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Transmission disequilibrium testing of the chromosome 15q11-q13 region in autism

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Abstract

Evidence implicates the serotonin transporter gene (*SLC6A4*) and the 15q11-q13 genes as candidates for autism as well as restricted repetitive behavior (RRB).

We conducted dense transmission disequilibrium mapping of the 15q11-q13 region with 93 single nucleotide polymorphisms (SNPs) in 86 strictly defined autism trios and tested association between SNPs and autism using the transmission disequilibrium test (TDT). As exploratory analyses, parent-of-origin effects were examined using likelihood-ratio tests (LRT) and genotype-phenotype associations for specific RRB using the Family-Based Association Test (FBAT). Additionally, gene-gene interactions between nominally associated 15q11-q13 variants and 5-HTTLPR, the common length polymorphism of *SLC6A4*, were examined using conditional logistic regression (CLR).

TDT revealed nominally significant transmission disequilibrium between autism and five SNPs, three of which are located within close proximity of the GABA_A receptor subunit gene clusters. Three SNPs in the *SNRPN/UBE3A* region had marginal imprinting effects. FBAT for genotype-phenotype relations revealed nominally significant association between two SNPs and one ADI-R sub-domain item. However, both TDT and FBAT were not statistically significant after correcting for multiple comparisons. Gene-gene interaction analyses by CLR revealed additive genetic effect models, without interaction terms, fit the data best.

Lack of robust association between the 15q11-q13 SNPs and RRB phenotypes may be due to a small sample size and absence of more specific RRB measurement. Further investigation of the 15q11-q13 region with denser genotyping in a larger sample set may be necessary to determine

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whether this region confers risk to autism, indicated by association, or to specific autism phenotypes.

Keywords

Autism; 15q11-q13; restricted repetitive behavior; 5-HTTLPR; association

Introduction

Autism is a neurodevelopmental disorder characterized by qualitative impairments in reciprocal social interaction, deficits in communication, and the presence of restricted repetitive behavior (RRB) with onset before 3 years of age (APA 1994). Autism is a severe form of autism spectrum disorders (ASDs), which also include pervasive developmental disorder-not otherwise specified (PDD-NOS) and Asperger disorder. ASDs are relatively common and highly heritable. The current estimate of the prevalence is 0.2% for autism and 0.6% for all ASDs (Chakrabarti and Fombonne 2005). Twin studies show increased concordance rates in monozygotic twins (60-91%) compared with those in dizygotic twins (0-10%) (Bailey et al. 1995; Steffenburg et al. 1989). Sibling recurrence rate has been estimated to be 4.5% (Jorde et al. 1991). Genetic studies suggest that autism is a complex genetic disorder with oligogenic inheritance, epistatic interactions among common susceptibility alleles, gene-environment interactions, and an undetermined proportion of rare variants, including inherited and de novo copy number variants (the first identified being chromosomal disorders). Estimates of the number of genes involved in autism range from 3-10 (Folstein and Rosen-Sheidley 2001; Pickles et al. 1995) to more than 15 (Risch et al. 1999), with each gene variant likely making a different contribution to the clinical symptomatology (Veenstra-Vanderweele et al. 2004).

Identification of autism susceptibility genes has been hampered by phenotypic heterogeneity (Spence et al. 2006), which is reduced by the application of the Autism Diagnostic Interview-Revised (ADI-R) and the Autism Diagnostic Observation Schedule (ADOS) (Hus et al. 2007; Veenstra-Vanderweele et al. 2004). The ADI-R is a semi-structured interview that is conducted with the primary caregiver designed to assess children's current and past behavior in three key domains of autism: social interaction, communication and restricted repetitive behavior (RRB) (Lord et al. 1994). The ADOS is a standardized semi-structured observational measure of the child, in which the examiner elicits socio-communicative behaviors that are delayed, abnormal, or absent in autism (Lord et al. 2000).

Neither the ADI-R nor the ADOS is designed as a rating scale, however, the individual items and sub-domain items on the ADI-R and ADOS provide critical information regarding behavioral severity within autism (Brune et al. 2006). Several studies have used the phenotypic data derived from the ADI-R and/or ADOS in order to further reduce heterogeneity and help identify potential autism susceptibility genes. For example, two studies have demonstrated increased linkage to chromosome 2q in families with probands who had delayed phrase speech at 36 months (Buxbaum et al. 2001; Shao et al. 2002a). Another study used savant skills factor (SSF) derived from the ADI-R and found an

increased linkage at D15S511, a marker linked to *GABRB3*, in families with individuals with high SSF (Nurmi et al. 2003b).

Various forms of RRB, such as flapping arms, lining up objects, peculiar fascination with odd objects or part of objects, a very narrow restricted interests, intolerance to changes of routines and insistence on sameness are common among individuals with ASDs (Lewis and Bodfish 1998; Lord et al. 1994; Rutter 1985; Szatmari et al. 2006; Turner 1999). Recently, Cuccaro and colleagues derived two RRB factors from the ADI-R, Repetitive Sensory Motor Action (RSMA) and Insistence on Sameness (IS) (Cuccaro et al. 2003). Using these factors, Shao and colleagues found a higher linkage signal to the 15q11-q13 region among families sharing high IS factor scores (Shao et al. 2003). Interestingly, a recent factor analysis of the ADI-R in 209 children with ASDs found two of three factors derived from the ADI-R sub-domains directly related to RRB: Repetitive Sensory and Motor Behavior and Inflexible Language and Behavior (Georgiades et al. 2007). In accord with this finding, a recent revision of the ADOS, which formerly did not include RRB scores as part of the diagnostic classification of ASDs, found that including RRB improved the diagnostic specificity and sensitivity of the disorder (Gotham et al. 2007). Furthermore, among three domains of autism, only RRB at age 2 significantly predicts diagnosis at age 9 after controlling for IQ (Lord et al. 2006). These findings support the significance of RRB in ASD research and its applicability to genetic association studies.

