRPM1* has been established, the cellular, biochemical, and electrophysiological characteristics of the channel are not well understood.

Ryanodine receptors, such as *RYR3*, are intracellular ion channels that release Ca^{++} from intracellular stores after transduction of external stimuli (Hakamata *et al.*, 1992). *RYR3* is abundantly expressed in brain and skeletal muscle and faintly expressed in the heart (Nakashima *et al.*, 1997). Ryanodine receptors, including *RYR3*, have been localized to rat hippocampus, specifically in CA1 pyramidal neurons known to be involved in long-term potentiation (Van de Vrede *et al.*, 2007). Furthermore, enhancement of long-term potentiation by nicotine activation of $\alpha 7$ receptors is dependent on ryanodine receptor release of calcium from intracellular stores in the rat dentate gyrus (Welsby *et al.*, 2006). It is possible that a dysfunctional or nonfunctional *RYR3* gene may blunt or alter the proper downstream signaling of activated $\alpha 7$ receptors.

These three genes are located both centromeric (*TRPM1* and *KLF13*) and telomeric (*RYR3*) to the *CHRNA7* candidate gene. The rs10162727 SNP in *TRPM1* was of particular interest as it was highly significant in both ethnic populations. SNP rs10162727 (C/T) is located in intron 16 of the *TRPM1* gene. The C/C genotype was significantly more prevalent in schizophrenics in both ethnicities. In African-Americans, the association signal seems to be driven by a lack of heterozygotes in the case group. According to the HapMap database (www.hapmap.org), the region surrounding this polymorphism has multiple CNVs. Large recurrent copy number deletions in genes *TRPM1* and *KLF13* were positively associated with schizophrenia in the study by Stefansson *et al.* (2008). It is reasonable to propose that the association in African-Americans may represent individuals with deletion variations.

We found a disproportionately high number of SNPs not in conformance with HWE in *TRPM1*, *KLF13*, *CHRNA7*, and *RYR3* as compared with the region telomeric of these genes. In human populations, HWE is common and deviations are most likely because of genotyping error or population stratification (Wigginton *et al.*, 2005). Genotyping error seems unlikely to be a large contributing factor as quality control checks, and pedigree checks for Mendelian inconsistencies in family member data were performed. There was no strong stratification in case– control populations in this dataset, based on a screen of 176 ancestry informative markers (Stephens *et al.*, 2009).

The 15q11-q14 region is one of the most dynamic regions of the human genome, containing a wide range of clinically recognized rearrangements including deletions, duplications,

inversions, and translocations (Gault *et al.*, 1998; Locke *et al.*, 2004; Flomen *et al.*, 2008). An intriguing explanation for the lack of HWE in this region is the surprising number of CNVs and in particular, common deletion variants (Gault *et al.*, 1998; Miller *et al.*, 2009; Van Bon *et al.*, 2009; Szafranski *et al.*, 2010). CNVs, genomic sequences which can encompass entire genes, are uncommon (< 3%), and their occurrence varies between populations (Redon *et al.*, 2006). Regions flanking segmental duplications (blocks of DNA ranging from 1 to 400 kb which occur at more than one site in the genome and share > 90% sequence identity) are susceptible to rearrangement by nonallelic homologous recombination. These regions represent hotspots of genomic instability which are prone to CNV (Sharp *et al.*, 2008; Szafranski *et al.*, 2010). A segregating deletion may leave 'footprints' in SNP genotype data by causing runs of homozygosity, Mendelian inconsistencies, and Hardy–Weinberg disequilibrium (McCarroll *et al.*, 2006).

