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ORIGINAL RESEARCH ARTICLE

Analysis of the *RELN* gene as a genetic risk factor for autism

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Several genome-wide screens have indicated the presence of an autism susceptibility locus within the distal long arm of chromosome 7 (7q). Mapping at 7q22 within this region is the candidate gene *reelin* (*RELN*). *RELN* encodes a signaling protein that plays a pivotal role in the migration of several neuronal cell types and in the development of neural connections. Given these neurodevelopmental functions, recent reports that *RELN* influences genetic risk for autism are of significant interest. The total data set consists of 218 Caucasian families collected by our group, 85 Caucasian families collected by AGRE, and 68 Caucasian families collected at Tufts University were tested for genetic association of *RELN* variants to autism. Markers included five single-nucleotide polymorphisms (SNPs) and a repeat in the 5′-untranslated region (5′-UTR). Tests for association in Duke and AGRE families were also performed on four additional SNPs in the genes *PSMC2* and *ORC5L*, which flank *RELN*. Family-based association analyses (PDT, Geno-PDT, and FBAT) were used to test for association of single-locus markers and multilocus haplotypes with autism. The most significant association identified from this combined data set was for the 5′-UTR repeat (PDT *P*-value = 0.002). These analyses show the potential of *RELN* as an important contributor to genetic risk in autism.
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Autism is a severe genetically influenced neurodevelopmental disorder characterized by significant disturbances in social, communicative, and behavioral functioning. Autism is the most common of a larger clinical group of pervasive developmental disorders (PDDs: Autism, Asperger syndrome, Rett syndrome, childhood disintegrative disorder, and pervasive developmental disorder—not otherwise specified). The onset of autism occurs before the age of 3 years with symptoms continuing for life. The most recent review of multiple epidemiologic surveys estimates the prevalence of autism at approximately 1 per 1000 children, and prevalence for all PDDs at 6–7 per 1000.[1] A more recent autism survey of a US metropolitan area found a prevalence of 3.4 per 1000.[2] Several twin and family studies have shown strong evidence for genetic factors in the etiology of autism.[3–6] The concordance rate for monozygotic twins (75%) is much higher than that of dizygotic twins (3%); in addition, calculations of the sibling recurrence risk ratio (λ_s) give results from 50 to 150,[4,7–9] considerably higher than λ_s estimates for other complex disorders.

Estimates have been made that several chromosomal loci contribute to genetic susceptibility in autism.[10] It is likely that genetic effects are conferred by multiple, possibly interacting, genes, as combinations of alleles with modest functional effects. Traditional linkage methods may not have enough power to detect such small effects in most studies, given the typical sample sizes. A promising alternative is to examine specific candidate genes and take advantage of the power of genetic association.

Numerous studies have examined candidate genes for involvement in autism. One promising category of candidates encodes proteins involved in relevant neurodevelopmental processes. One such gene is *reelin* (*RELN*), a large extracellular matrix protein that orchestrates neuronal positioning during corticogenesis. One of the most notable effects of *RELN* deletion is abnormal formation of the cerebral cortex in *reeler* mice, with inversion of cells in the horizontal laminations.[11] Further studies have

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identified *RELN* as necessary for proper formation of brain structures by directing migration of neuronal precursors.[12–19]

These suggestive developmental roles for *RELN* correlate with *RELN* abnormalities in several neurogenetic diseases. *RELN* mRNA and protein levels are significantly reduced in multiple brain areas of patients with schizophrenia[20,21] and bipolar disorder with psychosis.[21] These results are consistent with a role for *RELN* in the 'two-hit' model (neurodevelopment/vulnerability) of schizophrenia; low levels of RELN protein result in abnormal neuronal development, and the persisting low RELN levels in the developed brain increase vulnerability to schizophrenia-inducing damage. Similar *in vivo* abnormalities have been seen for *RELN* levels in autistic individuals; Fatemi *et al*[22] have shown a significant reduction in circulating levels of unprocessed *RELN* in autistic individuals.