Several lines of evidence provide support for the role of the serotonin transporter gene (*SLC6A4*) in the pathogenesis of autism as well as RRB. *SLC6A4* is a strong candidate gene for autism, based on reports of hyperserotonemia in autism (Cook et al. 1993; Coutinho et al. 2007; Hranilovic et al. 2006) and efficacy of selective serotonin reuptake inhibitors (SSRIs) targeting the serotonin transporter for reducing RRB and related aggression in autism (Kolevzon et al. 2006). 5-HTTLPR, the promoter-linked 44-bp insertion/deletion polymorphism of *SLC6A4*, has been widely studied in autism with conflicting results. To date, 8 of 15 family-based association studies reported over-transmission of either the short or long allele of 5-HTTLPR from the parents to their child with autism (Cho et al. 2007; Devlin et al. 2005; Guerini et al. 2006; Ramoz et al. 2006). In addition, our laboratory recently reported significant relations between 5-HTTLPR and specific behavioral phenotypes including a specific form of RRB, stereotyped and repetitive motor mannerisms as measured on the ADI-R (Brune et al. 2006).

Chromosome 15q11-q13 region has been also implicated in autism and RRB, based on the following observations: 1) maternal duplication of this region is the most common chromosomal abnormality associated with autism (Bolton et al. 2001; Bolton et al. 2004; Browne et al. 1997; Cook et al. 1997; Gillberg 1998; Schroer et al. 1998; Sebat et al. 2007; Szatmari et al. 2007; Wolpert et al. 2000a; Wolpert et al. 2000b); 2) genetic markers near *GABRB3* within the 15q11-q13 region have been implicated in autism through both linkage and association studies (Buxbaum et al. 2002; Cook et al. 1998; Curran et al. 2005; Kim et al. 2007; Liu et al. 2001; Martin et al. 2000; McCauley et al. 2004; Philippe et al. 1999; Shao et al. 2003; Shao et al. 2002b), and 3) clinical and genetic overlaps between Prader-Willi syndrome (PWS) and ASDs. PWS is a rare genetic disorder caused by structural or functional absence of paternally inherited genes in the 15q11-q13 region. The

majority of PWS individuals suffer from high levels of RRB, which is a diagnostic feature of ASDs (Bittel and Butler 2005; Dykens and Shah 2003; Dykens et al. 1999; State and Dykens 2000). Furthermore, an increased rate of ASD symptoms has been reported among individuals with PWS (Veltman et al. 2005).

Based on these observations, we examined the 15q11-q13 region using 93 single nucleotide polymorphisms (SNPs) in 86 autism trios and investigated transmission disequilibrium between these SNPs and autism. We also explored possible parent-of-origin effects using likelihood-ratio tests (LRT). In addition, we carried out exploratory analyses for genotype-phenotype associations between the 15q11-q13 SNPs and RRB phenotypes as measured by the ADI-R and ADOS. Based on a previous report (Nurmi et al. 2003b), we evaluated correlations between these SNPs and savant skills as measured on the ADI-R. As epistatic interactions among common susceptibility alleles may further increase susceptibility to autism, we examined interaction terms between the 15q11-q13 SNPs and 5-HTTLPR, that has previously shown positive correlations with specific ASD phenotypes (Brune et al. 2006), using conditional logistic regression (CLR).

Methods

Subjects

Recruitment, assessment, and inclusion/exclusion criteria of subjects were described in Kim et al (2002). Only one sibling was randomly selected from each affected sibling pair. In addition, subjects for the present study also met following criteria: 1) at least 3 years old, 2) had sufficient blood or DNA available for fine mapping studies, and 3) met the ADI-R and ADOS classification for autistic disorder. All subjects had a best estimate diagnosis of autistic disorder by a clinical psychologist or a psychiatrist. 86 children with autistic disorder and their biological parents (72 trios from the University of Chicago, and 14 trios from the University of California at San Diego) were included in the present study. As expected, the male to female ratio was approximately 4:1 (79.1%, n=68 vs. 20.9%, n=18). Detailed phenotypic data measured on both the ADI-R and ADOS were available only for the 72 subjects from the University of Chicago Developmental Disorders Clinic; they are referred to as “Core Sample” in the present study. Clinical Characteristics of Core Sample are summarized in Table 1.

Phenotypic Assessment: ADI-R and ADOS

The ADI-R is a semi-structured interview that is conducted with the primary caregiver (Lord et al. 1994). Parents were interviewed using the research version of the ADI-R (Lord et al. 1994). The ADOS is a direct observational measure of the child, which is conducted in an interview or play-like setting (Lord et al. 2000). An appropriate module for ADOS (1-4) was determined by the age and language level of subjects. Eight subjects received the Pre-Linguistic Autism Diagnostic Observation Schedule (PL-ADOS) (DiLavore et al. 1995), which was treated as Module 1 for analyses.

After complete description of the study to the parents, written informed consent was obtained. Assent was obtained from the children and adolescents. The study protocol was

reviewed and approved by the Institutional Review Boards at the University of Chicago and University of California at San Diego.

The 15q11-q13 SNP Genotyping

A total of 116 SNPs were selected from the 15q11-q13 region of an approximately 5.5 million base-pair-interval based on availability of TaqMan® SNP genotyping assays in 2001 (www.appliedbiosystems.com), position (denser SNPs were placed between *UBE3A* and *GABRG3*, the interval previously associated with autism), and minor allele frequency (MAF 0.2) (Table 2). Figure 1 shows the position of the 116 SNPs across the 15q11-q13 region drawn by Haploview program version 3.32 (<http://www.broad.mit.edu/mpg/haploview/>). The SNPs are focused largely around known genes or transcripts, leading to the appearance of higher linkage disequilibrium (LD) across the region unless the typical physical map overlay is considered where SNPs are clustered along the region. SNPs were genotyped by TaqMan® SNP genotyping assays. The standard TaqMan® SNP genotyping assay protocol was observed for polymerase chain reactions (PCRs), which contained 10 ng of dry DNA, 2.5 µL of 2x TaqMan Universal Master Mix (Applied Biosystems), 0.25 µL of 20x SNP Genotyping Assay Mix, and 2.25 µL of water, for a total volume of 5 µL. All PCRs were performed using a PerkinElmer 9700 Thermocycler (Applied Biosystems) under the following conditions: 1 enzyme activation step at 95.0°C for 10 minutes, and 40 to 50 alternating cycles of denaturation at 92.0°C for 15 seconds and reannealing and extension at 60.0°C for 60 to 90 seconds. The fluorescence intensity of the final reaction product was measured using an LJI Analyst AD fluorescence microplate reader and LJI Criterion Host Software (LJI Biosystems, Sunnyvale, CA).

