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Replication of Association Between Working Memory and *Reelin*, a Potential Modifier Gene in Schizophrenia

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Abstract

Background: The challenges in gene identification for psychiatric disorders have awakened interest toward quantitative traits and endophenotypes that are potentially more closely related to the underlying biology and provide more power in the linkage and association analyses. Previously, we successfully replicated schizophrenia linkage on chromosome 7q21-32 in Finnish families and demonstrated that an intragenic short tandem repeat (STR) allele of the regional *Reelin* (RELN) gene is associated with multiple cognitive traits representing central cognitive functions regarded as valid endophenotypes for schizophrenia.

Methods: Here, we used an extended sample of 290 Finnish families with schizophrenia and 375 control subjects in an association analysis between 96 SNPs and three STRs in *RELN* and diagnostic categories, clinical disorder features, as well as central cognitive functions impaired in schizophrenia.

Results: We replicated the original association between *RELN* intragenic STR allele and working memory in individuals ($n = 342$) not overlapping with the previous study. This risk allele remained central in the whole study sample by being associated with impaired cognitive functioning and more severe positive and negative symptoms of schizophrenia ($p = .0005$ – $.00002$). Additionally, multiple SNPs indicated association with the severity of positive symptoms of schizophrenia and together showed potential additive effect on the severity of the symptoms ($p = .0000001$). However, no significant associations with clinical diagnostic categories emerged.

Conclusions: The strongest effects on cognitive functions were detected among the affected individuals. We thus propose a particular role for *RELN* as a modifier gene of the pathogenesis of schizophrenia.

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Keywords

Endophenotype; memory; neuropsychology; psychiatry; RELN; schizophrenia

Identification of genetic risk variants has proven tedious for schizophrenia (MIM 181500) (1), a severe mental disorder with lifetime prevalence of .2%–1.2% worldwide (2), despite its neurodevelopmental origin (3) and heritability estimates as high as 83%–88% (4). Probably, only very few patients share identical genetic liability, and the predisposing variant combinations might differ substantially, especially in families not sharing the same ancestry. Furthermore, different amount and/or combinations of the variants might result in distinct phenotypes (1,3,5).

A special challenge in finding predisposing variants for complex disorders is the phenotypic heterogeneity within the core diagnosis (6). This variation, for instance in positive and negative symptoms of schizophrenia, might stem from effects of modifier genes, which supposedly have little or no effect on the disease liability itself (7). Although these modifier genes might affect some features in unaffected individuals as well (8), they are likely to display more significant effects in combination with the liability-increasing variants, that is, among the affected individuals (9).

A strategy to decompose the disorder—independently of diagnostic criteria—is the use of endophenotypes, intermediate factors between clinical phenotype and genotype, which are measurable but not directly detectable without “an aided eye” (10,11). The endophenotypes are assumed to involve the same biological pathways as the disorder but to have a simpler etiologic background and to relate more closely to relevant gene effects (10,11). In schizophrenia, general cognitive impairment is a core feature, and cognitive deficits precede the onset of the disorder (12). Most importantly, they appear in milder forms in healthy relatives of the patients, supporting their genetic background (13). Quantitative scores derived from neuropsychological test performance measures have been suggested as valid endophenotypes for schizophrenia (14) and other psychotic disorders (15), supported by their relatively high heritability estimates (16,17).

Our previous study replicated schizophrenia linkage on chromosome 7q21-32 harboring *reelin* (*RELN*) (MIM 600514) gene and detected association between a *RELN* intragenic short tandem repeat (STR) and multiple cognitive traits (18) covering the central cognitive functions impaired in schizophrenia (19). The *RELN* encodes for a glycoprotein essential in development and synaptic plasticity of central nervous system and evidently plays a role in various neurodevelopmental disorders (20).

Here, we determined the allelic variation of *RELN* by using 99 intragenic and flanking markers in a nationwide sample of 290 nuclear schizophrenia families and 375 independent control subjects from the isolated Finnish population (21) with limited genetic diversity (22) and high linkage disequilibrium (LD) between genetic markers (23). This study aimed to replicate our previous association between *RELN* and cognitive functions in an independent subsample and by considerably expanding the previous marker coverage and using several novel traits, to investigate further whether *RELN* variants modify clinical features of schizophrenia or are associated with cognitive disturbances in schizophrenia families and in general population (18).