A role for *RELN* is supported not only by relevant functional observations but also by multiple studies that detected autism linkage peaks in the region of 7q that contains *RELN*.[23–28] Specific linkage peaks for this region have been identified for D7S477 (110.4 cm multipoint MLS = 3.20),[24] D7S495 (144.7 cm, MLOD = 1.38),[25] D7S523 (123.0 cm, LOD = 1.0),[26] and D7S1813 (102.8 cm, M-HLOD = 1.40,[29] MMLS/het = 2.2[30]). This region has been hypothesized to contain more than one autism susceptibility gene.

Recently, studies from four groups have examined genetic associations of *RELN*. A polymorphic trinucleotide repeat (GGC) located in the 5′ untranslated region (UTR) of the *RELN* gene, and specific haplotypes of the 5′ UTR with two single-base substitutions in *RELN* exons were found associated with autism in both family- and population-based association studies.[31] It has also been reported that the larger 5′ UTR *RELN* alleles were transmitted more often than expected to affected children in a sample of 126 multiplex families.[32] However, other association studies on *RELN* in independent data sets did not see any such associations.[33–36] We report here the results of an association study in which *RELN* was examined as a contributing factor for autism in our data set, with particular attention given to the 5′ UTR, given its history of conflicting results.

Materials and methods

Subjects

A total of 371 families were ascertained through three centers. As part of a linkage study to identify genetic factors associated with autism, 217 Caucasian families (Duke samples) were ascertained through the Center for Human Genetics at Duke University Medical Center, or through collaboration with the University of South Carolina. A total of 86 Caucasian families were ascertained through the Autism Genetics Resource Exchange or AGRE, and 68 Caucasian families were ascertained through Tufts University. One AGRE family was not analyzed due to genotyping problems. Participants were recruited to the autism genetics studies via support groups, advertisements, and clinical and educational settings. Participants were from both multiple incidence families (more than one affected individual) and trios (parents who have one child with autism). All participants met research diagnostic criteria for autism, which included a clinical diagnosis of autism based on DSM-IV[37] and supported by the Autism Diagnostic Interview (ADI) or its revision (ADI-R).[38,39] Designation of individuals as affected for this study required meeting the cutoff scores for all three ADI domains as well as evidence of onset prior to 36 months of age. Additional inclusion criteria were as follows: (1) a minimal developmental level of 18 months as measured by the Vineland Adaptive Behavior Scales;[40] and (2) absence of severe sensory (eg, visual impairment or hearing loss) or significant motor impairments (eg, failure to sit by 12 months or walk by 24 months) based on screening by clinical staff. Individuals with neurologic or known genetic conditions that present with autistic features (eg, Fragile X Syndrome or Tuberous Sclerosis Complex) were excluded.[25,26,30] A total of 1507 individuals (including 577 affected individuals) from these 352 families were genotyped. Of the 577 affected individuals, 435 are from the 229 multiplex families and 142 are from singleton families. Table 1 shows descriptive features of the participants.

Molecular analyses

Genomic DNA extraction from isolated lymphocytes was performed according to established protocols.[41] Using previously described quality control procedures,[42] two CEPH standards were included on each 96-well plate, and samples from six individuals were duplicated across all plates as quality controls (QCs), with the laboratory technicians blinded to their identities. Analysis required that identical QC samples within and across plates had matching genotypes, in order to identify errors in loading and reading, and thus minimize the error rate in genotypes assignment. After QC verification, genotypes of the samples were uploaded into the PEDIGENE® database and merged into the Lapis management system for creating analysis input files.[43]

RELN single-nucleotide polymorphisms (SNPs) in a splice site junction for exon 6, in exons 44, 45, 50, in intron 59, and a polymorphic GGC repeat located immediately 5′ of the *RELN* transcription initiation site were genotyped in the samples from all three centers. The 5′ UTR triplet repeats and markers for exons 6 and 50 have been previously tested.[31] None of the SNP variations cause coding changes, but the exon 6 splice site SNP is predicted to have an effect on splice site choice.[31] The 5′ UTR marker was genotyped using a modification of the gel-based oligonucleotide ligation assay (OLA),[44] as described by Martin *et al*.[45] Genotyping of all SNPs was carried out with TaqMan assays from Applied Biosystems.[46]

Table 2 gives positional information about the *RELN* markers, most of which are located in GenBank

cosmid F19374. Allele designations and frequencies for all SNPs are also given in Table 2. The allele frequencies for the 5′ marker are given in Table 3.