5-HTTLPR Genotype data

5-HTTLPR was previously genotyped in the same sample set and reported in Kim et al (2002). As we previously found an evidence of allelic association between 5-HTTLPR genotype and specific autism phenotypes measured on the ADI-R and ADOS (Brune et al. 2006), we focused on 5-HTTLPR, the 44-bp insertion/deletion polymorphism in the promoter region of the serotonin transporter gene (*SLC6A4*) for gene-gene interaction analyses.

Statistical Analysis

1. Transmission Disequilibrium Test (TDT): Among 116 SNPs, 23 SNPs were dropped: 19 markers had more than one Mendelian error, 2 markers were not in Hardy Weinberg equilibrium (HWE), and 2 markers had more than one Mendelian error and were not in HWE (Table 2). In order to examine association between the remaining 93 SNPs and autism in 86 autism trios, we carried out TDT using Haploview version 3.32. (<http://www.broad.mit.edu/mpg/haploview/>). Power was calculated using Genetic Power Calculator (Purcell et al. 2003).
2. Parent-of-origin analyses: As a part of exploratory analyses, we examined possible parent-of-origin effects using the likelihood-ratio test (LRT) for the 93 SNPs (Weinberg 1999). We first tested for imprinting in the absence of effect of maternal genotype given the evidence for imprinting in this region and lack of evidence for

maternal genotype effects (Hogart et al. 2007). For SNPs with missing parental genotypes, the log-linear model was used to test imprinting effects (Weinberg 1999). Whereas for SNPs with no missing parental genotypes, the logistic model was used to test imprinting effects (Weinberg 1999). These tests provide a chi-square statistic with 1 degree of freedom of the imprinting effect. To check whether maternal genotype influenced these results, follow-up tests which conditioned on maternal genotype were conducted. Inclusion of the maternal genotype effects in the model reduces the power of the parent-of-origin test.

3. **Genotype-Phenotype analyses:** For the exploratory genotype-phenotype analyses, family-based association analyses were conducted to eliminate effects of population admixture. We used the Family-Based Association Test (FBAT) program (www.biostat.harvard.edu/~fbat/default.html) developed by Laird and colleagues (Laird et al. 2000; Rabinowitz and Laird 2000) to test whether transmission at each of the five SNPs of the 15q11-q13 that showed nominally significant transmission disequilibrium was related to phenotype. In order to control for effects of age and non-verbal IQ, each phenotype measure was modeled as the dependent variable of these covariates and the residuals were used as the adjusted phenotype in the FBAT analyses. Additive, dominant and recessive models were tested. The following ADI-R scores were separately tested: RRB domain total, RRB sub-domains (i.e., Preoccupations and circumscribed interests, Routines and rituals, Stereotyped and repetitive mannerisms, and Preoccupations with parts or materials), verbal sub-domain that include stereotyped behavior (i.e., Failure to initiate or sustain conversation, and Stereotyped, repetitive, or idiosyncratic speech), and the sum of the sub-domains from the Inflexible language and behavior and Repetitive sensory motor behavior factors derived in Georgiades et al. (2007). We also tested the RRB domain score of the ADOS with module (1-4) as an additional covariate. Because the majority of our subjects were missing at least one item from the RSMA and IS factors derived in Cuccaro et al. (2003), we did not use these measures. Of note, these items are not part of the ADI-R classification algorithm. For our exploratory analyses of savant skills, we calculated the weighted factor score Savant Skills used in Nurmi et al. (Nurmi et al. 2003b), based on the principal components analysis of Tadevosyan-Leyfer et al. (2003), which includes current and ever scores of visual-spatial, computational, mnemonic, and musical skills. A total of 11 phenotypes were tested. Power to detect a locus that contributes to RRB was calculated using QUANTO version 1.1 (Gauderman and Morrison 2006). In this study, the FBAT empirical variance ("-e") option was not used in the genotype-phenotype analysis to test association in the presence of linkage because the probands were not related (only one sibling was randomly selected from each affected sibling pair).
4. **Gene $\times$ Gene interaction analyses:** We examined gene $\times$ gene interaction between five SNPs of the 15q11-q13 that showed nominally significant transmission disequilibrium and 5-HTTLPR. Conditional logistic regression modeling was chosen for studying epistatic effects at the two unlinked loci, because it provides a natural and flexible framework for incorporating additional effects, such as parent-

of-origin effects (Cordell et al. 2004). For CLR, trios with incomplete data were dropped and the remaining complete case-parents were analyzed along with pseudo-siblings (see Table 4). We defined the 'risk alleles' as those alleles preferentially transmitted to the children with autism (e.g., the Short allele for 5-HTTLPR). Odds ratios, 95% confidence intervals, and p-values for transmission of each risk allele and co-transmission of both risk alleles were calculated. Additive effects were considered to be consistent with the TDT. All models were programmed using the PROC LOGISTIC procedure in SAS/STAT® software version 9.1.