Methods and Materials

Study Samples

The schizophrenia sample of 290 nuclear families was part of the Finnish schizophrenia family sample collected by the National Institute for Health and Welfare (formerly National Public Health Institute) using three nationwide medical registers (Hospital Discharge Register and Pension and Reimbursement Registers) and the Population Register Centre for pedigree information. The collected families were either from an internal isolate (IS) with higher lifetime morbid risk (3.2%) of schizophrenia (24) than in the general population (1.1%) (25) or outside the isolate (AF) (all Finland) with multiple affected family members. Each family included at least one affected individual born between 1940 and 1976, with the first schizophrenia diagnosis in any of the registers made between 1969 and 1998. Clinical data were collected from all mental health treatment contacts, and final diagnostic assessments was made independently by two or three—if necessary to gain consensus—psychiatrists or psychiatric residents according to the DSM-IV criteria (26). One of the psychiatrists also completed the Operational Criteria Checklist (OPCRIT) (27). Of this sample, altogether 983 affected and unaffected individuals were interviewed with the Structured Clinical Interview for DSM-IV (SCID) (28) and tested with comprehensive neuropsychological test battery (16). Additionally, the interview included the Scale for the Assessment of Positive Symptoms (SAPS) (29) for affected individuals and the Scale for the Assessment of Negative Symptoms (SANS) (30) for affected individuals as well as their healthy family members. In all, this series included 183 families from our previous study (18) (819 genotyped individuals), 84 new genotyped individuals belonging to those families, as well as 107 new independent families (356 genotyped individuals).

The schizophrenia sample was additionally divided into two genealogical subcategories: families from the internal isolate (IS), and families outside the isolate (AF). The inclusion criteria for families from the isolate were based primarily on identical-by-state (IBS) clustering information from families ($n = 198$) with a member genotyped with Illumina HumanHap300 platform (Illumina, San Diego, California) as part of the SGENE Consortium (<http://www.sgene.eu/>) or, secondarily, on at least one maternal and one paternal grandparent born in the geographic isolate region ($n = 92$). This division is justified by the genetic substructure of the Finnish population (31,32) and by our previous schizophrenia linkage and *RELN* association with cognitive traits observed only in the AF families (18) (Table 1).

The control sample, nested within the nationwide Health 2000 survey (<http://www.terveys2000.fi/>) of 9922 Finnish adults altogether from 80 municipalities, comprised 375 unrelated individuals altogether. Of these, 205 were chosen from the Psychoses in Finland (PIF) (25) and 82 from the Mental Health in Early Adulthood (33) subsamples on the basis of available neuropsychological test data and history of no psychotic disorder. The remaining 88 individuals, of which 43 were neuropsychologically assessed similarly as in the PIF study (25), were selected for regional control subjects living in or near the internal isolate (24) (Table 1).

This study has been approved by the Ethics Committees of the National Institute for Health and Welfare and the Hospital District of Helsinki and Uusimaa, Finland, and by the appropriate institutional review boards of the participating institutions. Written informed consent was obtained from all the participants.

Test Variables

Qualitative Traits.—In the schizophrenia sample, three diagnostic classes were used: CORESCH, consisting of schizophrenia only; SCHSPECT, adding schizophrenia spectrum psychotic disorders (schizoaffective, schizophreniform, delusional, brief psychotic, psychotic, and shared psychotic disorders); and ANYPSY, adding major depressive disorder with psychotic features and both bipolar type I and II disorders with psychotic features (Table 1).

Quantitative Traits.—In the schizophrenia and control samples, the selected test variables from the Wechsler Memory Scale-Revised (WMS-R) (34), the Wechsler Adult Intelligence Scale-Revised (WAIS-R) (35), the California Verbal Learning Test (CVLT) (36), the Trail-Making Test (TMT) (37), and the Stroop Task Interference Score (38) (Table 2) covered the central cognitive functions impaired in schizophrenia: attention, working memory, verbal learning and memory, information processing, and executive functioning (19). In the schizophrenia sample, the age of onset was defined as the earliest age when medical advice was sought for psychiatric reasons or when symptoms began to cause subjective distress or dysfunction on the basis of the OPCRIT rating (27). The severity of positive symptoms was assessed with the sum of all the SAPS items (29), and the severity of negative symptoms was assessed with the sum of all the SANS items (30). For all the traits, raw scores or transformed raw scores were used in the analysis. The normality of the trait value distributions were verified by using SPSS 16.0.1 (SPSS, Chicago, Illinois), and the raw scores were transformed if the skewness and/or kurtosis of the trait value distribution was < -1.0 or > 1.0 (Table 2).