Although small duplications or deletions and associated CNVs are primary mechanisms for the creation of new genes, such as *CHRFAM7A* (Gault *et al.*, 1998), recent data links them with susceptibility to common diseases including autoimmune disorders (Fanciulli *et al.*, 2007; Yang *et al.*, 2007), mental retardation (Sharp *et al.*, 2008), cancer (Lieberfarb *et al.*, 2003; Zhao *et al.*, 2004; Zhao *et al.*, 2005) and schizophrenia (Suturala *et al.*, 2007; Stefansson *et al.*, 2008; Stone *et al.*, 2008; Sinkus *et al.*, 2009). Importantly, genes associated with schizophrenia in this study and with a disproportionately large number of SNPs not in HWE are located in this region of genomic instability. Within these larger regions of instability, the smaller (300 kb) partial duplication of *CHRNA7* occurred (Gault *et al.*, 1998; Riley *et al.*, 2002) at some time during human divergence (Flomen *et al.*, 2008). An even smaller 2 bp deletion in exon 6 of the duplicated gene, *CHRFAM7A*, is associated with schizophrenia (Sinkus *et al.*, 2009) and is in linkage disequilibrium with an inversion (Flomen *et al.*, 2008). These reports lend further evidence to the possibility that increased risk of schizophrenia, associated with this region in this study, is partially a consequence of its chromosomal instability. Our current finding that multiple genes in this region are strongly associated with schizophrenia suggests that a combination of genetic mutations in this region and CNV may contribute to the development of mental illness.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1.

Nonparametric linkage (NPL) scores for 15q13-q14. NPL scores are plotted for seven linkage studies providing support for 15q13-q14. Specific variable number tandem repeat markers are shown along the x-axis. Genes in the region are shown at the top using GenBank nomenclature. For some studies, LOD scores were converted to NPL scores using the following formula, $LOD = (NPL_{stat}) / (2 \ln 10)$. The red line delineates the 15q13-q14 peak region selected for screening.

Fig. 2.

Proportion of single nucleotide polymorphisms (SNPs) in Hardy–Weinberg disequilibrium in genes surveyed across the 15q13–q14 linkage region for schizophrenia. BP4 and BP5 are breakpoints for Prader–Willi/Angelman syndromes. Approximate locations of deletions associated with mental retardation and schizophrenia as well as segmental duplications at 15q13–q14 are shown along the *x*-axis. HWE, Hardy–Weinberg equilibrium.

Fig. 3.

(a) Results of fine mapping for genes in the 15q13-q14 region in the Caucasian-non-Hispanic sample population. This scatter plot shows $-\log P$ values for single marker analyses with schizophrenia under a genotypic model of association. Scatter plots were generated with nominal P values. Adjusted P values for significant markers are displayed above the data point of interest and were generated with a Bonferroni correction. A separate plot was created for *B2M* because of its location relative to the linkage peak region. (b) Results of fine mapping for genes in the 15q13-q14 region in the African-American sample population. This scatter plot shows $-\log P$ values for single marker analyses with

schizophrenia under a genotypic model of association. Corrected P values for specific markers are indicated with *.

Table 1
Sample set for case-control and family-based association studies, with the a priori outcome of schizophrenia

Case-control	African-American	Caucasian	Totals
Total SZ	105	363	468
Denver SZ	56	234	290
NIMH SZ	47	73	120
COS	2	56	58
Total controls	45	99	144
NIMH families			
Schizophrenics	113	142	255
Family members	32	42	74

COS, childhood onset schizophrenics collected locally in Denver; Denver SZ, schizophrenics collected locally; NIMH, National Institute of Mental Health; NIMH SZ, schizophrenic probands from the NIMH Schizophrenia Genetics Initiative included in case-control association studies; SZ, schizophrenics; Family members, NIMH family members with a diagnosis of never mentally ill.

Table 2
Sample set for case-control and family-based association studies with the outcome of smoking status

Case-control	African-American	Caucasian	Totals
Total smokers	59	170	229
Denver smokers	54	160	214
NIMH smokers	5	10	15
Total nonsmokers	64	125	189
Denver nonsmokers	49	112	161
NIMH nonsmokers	15	13	28
NIMH families			
Smokers	17	45	62
Nonsmokers	25	51	76

Denver smokers, smokers collected locally; NIMH, National Institute of Mental Health; NIMH smokers, proband smokers from the NIMH Schizophrenia Genetics Initiative included in case-control association studies. Smoking information was available for most individuals utilized in case-control and family-based association studies. Former smokers were excluded from association studies with the outcome of smoking status.