Results

1. Transmission Disequilibrium Test (TDT): Five of 93 SNPs showed nominally significant association with autism (Table 3). None of these SNPs was statistically significant when using a Bonferroni correction for multiple testing ($\alpha = 0.05/93$), which yields a significance level of 0.0005. Three of the five SNPs were located within or in close proximity of three GABA_A receptor subunit gene clusters (*GABRB3*, *GABRA5*, and *GABRG3*). There was no significant linkage disequilibrium (LD) between these five SNPs (Figure 2). Power calculation revealed our sample size had approximately 33% power to detect association assuming a small to moderate effect size of each susceptibility allele (or odds ratio of 2) and minor allele frequency of 0.3.
2. Three SNPs showed marginal imprinting effects [rs2340625, χ^2 (df=1) = 7.1034, $p = .0078$; rs4906951, χ^2 (df=1) = 5.3131, $p = .0212$; rs5001649, χ^2 (df=1) = 3.9471, $p = .0469$]. The later two SNPs were in LD with each other, $r^2 = 0.75$. No LD was found with the 5 associated SNPs. These effects were no longer statistically significant when maternal genotype was included in the models.
3. Genotype-Phenotype analyses: In the present study, we also explored if the five associated SNPs are related to specific RRB phenotypes and savant skills measured on the ADI-R and ADOS. The distribution of the genotype groups for the associated SNPs in the Core Sample was as follows: rs8037745 (A/A: 18, A/G: 42, G/G: 12), rs3212337 (C/C: 20, C/T: 39, T/T: 13), rs2075716 (C/C: 37, C/T: 28, T/T: 7), rs7180500 (A/A: 18, A/C: 37, C/C: 13), and rs1382056 (A/A: 20, A/G: 34, G/G: 18). Our exploratory analyses revealed two SNPs (rs2075716 and rs1382056) have nominally significant association with an ADI-R subdomain (B2V=Relative failure to initiate or sustain conversational interchange) ($Z = -2.88$, $p = 0.0039$, and $Z = -2.22$, $p = 0.0264$, respectively) under a dominant model. The C allele of rs2075716 and the G allele of rs1382056 were each over-transmitted when subjects received less severe scores on the B2V subdomain adjusting for age and non-verbal IQ. In addition, the association between rs1382056 and the Savant skills factor is trending towards statistical significance (Tadevoysan-Leyfer et al., 2003) ($Z = 1.83$, $p = 0.0680$) under a dominant model. In this case, the G allele of rs1382056 was over-transmitted when subjects received more severe scores on the Savant skills factor adjusting for age and non-verbal IQ. However, none of these SNPs were

statistically significant after correcting for multiple comparisons ($\alpha=0.05/55=0.0009$). Power calculations revealed that our sample ($n = 72$) can detect a locus that explains 36% of variance in RRB ($R^2=0.22$) with 80% of power at a p-value 0.0009, when additive inheritance model and parent-offspring sample design were assumed.

4. Gene $\times$ Gene interaction analyses: We present odds ratios, 95 % confidence intervals, and p-values from the additive models in Table 4. There was no significant interaction term between each SNP and 5-HTTLPR genotypes in any of the models considered. That is, the best model contained only the two main effects of genotype for the SNP and 5-HTTLPR.

Discussion

Chromosome 15q11-q13 is highly vulnerable to structural rearrangement. This region has been considered as an autism candidate region primarily because of multiple reports of maternal interstitial duplication associated with autism and positive linkage and association studies in chromosomally normal autism families (Nurmi et al. 2003a). The 15q11-q13 region also contains imprinted genes that are only expressed from either paternally or maternally inherited chromosome. Absence of maternally inherited genes causes Angelman syndrome (AS), whereas absence of paternally inherited genes is responsible for Prader-Willi syndrome (PWS). Interestingly, increased rates of RRB as well as ASDs are reported among individuals with PWS, suggesting that the 15q11-q13 region may contain quantitative trait loci for RRB in addition to ASDs.

In this study, we examined the 15q11-q13 region of approximately 5.5 million base-pair interval using 93 SNP markers in 86 autism trios. We also explored parent-of-origin effects in these 93 SNPs. Because of the potential implication of the 15q11-q13 region in RRB pathogenesis, we also investigated the SNPs for association with specific RRB phenotypes measured on the ADI-R and ADOS. Among 93 SNPs, five SNPs showed nominally significant association with autism in our 86 autism trios before correcting for multiple testing. These SNPs were not in significant LD with each other. Interestingly, three of five SNPs were located in close proximity of the GABA_A receptor subunit gene clusters, located within 280Kb-interval. As genes of small to moderate effects may be responsible for autism phenotypes, genetic studies of autism often require a large number of samples in order to have adequate power. As an example, the current study had 33% power to detect genes of small to moderate effect, suggesting study in a larger sample would be necessary for a more conclusive study.

Two SNPs located in the *SNRPN* region (rs5001649, rs4906951) and one in *UBE3A* (rs2340625) showed detectable imprinting effects. These SNPs were in a region which spanned 500k base pairs and included an associated SNP (rs8037745) in *SNRPN*. Interpretation of this data requires substantial caution, as these effects were not robust to inclusion of maternal genotype effects. Further, given the number of SNPs tested these results may be false positives. However, we believe the parent-of-origin effect is still worth-considering in our future studies and in a larger sample set.

The FBAT for genotype-phenotype relations between specific RRB phenotypes and savant skills measured on the ADI-R and ADOS, and the five associated SNPs revealed nominally significant association between two SNPs (rs2075716 and rs1382056) and an ADI-R subdomain (B2V=Relative failure to initiate or sustain conversational interchange), which is a component of inflexible language behavior (Georgiades et al. 2007). The lack of relationships between genotype and phenotype may be due to Type II error, lack of more thorough measurement of RRB, and other confounding effects (i.e., concurrent psychotropic medication use). In addition, our phenotypic measures were derived from the ADI-R and ADOS, which were not designed as rating scales. An instrument designed to assess RRB, such as the Repetitive Behavior Scale-Revised (Bodfish et al. 1999; Bodfish et al. 2000) may increase the power to detect susceptibility alleles for RRB. Lastly, we also need to consider that the 15q11-q13 region may not contain quantitative trait loci for RRB or savant skills, but it may interact with genes in other chromosomal regions to increase ASD susceptibility. For example, one of the small nucleolar RNA (snoRNA), HBII-52, in the 15q11-q13 has been shown to regulate the processing of the mRNA of the serotonin 2C receptor gene (*HTR2C*), which may be related with the pathogenesis of RRB (Kishore and Stamm 2006).