Genotyping

The single nucleotide polymorphism (SNP) genotyping was performed with the Sequenom MassARRAY iPLEX Gold platform (Sequenom, San Diego, California). The *RELN* intragenic and flanking tagging SNPs (tagSNPs) were selected on the basis of CEPH genotype data of the International HapMap Project (39) by using pairwise tagging method with r^2 threshold of .8 in Tagger (40) implemented in Haploview 4.0 (41). The selected tagSNPs were biallelic with a minor allele frequency (MAF) $\geq 10\%$. Their flanking DNA sequences were derived from SNPper (42), and polymerase chain reaction (PCR) and extension primers were designed with MassARRAY Assay Design 3.1. Genotyping was performed according to manufacturer's instructions in 24- to 31-plex reactions in 384-well plates with a total reaction volume of 5 μ L, including 12.5 ng of genomic DNA. The genotypes were automatically called by MassARRAY Typer 4.0 and verified manually. As quality controls, eight water controls and eight duplicated DNA samples were included in each plate. From the original 99 SNPs, 96 in the schizophrenia sample and 95 in the control sample passed the quality controls of genotyping success rate $\geq 95\%$, MAF $\geq 5\%$, Hardy-Weinberg equilibrium (HWE) p value $\geq .01$ calculated from nonrelated individuals by using PEDSTATS 0.6.10 (43), and the number of Mendelian errors ≤ 5 according to PedCheck 1.1 (44). As exceptions, SNP rs362732 with HWE p value .007 in the schizophrenia sample and SNP rs12671505 with MAF 4% in the control sample were included in the analysis (Table S1 in Supplement 1).

The STR genotyping was performed with the ABI 3730xl DNA Analyzer platform (Applied Biosystems, Foster City, California). The *RELN* intragenic STRs RELNSAT2 and RELNSAT6, originally identified by sequencing by Millennium Pharmaceuticals (Cambridge, Massachusetts), were selected on the basis of our previous schizophrenia linkage and cognitive trait association results, respectively (18). Their flanking DNA sequences were derived from the UCSC Genome Browser (45), and PCR primers were designed with Primer3 (46). The *RELN* promoter-region STR D7S3120 was selected on the

basis of previous studies (20). Its PCR primers were derived from Persico *et al.* (47), and the used allele coding corresponds to its trinucleotide repeat length similarly as in Persico *et al.* (47). Genotyping was performed in single-plex reactions in 96-well plates with a total reaction volume of 15 μ L, including 10 ng of genomic DNA (Table S2 in Supplement 1). The genotypes were automatically called by ABI GeneMapper 4.0 and verified manually. As quality controls, two water controls and two duplicated DNA samples were included in each plate. All three STRs passed the quality controls of genotyping success rate 95%, HWE p value .01 calculated from nonrelated individuals by using PEDSTATS 0.6.10 (43), and the number of Mendelian errors <5 according to PedCheck 1.1 (44) (Tables S1 and S3 in Supplement 1).

In case of occasional Mendelian errors, all the genotypes were removed for the corresponding marker and nuclear family. All the families met the criteria of 3 Mendelian errors. A total of 32 individuals with >3 Mendelian errors and/or genotyping success rate <90% were excluded from the analysis.

Statistical Analysis

The LD between SNPs was estimated among nonrelated individuals (one/family) by using Haploview 4.1 (41). Haplotype blocks were defined according to the “solid spine of LD” algorithm, in which the first and last SNP in every haplotype block are in strong LD with all the intermediate SNPs, which might or might not be in strong LD with each other. All the markers, including the STRs, within the haplotype blocks were used to construct the haplotypes with Merlin 1.1.2 (48) according to the most likely pattern of gene flow, and the haplotypes were coded as numeric alleles for association analysis by using a custom-made computer script.

The qualitative association analyses were performed with FBAT 2.0.2c (49) on affected-only basis by using an additive model with empiric variance, as recommended in the presence of linkage (50), because *RELN* is located within the schizophrenia-linked chromosomal region (18). In the gender-specific analyses, the phenotypes of the other gender were set as unknown.