Genotyping

TaqMan PCR amplifications were performed on GeneAmp PCR Systems 9700 thermocyclers. 5′ nuclease activity on fluorescently labeled probes during amplification is the basis for allelic discrimination by Taqman, with released labels detected by an ABI Prism 7900 Sequence Detector. In total, 2.7 ng of genomic DNA was amplified in 5 or 10 μl reactions containing 900 nM primers, fluorescently labeled probes specific to each SNP variation at 200 nM, and

Table 1 Descriptive characteristics of participants from each of three sites

Site	*Gender*	*Age (months) mean (SD)*	*VABS mean (SD)*	*ADI-R language*
Duke	Male 75% Female 25%	105 (59)	59 (21)	0 72% 1 12% 2 16%
AGRE	Male 76% Female 24%	84 (44)	48 (22)	0 57% 1 15% 2 28%
Tufts	Male 79% Female 21%	105 (63)	53 (17)	0 79% 1 10% 2 11%

VABS = Vineland Adaptive Behavior Scale composite; ADI-R Language = Autism Diagnostic Interview-R language ratings: 0 = verbal, 1 = some speech, 2 = very limited speech.

Table 2 Marker characterization for panel of single-nucleotide polymorphisms (SNPs) genotyped in each data sets

Polymorphism region	*Appr. kb position on chr7*	*bp change*	*Minor allele*[a]	*Frequency*[b] *DUK*	*Frequency*[b] *AGR*	*Frequency*[b] *TUF*
PSMC2	102572	A>G	A (1)	0.43	0.46	NA
RELN intron 59	102691	T>C	C (2)	0.22	0.21	0.01
RELN exon 50	102716	T>C	C (1)	0.36	0.36	0.29
RELN exon 45	102740	A>G	G (2)	0.02	0.03	0.21
RELN exon 44	102741	A>G	G (2)	0.06	0.10	0.02
RELN exon 6	102950	A>G	A (1)	0.45	0.46	0.46
RELN 5′UTR	103190	Triplet	GGC (8)	0.44	0.44	0.44
ORC5L 1	103326	C>T	C (1)	0.23	0.27	NA
ORC5L 2	103337	A>G	G (2)	0.46	0.49	NA
ORC5L 4	103346	C>G	G (2)	0.42	0.44	NA
ORC5L 5	103367	A>C	C (2)	0.22	0.26	NA
ORC5L 6	103376	C>T	T (2)	0.49	0.49	NA

[a]Numeric designation used in text are given in parentheses.
[b]Estimated from genotyped founders with unknown or unaffected autism phenotype, PMSC and ORC5L SNPs were not typed for the Tufts samples.

Table 3 Allelic distributions of GGC triplet repeats assessed in genotyped founders with unknown or unaffected autism phenotype in each data set

Founder samples (N = chromosomes)	*5′ UTR triplet repeat alleles*								
	4	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*
DUK (368)	0	0.44	0.01	0.46	0	0.02	0.04	0	0
AGR (140)	0	0.44	0.01	0.47	0.01	0.02	0.02	0	0
TUF (108)	0	0.44	0.01	0.47	0	0	0.02	0	0

TaqMan Universal PCR Master Mix with AmpErase UNG (P/N 4326708). TaqMan MGB probes or TAMRA probes were used. MGB probes were labeled with 6-FAM or VIC while TAMRA probes were labeled with 6-FAM or TET. The reaction mixture underwent two preincubations, first at 50°C for 2 min to optimize AmpErase UNG activity to prevent carryover DNA contamination, and then at 95°C for 10 min to activate the AmpliTaq Gold enzyme. Then 40 amplification cycles were performed, each cycle consisting of denaturation at 95°C for 15 s followed by annealing and extension at 60°C (for MGB probes) or 62°C (for TAMRA probes) for 1 min.