Previously, we have reported positive correlations between a specific RRB phenotype and 5-HTTLPR, a common length polymorphism in the serotonin transporter gene (*SLC6A4*), the main target site of SSRIs that are often used to treat RRB (Brune et al. 2006). As 5-HTTLPR genotype data were available for the full set of 86 trios, we conducted exploratory analyses to examine gene $\times$ gene interaction between five SNPs and 5-HTTLPR using conditional logistic regression (CLR). CLR is one possible method to examine epistatic effects. Another method used to explore gene-gene interactions in autism is multifactor dimensionality reduction (MDR) (i.e. Ma et al. 2005). We chose CLR over MDR, because we were examining a total of 6 markers that showed significant main effects by TDT. When used in the presence of main effects or known important covariates, MDR does not disentangle the main effects in the final model. In addition, MDR does not assume genetic (locus) heterogeneity, which may severely impact the power of study (Coffey et al. 2004). CLR analyses of additive genetic effects, however, did not identify significant gene $\times$ gene interaction terms between 5-HTTLPR and the five SNPs (rs8037745, rs3212337, rs2075716, rs7180500, and rs1382056) suggesting independent effects of the markers.

Our study has several limitations. Small sample size and limited power to detect statistically significant transmission disequilibrium are the main limitations. In addition, we used phenotypic data derived from the ADI-R and ADOS, which may not provide sufficient phenotypic information for specific RRB phenotypes. Notably, these analyses were only exploratory given the main objective of this paper was extensive genotyping of the 15q11-q13 region in a strictly defined autism sample. In future studies, we may need to employ specific analysis methods, such as Restricted Partitioning Method (RPM), to evaluate epistatic effects for quantitative traits. Finally, the region was relatively poorly covered by SNPs in the current study and application of the current methods to a much denser set of SNPs, such as the density found in the current approximately 1 million genotype SNP chips will be necessary to provide full coverage. Of note, such chips may not cover all relevant regions in 15q11-q13 due to remaining gaps and common copy number variations (CNVs)

across the region. Finally, the current approach emphasizes SNPs and doesn't fully cover the possibility of autism susceptibility CNVs that may not be in LD with SNPs.

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Figure 1.

GOLD Heatmap of LD among 116 SNPs in the 15q11-q13.

The top white box represents the chromosomal interval (base-pair position from 20,405,438 to 25,939,830) of 5.5 million base-pairs. Each SNP position is drawn as a vertical line within the white box and the SNPs are focused largely around known genes (transcripts). The LD among SNPs is shown as GOLD heatmap (highest LD is shown in red, lowest LD is blue).

There are 10 haplotype blocks shown in triangles.

Figure 2.

LD plot of five SNPs.

The top white box indicates the chromosome 15q11-q13 interval, and vertical lines correspond to the location of each SNP. The numbers of 1, 20, 67, 69, 80, 105 and 116 are the serial numbers assigned to the original 116 markers. Markers of 20, 67, 69, 80 and 105 showed nominally significant TDT. The numbers in the boxes are r^2 calculated between SNPs, indicating very low level of linkage disequilibrium. For example, r^2 between rs2075716 and rs7180500 is 1. This LD plot is derived from the Haploview program.

Table 1
Clinical Characteristics of Core Sample

		Core Sample (n=72)	
Gender		Male (n=57)	Female (n=15)
Age (years)		7.2±4.0	5.9±2.3
VIQ		55.77±30.23	53.27±28.02
NVIQ		76.9±26.6	70.5±25.8
Ethnicity	Caucasian	46	13
	African-American	5	0
	Asian	5	1
	Hispanic	1	1
ADOS module	1 or PL-ADOS	25	7
	2	15	5
	3	10	2
	4	7	1

VIQ: Verbal IQ, NVIQ: Non-verbal IQ, ADOS: Autism Diagnostic Observation Schedule, PL-ADOS: Pre-Linguistic Autism Diagnostic Observation Schedule