The quantitative association analyses were performed with QTDT 2.5.1 (51) with the proportion of alleles shared identical by descent (IBD) calculated with multipoint computation of Merlin 1.1.2 (48) to extract maximal inheritance information from the families. The total association model, allowing more powerful analysis of samples including partly incomplete families, was used with “polygenic,” “non-shared environment,” “common environment,” and “nuclear family environment” as variance components, supposedly best describing the similarities in the analyzed traits between the family members. In the analysis of neuropsychological traits derived from WMS-R (34), WAIS-R (35), CVLT (36), TMT (37), and Stroop Task (38), the used covariates were gender, testing age, and affection status according to any psychotic disorder, all related to variations in the overall cognitive performance (12). In the analysis of clinical variables derived from SANS (30), the used covariates were gender and affection status according to any psychotic disorder, and from SAPS (29) and OPCRIT (27), the used covariate was gender. The population stratification, a potential source of false positive signals, was determined statistically insignificant in our sample with the “population stratification” model of QTDT 2.5.1 (51) and the same variance components and covariates as in the association analysis.

In the control sample, the haplotype association analyses were performed with PLINK 1.05 (52) with the “hap-assoc” option. The original trait values were adjusted by gender and testing age, fitted to a linear model in R 2.7.0 (53), and the resulting standardized residuals were used as phenotypes. The impact of this different analytic method was estimated

statistically insignificant by a comparison of additional SNP association analysis in the control sample with PLINK 1.05 (52), which revealed no significant differences in the results when compared with QTDT 2.5.1 (51).

The p value thresholds .0009 for significant and .00007 for highly significant trait associations—corresponding to type I error rate of .05—were estimated by using the conservative Bonferroni correction according to the number of independent markers and the product of the number of independent markers and the number of independent traits, respectively. Because both the analyzed markers and traits are not fully independent, their effective numbers were estimated with SNPSpD (54) and matSpD (54), respectively, and their MeffLi or VeffLi estimates (55) were used if smaller than Meff of Veff, respectively, as recommended by the author (<http://gump.qimr.edu.au/general/daleN/SNPSpD/>). The effective number of independent markers ($n = 56.74$) was the sum of the SNPSpD (54) estimate for SNPs and the number of the STRs. The effective number of independent traits ($n = 12.65$) was the sum of the matSpD (54) estimates for qualitative and quantitative traits. The Pearson correlation coefficient matrices of qualitative and quantitative traits were calculated by using SPSS 16.0.1 (SPSS). Overall, a p value threshold of .01 was used for suggestive association.

The significance of trait differences between the individuals positive (carrying one or two copies of the allele or haplotype) and negative (not carrying the allele or haplotype) for the STR alleles or haplotypes showing association with the trait were screened with the Generalized Estimation Equation Model (GEE) (56) of the package “gee” in R 2.7.0 (53). Gender, testing age (with cognitive traits [16]), and affection status according to any psychotic disorder (with cognitive traits [16] and SANS [30]) were used as covariates, and nuclear family status was used as a cluster division to take into account the within-family correlation.

The correlations between the severity of clinical symptoms and amount of risk alleles were estimated by fitting the values into a linear model and calculating the Pearson correlation coefficient in R 2.7.0 (53). The markers not in strong LD ($r^2 < .10$) with each other were selected by using Tagger (40) implemented in Haploview 4.1 (41).

Results

Association Analysis of Dichotomized Diagnostic Categories

Any significant associations of *RELN* variants with the clinical diagnostic categories (CORESCH, SCHSPECT, and ANYPSY) or in the gender-specific analyses remained absent (data not shown). The D7S3120 STR alleles and their frequencies resembled those reported previously (47). None of the alleles, however, exceeded the length of 15 trinucleotide repeats (Table S4 in Supplement 1).

Association Analysis of Clinical Disorder Features

In the schizophrenia sample, suggestive associations ($P_{QTDT} < .01$) emerged between the severity of positive symptoms and SNPs rs2299356, rs12705141, rs727708, rs540058, rs563264, rs16872603, and rs11761011 (Figure 1A). All the potential risk alleles increased the severity additively (data not shown), and the total number of the risk alleles of the SNPs not in strong LD with each other ($r^2 < .10$; rs563264, rs16872603, and rs11761011) (Figure S1 in Supplement 1) and the severity of positive symptoms showed positive correlation ($r = .24$, $p = .0000001$) (Figure 1B).