The 5′ repeat was typed using microsatellite methods. In total, 30 ng of genomic DNA was amplified in 10 μl reactions containing 3 mM $MgCl_2$, 600 nM dNTPs, one unlabeled primer at 4 ng/μl, one FAM-labeled primer at 4 ng/μl, 1× Platinum Taq buffer and 0.5 U Invitrogen Platinum Taq. Amplification cycles were 94°C for 4 min, five cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 30 s, 20 cycles of 94°C for 5 s, 55°C for 30 s, 72°C for 45 s, 15 cycles of 94°C for 5 s, 55°C for 45 s, 72°C for 80 s, and a final extension of 72°C for 7 min. The labeled products were separated on acrylamide gels, which were scanned to detect the fluorescent products. The detected products were compared to products of known sizes to determine the repeat numbers for the 5′ alleles.

Statistical analyses

Genotypes within each family were examined for Mendelian inconsistencies using PEDCHECK.[47] Inconsistency reports were re-read by laboratory technicians, without family identifying information. Families that had obligate recombinants between SNPs within one gene, based on haplotype analysis with SIMWALK2,[48] were re-read and re-genotyped if necessary. Allele frequencies were estimated from genotyped founders with unknown or unaffected phenotypes.

Hardy–Weinberg equilibrium (HWE) was assessed using exact tests implemented in the Genetic Data Analysis (GDA) program.[49] From each family, one affected and one unaffected individual were selected randomly to test for deviations from HWE for each marker. Each *P*-value was estimated using a permutation test with 3200 permutations for both affected and unaffected samples.

Linkage disequilibrium between markers was calculated as D'[50] and the squared correlation coefficient R^2,[51] using the software package GOLD.[52] This software used the haplotypes of the founder individuals generated by SIMWALK2 software to calculate LD measurements.

Family-based association tests for single-loci were conducted using the pedigree disequilibrium test (PDT),[53,54] geno-PDT,[55] and a family-based association test (FBAT).[56] The PDT is a test for association that examines transmissions from parents to affected offspring, and also compares genotypes of affected individuals with their unaffected siblings. The geno-PDT test, while not as powerful as the PDT under an additive model, is more powerful under a recessive or dominant model, and is able to test association of specific genotypes. While PDT can analyze only one locus at a time, FBAT is able to consider the transmission of haplotypes of multiple loci using the haploFBAT routine.[57] The FBAT option used was an empirical variance estimate test for association in the presence of linkage. Global and haplotype-specific associations were also investigated.

Results

Tests for HWE deviations were calculated for each marker in 352 affected individuals and separately in 97 unaffected individuals selected at random from each family, with most polymorphisms showing no evidence of deviation from HWE in all data sets. The only SNP showing deviation from HWE for the entire set of unaffected individuals was the *RELN* exon 50 SNP (*P*-value = 0.016), which also had deviations in the Duke data set alone (*P*-value = 0.035). In the affected samples, only the exon 45 SNP was not in HWE for the entire data set (*P*-value = 0.006).

Calculation of LD by D' and R^2 for each SNP pair showed significant LD generally only for short distances (Figure 2). The only SNP pairs that showed strong levels of LD by D' and R^2 were within *ORC5L*, as well as *ORC5L* SNPs with the *PSMC2* SNP. The LD between *PSMC2* and *ORC5L* is in spite of the fact that *PSMC2* and *ORC5L* are on opposite sides of *RELN* (Figure 1), and neither shows LD with any *RELN* marker.

Allelic association results from PDT and geno-PDT are presented in FBAT results were nearly identical to the PDT results for all markers, and are not shown. The 5′ UTR shows the strongest association with autism for the overall data set (*P*-value 0.002 by PDT), with the most significant contributions coming from the AGRE data set (*P*-value 0.004 by PDT). In addition, several of the *RELN* SNPs showed nominal associations. Exon 6 and 45 markers were associated when the Duke data set was considered alone, exon 50 was significant for the Tufts data set, and exon 44 had significant association for the entire data set, but not with any of the individual center sets. Pairwise marker FBAT was performed for the entire data set, and detected significant associations only for exon 44 when paired with the 5′ UTR, exon 45, or exon 50 (Figure 2).

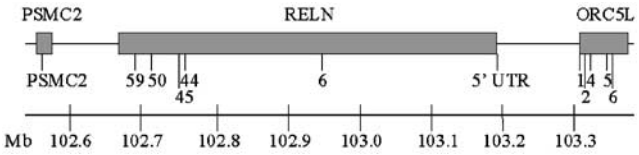

Figure 1 Relative locations of PSMC2, RELN, and ORC5L SNPs on chromosome 7q.