Table 2

The 15q11-q13 SNPs

No	dbSNP#	Position ¹	Distance ²	Gene ³	T:U ⁴	OA ⁵	OAF ⁶	Context Sequence ⁷
1	rs1544285	20405438		<i>TUBGCP5</i>	43:34	C	0.357	TGTCGTCATCATAGGCAAGTTCTATTC/TTCATTTTTACGGAAAGGCATCAGTAA
2	rs4293342	20455753	50315	<i>CYP11</i>	46:37	T	0.488	GAATAACTGATGTGTAAATTTAGAG[C/T]AGACTTGTGCATATGAACAAAACT
3	rs11855712	20465869	10116	<i>CYP11</i>	32:25	C	0.81	GGGGATGTTAGGAGGTTGTGAGAC[C/T]ACTTGGTCTTGGGAGACCCCTCCCA
4	rs2305094	20481346	15477	<i>CYP11</i>	NA			ACTCAGCGTACAAAGAGGTGAGCAC[C/T]GCTCGCGCACTCGCGGCCCTCCCG
5	rs1109036	20485132	3786	<i>CYP11</i>	36:35	A	0.313	GGTGTTCACACTCAGTTGATGG[A/G]CCAAAGCAGCCGGCCGGCGCGCGGC
6	rs1719820	20517165	32033	<i>CYP11</i>	35:33	G	0.734	TCAACACTGGCACCCTGTGCCAAC[A/G]CTGGCACCCCTGTGAGTCAGGCAAT
7	rs850817	21444782	927617		40:29	A	0.641	TTGTGGGAGGGAAATGTGGTTGCAC[T/A]GIGTCTTGAAGAACACAGGCATGTTA
8	rs850791	21487876	43094		18:11	G	0.098	GACACAACTCAGCAAAAACCGCTGC[A/G]CTGGAAGTTAAGTTTGGAAAGGTAA
9	rs1524842	21505342	17466		42:38	G	0.488	AGATTGGGCTTAGGGGTGTAGATGG[A/G]AAATCCAGCAGTTAATGAACCTTTA
10	rs729731	21725358	220016		50:33	T	0.383	AATTCACCTCAAGTGAAAACTTTGA[A/T]GTAACTCTTTACTATTCTATCTTC
11	rs3742950	22473323	747965	<i>C15orf2</i>	37:35	C	0.652	TGCCCTTCTTCTCCCAACCTGTG[C/G]AGACACAGACTCCCTGCCCCCTGAC
12	rs12902137	22475756	2433	<i>C15orf2</i>	NA			ACAACATCCCTGTGCTCCCTTCTC[T/G]TCAGTACACATGGGTCCCACTGG
13	rs12905620	22481471	5715	<i>C15orf2</i>	31:31			CCATTAAACCATCATTTTACTCGATA[C/T]AGAGAGCAACTCTATCTTCAATGCA
14	rs1463292	22551998	70527		44:36	G	0.426	AGAGAAAAATCGATAATAAACTCC[C/A/G]TTTGTAAACATAAAGGGAAGATCAT
15	rs11161139	22614603	62605		40:31	C	0.667	GTGATGTCACAGGCACAGAGACAC[C/T]GTGCTGAGCAGCCGAGTTCCCAACA
16	rs736008	22643359	28756		38:36	C	0.43	ATCCTCTACCTAGAAAAACGACATTT[C/T]GGCACCAAGCATAGGTTACTTTTCAT
17	rs5001649	22659931	16572		32:23	C	0.729	ATTGCCCTGCTTTGTATATGTTTGC[C/T]TAATCATTCCTGAAAGATTTCATGTCT
18	rs2047433	22679431	19500		NA			TCTGGACAGTCAAGAAAGGAAGCCAG[G/T]AAAAATCAGAAACACAGCGGCTTTGCA
19	rs11634496	22690296	10865		41:27	C	0.753	GCCAAAGAAAGGAGGCGCTAGATA[A/C]TCCCTCTGAGAGCTGGCAATAGATG
20	rs8037745	22698398	8102		50:29	G	0.394	AGTGATCATGTTCTTTGTGCAATGT[A/G]GTAGATGGAGAGGTGAATGAAGAT
21	rs705	22770605	72207	<i>SNURF-SNRPN</i>	46:34	T	0.509	AGCTTGCAATTGTTCTAGGAGAAC[C/T]GCCGTCAACCTTTATCTATAGCCTT
22	rs4906699	22872122	101517		50:34	C	0.408	CCTCAGGATGGTAAAGTATGGTTT[C/T]CAAAAGGCACCTAAATGTTCCATGTTT
23	rs11161166	22875770	3648		41:32	A	0.446	AACAGTGGAGTTCCAAATCACTGG[C/A/T]TAGCTTTTCATGTTGTGTCCATGCA
24	rs1549478	22883293	7523		45:34	T	0.423	GCCTTTTGCCGCATTTCCATGTCA[C/T]ATCCCATATGTTATGAGGATTTGTG
25	rs7162559	22903434	20141		43:33	A	0.325	AAATTTCTAATCTAGGATTTTAGI[A/G]TGGATATTGTTGGCTAATAGGAATA
26	rs2714758	23030430	126996		10:07	A	0.932	CCGTGGTCTCTCTGCACCTGAGCTGTG[A/G]TGACCATATCCAGGTCTCTGTAGAT