Table 3
Single nucleotide polymorphisms (SNPs) genotyped in the 15q13-q14 linkage peak region

Gene	Locus	Size (kb)	Function	Number of TagSNPs
<i>MTMR15</i>	28983421–29022600	39.2	Myotubularin related protein 15	25 ^a
<i>MTMR10</i>	29018436–29071099	52.7	Myotubularin related protein 10	32 ^a
<i>TRPM1</i>	29080844–2918216	100.4	Transient receptor potential cation channel	100
<i>KLF13</i>	29406374–29457393	51	Transcription factor	25
<i>CHRNA7</i>	30110017–30248525	138.5	Neuronal nicotinic acetylcholine receptor subunit	83
<i>ARHGAP11A</i>	30694982–30719160	28.2	Rho-GTPase activating protein 11A	4
<i>SGNE1</i>	30721251–30776590	55.3	Secretory granule, neuroendocrine protein 1	70
<i>GREM1</i>	30797593–30814158	16.6	Cystein knot superfamily 1	15
<i>RYR3</i>	31390468–31945593	555.1	Ryanodine receptor 3	718
<i>AVEN</i>	31945720–32118584	172.9	Cell death regulator	127
<i>CHRM5</i>	32097719–32144579	46.9	Cholinergic receptor, muscarinic 5	21
<i>SLC12A6</i>	32312957–32398222	87.3	Potassium chloride cotransporter KCC3	41
<i>GOLGA8B</i>	32458563–32466,982	10.7	Golgin-67	1
<i>CX36</i>	32831970–32833981	2	Connexin 36	2
<i>ACTC</i>	32869723–32875153	5.4	Cardiac muscle a actin proprotein	5
<i>B2M</i>	42790976–42797649	6.67	Beta 2 microglobulin precursor	6

^aGenes MTMR15 and MTMR10 partially overlap and share 11 tagSNPs (March 2006 NCBI36/hg18).

Table 4
Significant *P* values (genotype tests) for the case-control analysis in Caucasian-non-Hispanics

Gene	SNP	Schizophrenia vs. no diagnosis	Smokers vs. nonsmokers	SZ smokers vs. SZ nonsmokers
<i>MTMR15</i>	rs7171212	7.6E ⁻⁵ , 0.042 [*]	0.086	0.824
<i>TRPM1</i>	rs1035706	3.4E ⁻⁵ , 0.019 [*]	0.007, 1 [*]	0.062
<i>TRPM1</i>	rs10162727	5.4E ⁻¹² , 3E ⁻⁹ [*]	0.282	0.543
<i>KLF13</i>	rs12439853	4E ⁻⁶ , 0.002 [*]	0.603	0.394
<i>CHRNA7</i>	rs8028396	0.762	0.0002, 0.112 [*]	0.0001, 0.056 [*]
<i>RYR3</i>	rs16957135	3.7E ⁻⁵ , 0.02 [*]	0.641	0.017, 1 [*]

SNPs, single nucleotide polymorphisms; SZ, schizophrenic.

^{*} *P* values with a Bonferroni correction.

Table 5
Significant *P* values (genotype tests) for the case-control analysis in African-Americans

Gene	SNP	Schizophrenia vs. no diagnosis	Smokers vs. nonsmokers	SZ smokers vs. SZ nonsmokers
<i>TRPM1</i>	rs10162727	4.4E ⁻⁹ , 4.6E ⁻⁶	0.197	0.595
<i>KLF13</i>	rs16956707	9E ⁻¹³ , 9.4E ⁻¹⁰ *	0.805	0.805
<i>CHRNA7</i>	rs11071532	3E ⁻⁶ , 0.003*	0.154	0.154
<i>CHRNA7</i>	rs2651415	7E ⁻⁷ , 7.3E ⁻⁴ *	0.822	0.981
<i>SGNE1</i>	rs11071897	1.9E ⁻⁵ , 0.02*	0.028, 1*	0.005, 1*
<i>GREM1</i>	rs16973259	2E ⁻¹² , 2.1E ⁻⁹ *	0.268	0.312
<i>RYR3</i>	rs7173813	7E ⁻⁶ , 0.0073*	0.679	0.996
<i>RYR3</i>	rs12917360	6E ⁻⁶ , 0.0063*	0.085	0.569
<i>RYR3</i>	rs9672919	0.0008, 0.834*	0.811	0.212
<i>RYR3</i>	rs8035184	0.0004, 0.417*	4E ⁻⁵ , 0.041*	0.034, 1*
<i>AVEN</i>	rs527834	3E ⁻⁶ , 0.0031*	0.946	0.314

SNPs, single nucleotide polymorphisms; SZ, schizophrenic.