Among the affected individuals, the longest allele of RELNSAT6 (19 allele positives with trait data available) showed significant association with both more severe positive symptoms

($P_{GEE} = .0005$; β : 13.28, 95% CI: 3.74 to 22.82) and more severe negative symptoms ($P_{GEE} = .00006$; β : 16.50, 95% CI: 7.17 to 25.83) of schizophrenia. Additionally, the D7S3120 allele 13 showed suggestive association ($P_{QTD\tau} = .002$) with the age of schizophrenia onset, the allele positives ($n = 23$) having earlier age of onset (mean 20.3, median 18 years) than the allele negatives ($n = 432$) (mean 24.6, median 22 years).

Association Analysis of Quantitative Neuropsychological Test Measurements

Among the subset of individuals ($n = 342$) not overlapping with our previous study (18) and with genotype and trait data available, the previous STR association was replicated, because the individuals positive for the longest RELNSAT6 allele ($n = 20$) showed similarly impaired cognitive performance as previously (Table S5 in Supplement 1).

In the whole schizophrenia sample, the individuals positive for the longest RELNSAT6 allele ($n = 43$) again showed generalized cognitive impairment. This effect was stronger among the individuals affected with psychosis. The allelic association between the longest RELNSAT6 allele and verbal working memory was highly significant ($P_{QTD\tau} = .00002$) (Table 3).

The individuals affected with psychosis showed poorer average cognitive performance than their unaffected relatives who, in turn, showed poorer performance than the control subjects (Figure S2 in Supplement 1). The neuropsychological traits and the SAPS (29) and SANS (30) symptom ratings showed correlation (Table S6 in Supplement 1). With the SAPS (29) and SANS (30) sum scores included as covariates (Methods and Materials in Supplement 1), the association between the longest RELNSAT6 allele and verbal working memory remained suggestive ($P_{QTD\tau} = .003$) and significant ($P_{QTD\tau} = .0003$), respectively, despite the lower number of informative individuals ($n = 461$ and $n = 627$, respectively).

In the AF subsample, the longest RELNSAT6 allele (36 allele positives with trait data available) showed significant association with poorer performance in verbal working memory ($P_{QTD\tau} = .0003$). Additionally, one haplotype—not harboring RELNSAT6—showed highly significant association with poorer performance in verbal memory traits ($P_{QTD\tau} = .00005$ – $.00006$) (Results in Supplement 1).

In the IS subsample, any significant associations remained absent, in line with our previous results (18).

In the control sample, two haplotypes—not harboring RELNSAT6—showed association with verbal working memory ($P_{PLINK} = .0005$) and verbal memory ($P_{PLINK} = .0009$) (Results in Supplement 1).

Discussion

Here, we performed a two-stage analysis: first, attempting to replicate the previous association between *RELN* and cognitive functions in an independent subsample; and second, extending the *RELN* investigation by using larger sample size, considerably extended marker coverage, and several novel traits (18). The new independent carriers of the longest RELNSAT6 STR allele showed a similar trend of poorer cognitive functioning, as previously (18). Despite the reasonably large number of markers genotyped, this same risk allele remained central in the whole sample, showing association with impaired cognitive functioning, and with more severe positive and negative schizophrenia symptoms. As expected, these traits showed correlation, but the association remained while covarying for the symptom ratings. Although these ratings are prone to changes according to disorder phase, in the study subject interviews all the available clinical data were evaluated, and

lifelong information was taken into account. Whether RELNSAT6 itself is the causative variant here or merely reflects effects of another variant(s) remains unclear. Interestingly, however, the longest variant of this STR revealed the associations (57). Additionally, we detected multiple *RELN* SNPs associated with the severity of positive schizophrenia symptoms, the total number of the risk alleles revealing a positive correlation with the severity.

We detected a gradually ascending trend in overall neuropsychological functioning from individuals affected with psychosis to their unaffected family members, followed by control subjects from general population. These results are not only in line with previous evidence (13) but also fit well within the endophenotype concept (10,11). Because psychosis itself affects cognitive functions (12), we assumed that *RELN* effects could be more distinct among unaffected individuals. The effect of the risk allele on cognition was larger among the cases, however, whereas any effect among the control subjects remained negligible, fitting the modifier gene hypothesis (7,9).