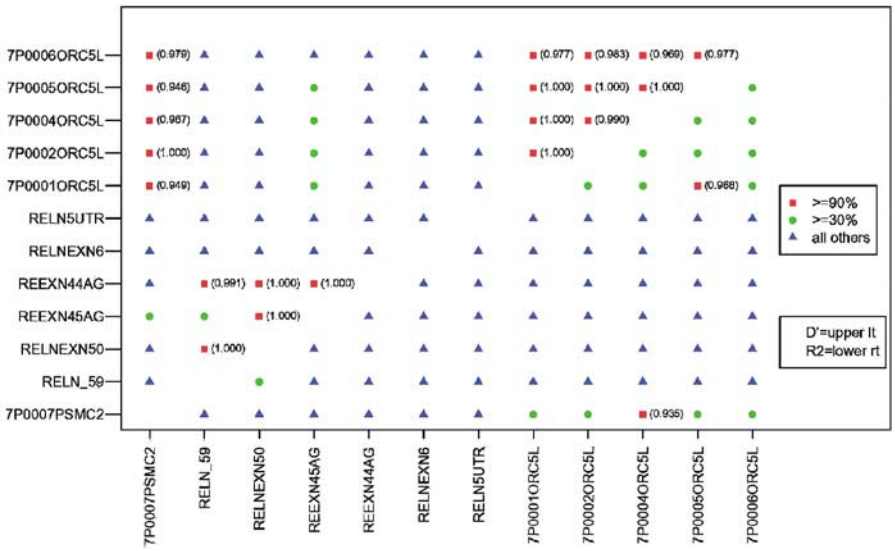

Figure 2 Linkage disequilibrium between all SNPs typed, calculated by D′ and R^2.

Multilocus FBAT analysis was performed to try to identify specific haplotypes of the *RELN* markers that were associated with autism (the 'haplotypes' referred to here are multilocus genotypic combinations). As over 90% of the 5′ UTR markers typed had eight or 10 GGC repeats, genotypes for this marker was collapsed into two alleles: allele 1 is defined as $\geq$10 repeats, and allele 2 as $\leq$8 repeats. The rarer haplotypes, those occurring in fewer than 10 families, were not included in the analysis. The most significant result was found in the AGRE data set for a haplotype of SNPs 59, 50, 45, 44, and the 5′ UTR (alleles 1-2-1-1-1, respectively), which showed significant association with autism ($P=0.002$). This is the most common haplotype for these SNPs in this data set, with a frequency of 0.33. A significant result was also found in the entire data set for a haplotype of all *RELN* markers (allele composition 1-2-1-1-1-1 for SNPs 59, 50, 45, 44, 6 and the 5′ UTR); this haplotype is the most common for these markers in the entire data set (frequency 0.17, $P=0.025$).

Discussion

In the overall data set, the PDT and FBAT (and to a lesser degree, the geno-PDT) analyses showed that the association of the *RELN* 5′ UTR to autism is statistically significant. In the 5′ UTR, the most common repeat, 10, was over-represented in autism. Allele 10 is overtransmitted (193 transmitted *vs* 160 not transmitted) with an allele-specific *P*-value of 0.003 and a global *P*-value of 0.003. When the results were broken down by individual data sets (Table 4), it was clear that the most significant contributions to this association are from the AGRE data set.

A more stringent analysis of the association results, using a cutoff *P*-value of 0.004 from a conservative Bonferroni correction (from 12 markers tested), leaves the 5′ UTR as the only significant association. Under less strict criteria, several other markers have suggestive results. The exon 44 and 45 SNPs showed association with the entire data set (exon 44 PDT $P=0.028$, exon 45 FBAT $P=0.05$), but all of the *RELN* SNPs (except exon 44) showed association with at least one of the subsets. The 5′ UTR and exon 59 SNPs were significant in the AGRE set, the SNPs from exons 6 and 45 were significant in the Duke set, and the exon 50 SNP was significant in the Tufts set (Table 4). These significant results across *RELN*, combined with the nonsignificant results for the flanking genes *ORC5L* and *PSMC2*, support a *RELN*-specific association to autism.