No	dbSNP#	Position ¹	Distance ²	Gene ³	T:U ⁴	OA ⁵	OAF ⁶	Context Sequence ⁷
27	rs1977036	23074798	44368		18:13	C	0.881	GCATCCTGTCTTATGAGTCACTGTG[C/T]ACTTTAGTGTGCTAGTGACCAAG
28	rs4906951	23126764	51966		35:34	C	0.345	AGAAAAACAGATAGTCTTACTCTCA[C/T]GAGGCTTAAAAATTCAGGAGGAAC
29	rs12907375	23151415	24651		NA	NA		GCCAAACCGTTTTAAGAGAGTACAAAT[A/G]TATTTGATTTAAAGCAAAACAGGAAG
30	rs4906708	23169072	17657		32:30	T	0.246	CACACAGACAACTCTACCTCCATTG[C/T]GCCAAATATACCTTTATACATGTAG
31	rs2340625	23190278	21206		35:33	C	0.713	GGTGTTATTTCTTCCTGCTAGAAC[A/C]G/TATGATTTCTTAGCTATTCCCAAGCA
32	rs7496951	23222396	32118	UBE3A	NA	NA		AAATATTACTTAAGTGTTTTATT[A/C]G/ATGACTTACCAGCAATGGAAAGATC
33	rs2526025	23236559	14163		29:18	C	0.833	GAAAAACCAACAATAAGCTAGAGA[A/C]TTTAAAGTTATTCAGGACAACTGAT
34	rs1385388	23432131	195572		38:29	T	0.705	GGGGTGCAGGTTTCTGCCAGGCTGG[C/T]CCTTCAGGTTGCTGCTTTGCTCCT
35	rs7181116	23471769	39638		48:36	C	0.339	GGCCCGTGTAAACAGCAGCCACACCA[C/T]TGAGAGGCCCTGGAGATGGCTTTCCC
36	rs8041681	23480300	8531	ATP10C	NA	NA		CCAAATGACCTCATCAACATCGCTGG[C/A]G/GTGACCTGATTGTCCACGGGGACCT
37	rs2066705	23488097	7797	ATP10C	38:37	T	0.686	AACAGCACAGAAATCAAAATCAATGTG[C/T]TAGAATTTCTTTGGGAGTCCCGGTG
38	rs2291355	23504161	16064	ATP10C	43:42	A	0.432	TGACAAAGAGCACTCACCCCTCCAGG[A/G]CCGTCCCTGC AAACTCCACACTGCC
39	rs2014053	23515138	10977	ATP10C	49:42	T	0.5	GCACAGGGGGGCTCACACTCCACAG[A/C]TGACACAGCTTGGACCCCTACAGCTA
40	rs12901627	23539308	24170	ATP10C	38:30	C	0.666	GAAAAAGTGTCTCTCTGTGCACAG[C/T]GTAAAGAGGGGTCTGGAGCCTCAGTG
41	rs11161217	23561966	22658	ATP10C	48:38	C	0.462	TCTTCCAGCTCTGTGGATTAGACA[G/A]CGGTATTTGATTTTCATGATCAAGTTCT
42	rs7165728	23569325	7359	ATP10C	30:25	T	0.786	CACATCCCACTAGGCAATAAGTGTTC[C/T]CTGAAAGTCGAGTTCCTGCTTTCTT
43	rs11632263	23579795	10470	ATP10C	44:37	T	0.434	ATGCTGAAGAGAGAGGAGTGTCCCTG[C/T]CATTTTAAAGCCTCTACTTTTTTTAA
44	rs12439329	23588937	9142	ATP10C	47:45	A	0.52	GCACGAAAGCACGCACTGCACCAGGC[A/T]CCTCCAGAGTGTAAATTCACCTCCA
45	rs872537	23599298	10361	ATP10C	42:42			CAGGTGCTCTTTATGGCCAAAGGGCTG[A/T]GTCTTTGTGAGGTGAGATAATTCTCT
46	rs1345099	23609105	9807	ATP10C	43:39	A	0.509	TAAAGATAGAATAAAAGTGCAGTGCA[A/G]GATTTACTCTTCTGCATCAATAAG
47	rs11161232	23619364	10259	ATP10C	32:30	T	0.251	AAAAATCTCAAGAGTGAACACAGAAA[C/T]JGGGGTTTGGGGCTTGGAAAAGCACAC
48	rs11630555	23627010	7646	ATP10C	43:40	C	0.429	ATTTAAGATGGTCTATATTAACCC[C/T]GTTTTCAAAAAGAACAAAGTGCGCTTTA
49	rs11633552	23638323	11313	ATP10C	29:24	G	0.203	TGTTGCTGTGTGTGGGTGGATGGG[C/G]AAAAAGCCCTGGGGTCTGGGTCTCCT
50	rs34704627	23649630	11307	ATP10C	28:24	T	0.812	GAGTCTAACAGTCAACCCAGCCCCCTT[C/T]TCAACCTTCCCAAAACGTGGGGAAG
51	rs8025575	24339176	689546		42:41	C	0.415	AAAGAAATGAAAACAGGACCTGTAAA[C/G]JAATTTCCCAAGAAATCTGCATTATTT
52	rs11637141	24343508	4332	GABRB3	24:22	T	0.207	AGAAAGCCTTTGCTTACTAAACTGAA[C/T]GAGAGGATATGAAGTAAAGTACTCA
53	rs2081648	24349292	5784	GABRB3	NA	NA		GATTGTATTAGAAATGCCAGCATCT[C/T]JAAACAAGTTCTACTTAAATGGTAAAGC
54	rs1426217	24372218	22926	GABRB3	39:34	A	0.586	AATCTTCCGTCAAAGACAACTCT[A/G]JAAAGTGACAAAGTAAATTTTCAGATTTG
55	rs8024564	24384306	12088	GABRB3	40:40			CAGTGTAGGGTCTCTGTGACTAC[A/G]JAGGTATGTTTCTGTATAGAGAAAGCAG