In our previous study, the linkage signal on chromosome 7q21-32—harboring *RELN*—increased when the phenotype included all the schizophrenia spectrum disorders (18). Similarly, among the Scottish pedigree with several major mental disorders, caused by inherited balanced translocation (58) disrupting *DISC1* (MIM 605210), *DISC2* (MIM 606271), and *TSNAX* (MIM 602964) genes in chromosome 1q (59,60), the linkage on the translocation region strengthens when the phenotype is broadened beyond schizophrenia (61). The partly common pathophysiological background of the schizophrenia spectrum disorders is further supported by their broadly dispersed liability among the close relatives of schizophrenia patients (62,63). Thus, in analyses of both clinical diagnostic categories and more fine-tuned disorder characteristics, the additional inclusion of subjects affected with psychoses other than schizophrenia is arguable (64).

The low number of original settlers has affected the Finnish gene pool (22), causing enrichment of several monogenic disorders (65), and similar enrichment of genetic variants behind complex disorders is plausible (66). The high LD between genetic markers in Finns (23), together with the familial enrichment of the disorder in our sample, diminishes the genetic variation and might thus assist in the identification of risk variants (67). By contrast, the use of family samples might diminish the variation in cognitive traits (14), and thus the results obtained from our familial cases might not be generalized to all cases, and vice versa (1).

Previously, we observed no schizophrenia linkage in families originating from the internal isolate, and interestingly, the RELNSAT6 risk allele was also absent in this subsample (18). Here, the risk allele appeared only in four families from the isolate, yet only one of them fulfilled the previous sole criterion of at least one maternal and paternal grandparent born in the municipality, and again, no associations were detected in this subsample. Thus, the geographic distribution of *RELN* associations remained uneven, supporting the evidence of different risk variants and/or their different frequencies affecting the schizophrenia liability in and outside the internal isolate (18,24). That the genetic architecture outside the isolate is more closely related to other Western European populations and the *RELN* associations were mostly contributed by these families supports the generalizability of our results (31).

RELN (68) encodes for a large glycoprotein of 388 kDa (69). Functionally, the most important regions of Reelin are the conserved C-terminus, crucial for downstream signaling (70), and reelin repeats five and six of a total of eight, forming an essential binding site (71) for Very-Low-Density Lipoprotein Receptor (VLDLR), Apolipoprotein E Receptor 2 (ApoER2), and Alpha-3-Beta-1 integrin Receptor ($\alpha 3\beta 1$) (72,73). Although our associated

markers appeared outside them, present data are insufficient to exclude the importance of other *RELN* regions (20,74). That *RELN* is expressed in brain areas showing synapse remodeling (75), a key feature of cognition (76), is especially interesting with regard to our association results with cognitive traits. The *reeler* phenotype in *RELN* null mice involves malformation of the brain cortex and severe phenotypic changes (77), whereas heterozygous *RELN* null mutation as well as homozygous null mutation of reelin receptor ApoER2 or VLDLR result in cognitive disturbances (78,79).

In human brain, reduced reelin protein and/or messenger RNA levels are detected not only in schizophrenia but also in autism (MIM 209850) (80), lissencephaly (MIM 257320) (81), bipolar disorder (MIM 125480) (82), and major depression (MIM 608516) (83). The *RELN* expression is reduced by its promoter region hypermethylation (84), and in post-mortem brain studies, *RELN* promoter regions important for regulation have usually been hypermethylated in persons with schizophrenia (85). The *RELN* methylation might, however, increase with age as well (86). *RELN* expression is also reduced by its promoter-region trinucleotide repeat lengthening (87), which in turn is associated with treatment-resistant schizophrenia (88). Interestingly, a long allele of this promoter-region STR here showed association with earlier age of schizophrenia onset. In addition to this *RELN* promoter-region, however, only a few direct genetic associations with schizophrenia liability exist (89,90).