The results for *RELN* associations in each of the subsets correspond to results of previous screens of these samples that have shown linkage of chromosome 7q to autism. A screen of the Duke data set identified a peak at D7S495 (144.7 cm).[25] The AGRE and Tufts samples used in this study have been part of larger data sets previously screened for linkage, but the subsets used here have not specifically been tested for linkage. The screens of the larger data sets did show linkage peaks in 7q;[26,29] therefore, it is possible that the samples included in this work contribute to the observed linkages.

Using FBAT, associations have been detected for the exon 44 SNP when paired with the 5′ UTR, exon 45, or exon 50; however, these pairings did not show any significant LD by R^2. In fact, R^2 results indicated that none of the *RELN* markers are in LD with each other, and are also not in LD with the *PSMC2* or *ORC5L* SNPs (Figure 3). The only markers for which LD was seen were the *ORC5L* SNPs, several of which showed LD within *ORC5L*, as well as to *PSMC2* (Figure 2). These LD results, combined with significant geno-PDT association scores for the *ORC5L* SNPs (data not shown), indicate that further study on

Table 4 *P*-values from PDT and FBAT analysis of three data sets

	Overall (N = 371)	
	PDT	*Geno-PDT*
(a) Associations for the entire data set		
Intron 59	0.156	0.314
Exon 50	0.158	0.347
Exon 45	0.061	0.091
Exon 44	**0.028**	**0.033**
Exon 6	0.727	0.734
5′ UTR	**0.002**	0.177

	AGRE (N = 85)		*Duke (N = 218)*		*Tufts (N = 68)*	
	PDT	*Geno-PDT*	*PDT*	*Geno-PDT*	*PDT*	*Geno-PDT*
(b) Associations for the individual data sets						
Intron 59	**0.018**	**0.038**	0.929	0.993	0.414	0.414
Exon 50	0.086	0.218	0.375	0.620	**0.022**	0.100
Exon 45	0.564	0.564	**0.033**	**0.033**	0.399	0.654
Exon 44	0.197	0.305	0.121	0.083	0.317	0.317
Exon 6	0.053	0.159	**0.042**	0.094	0.307	0.442
5′ UTR	**0.004**	0.083	0.228	0.607	0.362	0.437

Bold type indicates $P<0.05$.

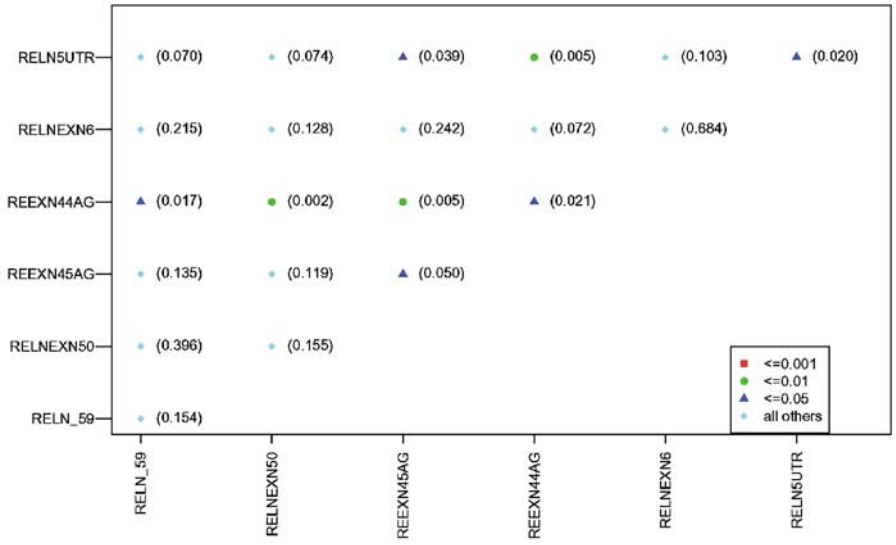

Figure 3 Pairwise FBAT of RELN markers.

ORC5L may be merited, even though there were no significant PDT or FBAT scores for *ORC5L* markers (data not shown). The deviations from HWE observed in exons 45 and 50 are potentially of interest. These deviations may be significant for autism susceptibility, as they could indicate a disease-related association. Further analysis including analysis of additional markers in these areas could help clarify this finding.