* *P* values with a Bonferroni correction.

Table 6
Odds ratios (ORs) for single nucleotide polymorphisms (SNPs) significant in the case-control analysis

Gene	SNP	Position	Major/minor	MAF	Risk allele	OR	95% CI
Caucasian-non-Hispanic sample population							
<i>MTMR15</i>	rs7171212	28995003	C/T	0.236	C	0.56	0.36–0.88
<i>TRPM1</i>	rs1035706	29130367	G/A	0.167	G	2.42	1.58–3.69
<i>TRPM1</i>	rs10162727	29131168	C/T	0.102	C	3.97	2.68–5.88
<i>KLF13</i>	rs12439853	29422025	G/A	0.155	G	2.14	1.43–3.21
<i>RYR3</i>	rs16957135	31724161	A/G	0.155	A	2.63	1.72–4.02
African-American sample population							
<i>TRPM1</i>	rs10162727	29131168	C/T	0.309	C	2.01	1.26–3.21
<i>KLF13</i>	rs16956707	29428915	G/A	0.226	G	4.04	2.27–7.07
<i>CHRNA7</i>	rs11071532	30148583	T/A	0.441	T	1.66	1.09–2.53
<i>CHRNA7</i>	rs2651415	30230746	C/T	0.435	C	2.57	1.67–4.09
<i>SGNE1</i>	rs11071897	30758430	G/A	0.221	G	2.39	1.45–3.94
<i>GREM1</i>	rs16973259	30807915	C/G	0.162	C	11.89	5.2–27.16
<i>RYR3</i>	rs7173813	31477080	G/A	0.321	A	2.6	1.47–4.62
<i>RYR3</i>	rs12917360	31819956	C/G	0.113	C	4.09	1.91–8.77
<i>RYR3</i>	rs9672919	31835138	A/T	0.151	A	3.09	1.67–5.74
<i>AVEN</i>	rs527834	32060559	C/T	0.486	T	2.84	1.68–4.81

CI, confidence interval; MAF, minimum minor allele frequency.

ORs were obtained utilizing logistic regression with an additive model.

Table 7
Results of gene-based set-tests in Caucasian-non-Hispanic participants with the outcome of schizophrenia

Gene	# SNPs	# Sig SNPs	Significant SNPs	Emp <i>P</i> value
<i>MTMR15/MTMR10^a</i>	37	2	rs4779794, rs7171212	0.086
<i>TRPM1</i>	41	2	rs10162727, rs1035706	0.002
<i>KLF13</i>	9	1	rs12439853	0.03
<i>CHRNA7</i>	31	2	rs1909884, rs3087454	0.05
<i>ARHGAP11A</i>	2	0	NA	1
<i>SGNE1</i>	37	0	NA	1
<i>GREM1</i>	5	0	NA	1
<i>RYR3</i>	322	3	rs11852941, rs16957135, rs16958081	0.03
<i>AVEN</i>	39	1	rs591036	0.104
<i>CHRM5</i>	9	0	NA	1
<i>SLC12A6</i>	16	0	NA	1
<i>GOLGA8B</i>	1	0	NA	1
<i>CX36</i>	2	0	NA	1
<i>ACTC</i>	4	0	NA	1
<i>B2M</i>	5	0	NA	1

Emp *P* value, gene-based empirical *P* value permuted 10 000 times; NA, not available; SNPs, single nucleotide polymorphisms; # Sig SNPs, number of significant SNPs for each gene; # SNPs, number of SNPs surveyed in this gene.

P values < 0.05 are italicized in bold.

^a Genes *MTMR15* and *MTMR10* were considered as one set as they partially overlap and share tagSNPs.