Although future studies are required to elucidate the role of *RELN* variants in schizophrenia and other neurodevelopmental disorders, our study provides evidence for *RELN* in the pathogenesis of schizophrenia by revealing associations of *RELN* variants with both clinical features of schizophrenia and cognitive impairment. Our results, in addition to strong functional evidence (20), suggest a role for *RELN* as a genetic modifier of disorder-related characteristics.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(A) Single-nucleotide polymorphism (SNP) associations with the severity of positive symptoms (sum of all Scale for the Assessment of Positive Symptoms [SAPS] [29] items) of schizophrenia. The SNPs rs2299356, rs12705141, rs727708, rs540058, rs563264, rs16872603, and rs11761011 with p values $< .01$ are framed. Of these, rs2299356, rs12705141, rs727708, and rs540058—presented as circles—are in linkage disequilibrium (LD) ($r^2 > .10$) with rs563264 and were excluded from further analysis. The SNP rs563264 was selected by using the tagger algorithm (40) implemented in Haploview 4.1 (41) (Figure S1 in Supplement 1). **(B)** The severity of the positive symptoms compared with the total amount of risk alleles of the three SNPs not in strong LD with each other ($r^2 < .10$; rs563264, rs16872603, and rs11761011). Only the individuals with genotypes available for all these SNPs were included in the analysis. The number of individuals in each category (category indicated in brackets): 25 [2]; 106 [3]; 186 [4]; 125 [5]; 30 [6]. Categories 1 and 2 are combined, due to the low number of individuals. In the box plots, the rectangle ends represent the lower (Q1) and upper (Q3) quartiles; the horizontal lines in the box plots the median values, the whiskers the smallest and largest nonoutlier values (lower limit: $Q1 - 1.5 \times [Q3 - Q1]$; upper limit: $Q3 + 1.5 \times [Q3 - Q1]$), and the circles the outlier values.

Table 1

Study Samples: Schizophrenia and Control

	Families	Individuals	Affected			Additional Phenotype Data			
			CORESCH	SCHSPECT	ANYPSY	NP	SAPS	SANS	OPCRIT
Schizophrenia Total	290	1259	297	409	464	892	476	709	474
Male		640	191	250	271	457	274	381	282
Female		619	106	159	193	435	202	328	192
AF	109	518	135	189	210	367	192	259	214
Male		268	83	109	119	185	107	139	125
Female		250	52	80	91	182	85	120	89
IS	181	741	162	220	254	525	284	450	260
Male		372	108	141	152	272	167	242	157
Female		369	54	79	102	253	117	208	103
Control			Additional Phenotype Data						
Individuals			NP						
Control Total		375	330						
Male		180	150						
Female		195	180						

AF, all Finland (outside internal isolate); IS, internal isolate; CORESCH, core schizophrenia; SCHSPECT, schizophrenia spectrum; ANYPSY, disorders with psychotic features; NP, neuropsychological tests; SAPS, Scale for the Assessment of Positive Symptoms (29); SANS, Scale for the Assessment of Negative Symptoms (30); OPCRIT, Operational Criteria Checklist (27).

Table 2

Analyzed Neuropsychological Test and Clinical Disorder Feature Variables

Trait (Subset/Definition)	Test	Scale	Transformation	Schizophrenia Sample		Control Sample	
				Skewness	Kurtosis	Skewness	Kurtosis
Attention and Working Memory							
Verbal attention (digit span forward)	WMS-R	Raw test score	None	.289	-.148	-.102	-.430
Verbal working memory (digit span backward)	WMS-R	Raw test score	None	.296	.265	.110	.175
Visual attention (visual span forward)	WMS-R	Raw test score	None	.126	.353	.073	.154
Visual working memory (visual span backward)	WMS-R	Raw test score	None	-.485	.394	-.471	.146
Mental tracking (trail making A)	TMT	Time (sec)	Log10	.542	.599	.511	.561
Verbal Learning and Memory							
Verbal learning (recalling word list during five trials)	CVLT	Raw test score	None	-.180	-.630	-.724	.706
Recalling words after short delay (5 min)	CVLT	Raw test score	None	-.146	-.498	-.600	.016
Recalling words after long delay (20 min)	CVLT	Raw test score	None	-.249	-.546	-.856	.332
Semantic clustering ratio (learning strategy)	CVLT	Raw test score ratio	None	.482	.086	.513	-.329
Ability Functions							
Verbal ability (vocabulary)	WAIS-R	Raw test score	None	-.188	-.627	-.168	-.219
Processing speed (digit symbol)	WAIS-R	Raw test score	None	.303	-.340	-.218	-.501
Executive Functions							
Executive functioning (trail making B)	TMT	Time (sec)	Log10	.653	.197	.569	.347
Stroop interference score	Stroop task	Raw test score index	Log10	.365	.378	NA	NA
Positive Symptoms							
Severity of symptoms (all SAPS items)	SAPS	Raw score sum		-.421	-.380	NA	NA
Negative Symptoms							
Severity of symptoms (all SANS items)	SANS	Raw score sum		-.046	-1.127	NA	NA
Age of Onset							
Earliest age of subjective dysfunction	OPCRIT	Age (yrs)	Log10	.165	2.452	NA	NA

WMS-R, Wechsler Memory Scale-Revised (34); TMT, Trail Making Test (37); CVLT, California Verbal Learning Test (36); WAIS-R, Wechsler Adult Intelligence Scale-Revised (35); other abbreviations as in Table 1.