A significant issue in interpreting the association between *RELN* and autism susceptibility is the disparity between different studies. In particular, the role of the 5′ UTR is controversial, as it has been observed that the longer alleles ($\geq$11 GGC repeats) are overtransmitted to affected individuals,[31,32] but other studies have shown no association between the number of 5′ UTR repeats and autism.[33–36] One explanation is that the longer alleles are less common; in the total data set used in this paper, only $\sim$5% of chromosomes have alleles with $\geq$11 repeats. Therefore, the sample population would need sufficient representation of these longer alleles, either

through a large sample size, or by an over-representation of these alleles in the sample population. In this study, while no significant overtransmission of 5′ UTRs with 11 or more GGC repeats was seen, the allele with 10 repeats was significantly overtransmitted to affected individuals.

Another difficulty in consistently detecting association of the 5′ UTR could be explained by genetic complexity. As it is recognized that contributions of multiple genes are probably required for autism, the contributions of *RELN* alone may not be sufficient, and may not even be necessary, for autism. Therefore, while *RELN* may have a large effect, there could be cases of autism in which it has no role. To identify a *RELN* specific contribution to autism, a study would require a population with a large representation of subjects where the *RELN* gene contributes to autism risk.

In those populations for whom *RELN* is significant, until the specific *RELN* susceptibility variation is identified, different regions of *RELN* may be significant in different subgroups because of the varying backgrounds of the populations. Another complicating factor could be that there are multiple variations across *RELN* independently contributing to autism susceptibility. This would mean that to detect 5′ UTR association, not only might a large sample set be required but also this sample set would need sufficient numbers of subjects for whom the 5′ UTR is significant.

The association results of this study, when broken down into subsets, suggest that there is a specific population in the AGRE set for whom the 5′ UTR is involved in autism, but not in the Duke or Tufts sets (Table 4). This strong 5′ UTR association, the overtransmission of the 10-repeat allele, and the significant FBAT association scores for haplotypes containing the longer 5′ UTR all support the findings of previous studies, which showed that longer lengths of the 5′ UTR are significantly associated with autism.

There are similar disparities among studies concerning associations of SNPs within *RELN* to autism. Of particular importance to this study are the results of Bonora *et al*,[34] in which none of the *RELN* SNPs examined showed any association to autism, unlike the associations of the 5′ UTR and some SNPs seen here. One explanation for this disparity could be that the data set used by Bonora *et al* did not contain sufficient numbers of subjects with meaningful variations, as each variation examined was only seen in a few affected individuals from the study population. As already shown here, some SNPs are seen to be significant only in one subgroup, presumably due to the composition of that group; SNP 6 was significant only for the Duke set, while SNPs 59 and 50 were significant only for the smaller AGRE and Tufts sets, respectively. This effect is more likely due to genetic background heterogeneity across sites than to sample size.

Another possibility for the differences between studies for detecting *RELN* significance is the consideration of haplotypes *vs* single marker mutations. We already hypothesized that different variations in *RELN* could contribute to autism susceptibility, but it may be that multiple changes working in unison are required. If this is the case, examination of single variations would be much less likely to produce significant results, unless a large proportion of subjects with the variation of interest also carried the other necessary changes. It is also possible that *RELN* has several different haplotypes that contribute to autism; again, the different SNP associations seen in the different subgroups are consistent with this hypothesis. The missense variants within *RELN* that were detected by Bonora *et al* in their sample set may also prove useful in analysis of our samples in this regard. If the allelic heterogeneity of these variants is significant for autism susceptibility, but does not contribute in all cases, there may be subgroup-specific associations, as well as frequencies that vary by subgroups.

The results presented here strongly suggest that *RELN* is involved in autism susceptibility, although further work is necessary to identify the specific variations with direct effects. This will likely require analysis of more markers on more subjects, but as different associations are affiliated with different data sets in this study, subdivision of data sets by ethnic origin or diagnostic criteria may prove more useful. Methods such as these should improve identification of important variations, and determine biological significance of the variations, thus providing understanding of how autism susceptibility is modified by the functions of *RELN* in neural development and signaling.

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