Table 8
Results of gene-based set-tests in African-American participants with the outcome of schizophrenia

Gene	# SNPs	# Sig SNPs	Significant SNPs	Emp <i>P</i> value
<i>MTMR15/MTMR10</i> ^a	35	1	rs11293	0.1
<i>TRPM1</i>	84	4	rs10162727, rs1035706, rs2042613, rs7178472	0.0002
<i>KLF13</i>	18	4	rs11858244, rs16956707, rs1865872, rs1989684	9.99E⁻⁵
<i>CHRNA7</i>	66	5	rs11071532, rs2651415, rs3087454, rs8026798, rs8041763	9.99E⁻⁵
<i>ARHGAP11A</i>	4	0	N/A	1
<i>SGNE1</i>	55	3	rs11071897, rs16964412, rs7169422	0.0075
<i>GREM1</i>	13	1	rs16973259	0.0002
<i>RYR3</i>	603	5	rs12917360, rs7162287, rs7173813, rs8035184, rs9672919	9.99E⁻⁵
<i>AVEN</i>	101	2	rs10519901, rs527834	0.09
<i>CHRM5</i>	22	0	N/A	1
<i>SLC12A6</i>	29	3	rs17817800, rs2615358, rs4780235	0.151
<i>GOLGA8B</i>	1	0	N/A	1
<i>CX36</i>	2	1	rs3743123	0.06
<i>ACTC</i>	5	2	rs3729755, rs7166484	0.106
<i>B2M</i>	5	0	N/A	1

Emp *P* value, gene-based empirical *P* value permuted 10 000 times; NA, not available; SNPs, single nucleotide polymorphisms; # Sig SNPs, number of significant SNPs for each gene; # SNPs, number of SNPs surveyed in this gene.

P values < 0.05 are italicized in bold.

^a Genes *MTMR15* and *MTMR10* were considered as one set as they partially overlap and share tagSNPs.

Table 9
Significant *P* values (genotype tests) for family-based analysis in Caucasian-non-Hispanics

Gene	SNP	Schizophrenia vs. no diagnosis	Smokers vs. nonsmokers	SZ smokers vs. SZ nonsmokers
<i>MTMR15</i>	rs7171212			
<i>TRPM1</i>	rs2911855	2E ⁻⁵ , 0.01 [*]	0.931	0.791
<i>TRPM1</i>	rs2288242	7E ⁻⁵ , 0.039 [*]	0.2	0.396
<i>TRPM1</i>	rs1035706	0.404	0.413	0.305
<i>TRPM1</i>	rs10162727	0.004, 1 [*]	0.027, 1 [*]	0.46
<i>KLF13</i>	rs12439853	0.152	0.101	0.394
<i>CHRNA7</i>	rs8028396	0.934	0.059	0.251
<i>SGNE1</i>	rs4396507	4E ⁻⁶ , 0.002 [*]	0.02, 1 [*]	0.359
<i>RYR3</i>	rs16957135	0.364	0.125	0.261

SNP, single nucleotide polymorphism; SZ, schizophrenic.

In addition, *P* values are provided for SNPs of interest from case-control results.

* *P* values were corrected for multiple tests with a Bonferroni correction.

Table 10
Significant *P* values (genotype tests) for the family-based analysis in African-Americans

Gene	SNP	Schizophrenia vs. no diagnosis	Smokers vs. nonsmokers	SZ smokers vs. SZ nonsmokers
<i>TRPM1</i>	rs10162727	0.149	0.181	0.589
<i>KLF13</i>	rs16956707	0.522	0.923	0.219
<i>CHRNA7</i>	rs11071532	0.07	0.212	0.138
<i>CHRNA7</i>	rs2651415	0.608	0.411	0.763
<i>SGNE1</i>	rs11071897	0.468	0.171	0.122
<i>GREM1</i>	rs16973259	0.138	0.33	0.336
<i>RYR3</i>	rs7173813	0.636	0.252	0.224
<i>RYR3</i>	rs12917360	0.523	0.085	0.569
<i>RYR3</i>	rs9672919	0.854	0.638	0.196
<i>RYR3</i>	rs8035184	0.177	0.008, 1*	0.071
<i>AVEN</i>	rs527834	0.210	0.867	0.847

SNP, single nucleotide polymorphism; SZ, schizophrenic.

In addition, *P* values are provided for SNPs of interest from case-control results.

* *P* values with a Bonferroni correction.