Neuropsychological Test Differences in the Schizophrenia Sample Between RELNSAT6 Risk Allele Positives and Negatives

Table 3

Trait	All Individuals (<i>n</i> = 921)				Affected Individuals (<i>n</i> = 385)			
	Allele Positives (<i>n</i> = 43)		Allele Negatives (<i>n</i> = 878)		Allele Positives (<i>n</i> = 19)		Allele Negatives (<i>n</i> = 366)	
	Mean ± SD		Mean ± SD		Mean ± SD		Mean ± SD	
Attention and Working Memory								
Verbal attention ^a	5.91 ± 1.84		6.35 ± 1.93	-.45 (-.95-.05)	5.05 ± 1.54		6.10 ± 1.85	-1.10 (-1.83-.38)
Verbal working memory ^a	4.19 ± 2.00		5.51 ± 1.93	-1.34 (-1.88-.80)	3.37 ± 1.64		4.98 ± 1.80	-1.72 (-2.48-.97)
Visual attention ^a	7.09 ± 1.87		7.90 ± 1.87	-.84 (-1.32-.36)	6.47 ± 1.78		7.49 ± 1.83	-1.10 (-1.84-.36)
Visual working memory ^a	5.74 ± 2.51		7.11 ± 2.20	-1.44 (-2.26-.62)	4.89 ± 2.51		6.65 ± 2.29	-1.93 (-3.28-.59)
Mental tracking ^{b,c}	58.82 ± 39.33		55.00 ± 30.35	4.21 (-5.68-14.10)	67.94 ± 29.80		64.08 ± 34.25	5.85 (-7.14-18.85)
Verbal Learning and Memory								
Verbal learning ^a	36.62 ± 14.66		42.59 ± 13.38	-5.31 (-9.60-1.02)	28.47 ± 10.65		36.46 ± 13.17	-7.88 (-12.89-2.87)
Recalling words after short delay ^a	7.17 ± 3.75		8.90 ± 3.51	-1.58 (-2.60-.57)	5.32 ± 3.02		7.49 ± 3.41	-2.18 (-3.60-.76)
Recalling words after long delay ^a	8.21 ± 3.90		9.35 ± 3.57	-.97 (-1.94-.00)	6.42 ± 3.40		7.83 ± 3.54	-1.38 (-2.75-.01)
Semantic clustering ratio ^a	1.63 ± .82		1.62 ± .78	.04 (-.20-.28)	1.29 ± .81		1.39 ± .69	-.06 (-.45-.32)
Ability Functions								
Verbal ability ^a	34.50 ± 13.33		38.20 ± 13.46	-3.74 (-8.06-.58)	27.21 ± 11.67		33.49 ± 13.99	-7.12 (-13.34-.90)
Processing speed ^a	34.46 ± 16.45		35.75 ± 14.67	-1.22 (-4.89-2.44)	25.74 ± 11.56		31.07 ± 12.61	-6.40 (-11.03-.176)
Executive Functions								
Executive functioning ^{b,c}	137.67 ± 81.90		125.35 ± 73.99	17.90 (-10.73-46.52)	195.83 ± 56.90		146.69 ± 88.10	57.86 (8.02-107.71)
Stroop interference score ^{b,c}	90.24 ± 32.95		84.51 ± 38.61	5.86 (-4.25-15.96)	100.23 ± 30.78		92.53 ± 39.71	9.50 (-7.82-26.81)

Neuropsychological test differences (raw test scores) in the schizophrenia sample between RELNSAT6 risk allele positives (individuals carrying one or two copies of the allele) and negatives (individuals not carrying the allele). Significance was estimated with the GEE (56) analysis by using gender, testing age, and affection status according to any psychotic disorder as covariates. The nuclear family status was used as cluster division to take into account the within-family correlation. All *p* values < .05 are shown. GEE, Generalized Estimation Equation Model (56); CI, confidence interval.

^aLower test score represents poorer performance.

^bHigher test score represents poorer performance.

^cSignificance was estimated by using the transformed raw test score.