No	dbSNP#	Position ¹	Distance ²	Gene ³	T:U ⁴	OA ⁵	OAF ⁶	Context Sequence ⁷
56	rs2873027	24418502	34196	GABRB3	38:36	C	0.622	CTTGGCCACAAAATTACCAATAATC[C/T]ACGTGATGGTTCTGCTTTCGTTTG
57	rs4542636	24419024	522	GABRB3	NA			AGGCTTAGGAGCTGATAGAGGACAT[C/T]GATTGTTCTTCAGATGCTTGCCACA
58	rs754185	24438972	19948	GABRB3	29:27	T	0.39	ACAAATTTTAAATATTGTGAGTTA[T/C/T]TGGGAGAAAGATTATCACTCAACAG
59	rs12912421	24453689	14717	GABRB3	42:35	A	0.482	CTAGTTTAGTAAACAGAGACCTGTAC[A/G]GTCTAAATCTACGGATGGATGTTAG
60	rs2315905	24464785	11096	GABRB3	44:30	T	0.334	TTGGATGTCATTTATGTTGTTTTTTT[C/T]TGTCAAATGGCCTGTCAAGCTCTTGC
61	rs890317	24473294	8509	GABRB3	29:23	C	0.748	AGAACTCTCCATGATTGAAATGGT[A/C]GCACATGGAATAACATCGATAAGTT
62	rs878960	24480029	6735	GABRB3	34:32	T	0.616	AAAACATATATAGGATTATACACTCT[C/T]ATACACTCATGAGTGGGACCTGGC
63	rs11631421	24502431	22402	GABRB3	49:37	T	0.624	CAATCAGAAAGATGCTTCAGGCTCTA[C/T]CCACCTTCGAAACTCAACGGGTCC
64	rs981778	24508333	5902	GABRB3	46:32	G	0.635	CAGCAGGTGGAGCACAGGGCTTA[A/G]TGGAGGCCAGGGAAGGTGGCAGAG
65	rs970408	24540078	31745	GABRB3	22:11	T	0.118	TTAGATTGGTCAACAGAGATGAATG[C/T]AGATGCACACAAAATATCAACAGCAA
66	rs2059574	24548136	8058	GABRB3	NA			TTGCTGATTTTCAGGCCAAACTATGT[A/T]JACATGGCTTTCAATGGGTGCTTGGC
67	rs3212337	24562204	14068	GABRB3	52:32	T	0.384	TGTCCTGCAGTAAGAGTAAGAAAAGT[C/T]JGGTGCTATTTCTGAGCCTATTTTCT
68	rs8179184	24570695	8491		23:19	T	0.14	CGTTTTTGTCTCTGTAGACTTCTT[C/T]JAGAATATTCAGGACTTAATGAATG
69	rs2075716	24665997	95302	LOC72772	50:30	C	0.624	TCAAAGTTGCCTTGAAAGCCAGGCC[C/T]ATGTCTACTTGGCATTGAAAAGGACT
70	rs28705902	24675174	9177	LOC72772	39:34	A	0.395	GCAGAGCAGGAGTGTCTTATCTCC[A/G]CTCTCTGGAGAGAGCCACGGAAAAACA
71	rs35586628	24683233	8059		39:35	T	0.568	AACTACTGGTGAATAAGGAATCA[C/T]AAGCAGCAATCAACCTGTGGTTGA
72	rs9745027	24693360	10127		NA			AGAGGTCCCTTCACTGCCATTATAG[A/C]JGAATACATGAGCGTGAATTTTAACC
73	rs35399885	24743281	49921	GABRA5	43:31	T	0.351	CACTCAGCATTTTAGGAAATATGTG[C/T]TGTCTTGAAACAAATAACAAATATT
74	rs140683	24771081	27800	GABRA5	41:40	A	0.429	CAGCACTGACCCCTGTTTCCGTTT[A/T]JCACTCTGCCCTGCTGAAACCCCGCA
75	rs140685	24771205	124	GABRA5	45:43	C	0.503	CCACCGCCATGGACTGGTTTCATAG[C/T]GTGTGCTATGCGCTTCGCTCTCTCGG
76	rs11263717	24780701	9496		44:41	A	0.435	AATGTAAGACCTCAGAAAGGAAAAAT[A/T]TGAAGTGAGCTCTAAATTGATCCAAAT
77	rs1432133	24811092	30391		40:35	A	0.506	GCCAAATTTCAATTTGCTATAACCAT[C/A/G]GGCCCTTCTCATCCCTTATCAAAGGTG
78	rs1432129	24814779	3687		47:34	C	0.547	AAGATCTGGACTCACATAATGAGTT[A/C]TCCCCACAGTTACACGACGGTTTCA
79	rs28431127	24833418	18639		46:31	T	0.488	ATAGGAAACAAAAATCATGTTATCCT[C/T]TTTTACCCCTTCTCTTATTCGTTGT
80	rs7180500	24835925	2507		45:25	A	0.497	CTCTTCTGGAGCATAATACAAACT[A/C]TTACTAAACATACTACAGCTAGTGT
81	rs6606855	24846903	10978		47:34	A	0.588	TGTCACGATAATCATGATGATG[A/G]TAAAAACAGTACTAATCCCATTTGA
82	rs7172534	24855745	8842		40:39	T	0.611	ATACATCATTTATTCAGTTGACTGCT[C/T]JAGAGTAACCCCTTCTTATGATGTT
83	rs4078843	24877209	21464		41:33	A	0.324	TTACATGGGTCCACAGACTACAGT[C/A/G]CACAAATCAGAGCTTCAATTTATTA
84	rs4555125	24922458	45249		32:29	A	0.738	GCCTGAGGAGGCACAGATTCTCGGG[G/A]CTCAGGAAACATAAATAGAGGGAAG

No	dbSNP#	Position ¹	Distance ²	Gene ³	T:U ⁴	OA ⁵	OAF ⁶	Context Sequence ⁷
85	rs4887536	24931274	8816		45:42	A	0.556	AAAGTAGGAGTGTCTGAACCAACAAG[A/C]CCCTGAGTATCCCCACAGCAGGTTC
86	rs208174	24962533	31259		34:32	C	0.664	CTCCTCTCCTAGTGTTCACGTCTC[C/T]CTCAGAAAGAACCCAGGTGCACGTTC
87	rs2286946	24985293	22760		NA			ATAAGCTGAAGAAAAAGCCAGTAA[T/C]GTTTGTCAATTTGACAGCAGTGGATTA
88	rs741121	24990517	5224		50:37	G	0.456	ATGAAAAAGGAGTACCTAGGTTT[G/T]CTAGAAATTGGAATAGGGAAAGAAAA
89	rs208129	25007653	17136		42:38	A	0.573	TGGTGTGAATTTTGAATGCTGTGGG[A/T]AAGATGGAAGCCAAAGATGCACCGG
90	rs208126	25009214	1561		39:38	T	0.582	GCCACAGGAGAGAAAAACAAGAG[G/T]TAGTTCTCTTACCCATGCACACCTT
91	rs12907392	25038596	29382		33:26	T	0.735	CACCTGCCATTTTGGAAAGTCAAGTGT[T/C]JAGCAGGGTGGGAGGTGTGTGTTC
92	rs897173	25052647	14051		35:26	A	0.722	ATTATTTTACATCCCTGCAATGA[A/G]CTTTTCTTGATGCTGTGTCATATAGG
93	rs6606891	25087882	35235		NA			TCTTCCTCAGTGACACTGGATGTGT[C/JAAGACAAAAGGCCCTGGGAAGATGTG
94	rs8043244	25091556	3674		NA			ATCTTGCAAAAGTTAATGGTATTC[A/G]ATTATGATTTCTTACACACATATTG
95	rs28564251	25161851	70295		38:30	A	0.546	CCTCATTCCTTAAAGTTGTTTGA[A/G]TAATTTGTTTCTTCTTGGTTTTCT
96	rs9672931	25279996	118145		36:29	G	0.71	GCAGAGCACTTGGAGTTTGAAATGC[A/G]GTGGCCACTGGAGCAAACTTCTCA
97	rs28378890	25287491	7495		34:27	A	0.738	CCTTCAAATTACTGAAAAAGGACAAAGT[A/G]GACATGCTTGGGAAGAAAAATCT
98	rs12440080	25318817	31326		37:32	A	0.459	ATTTAAATCTTTTATCATCTTGTCT[A/G]TGAAGGGATTGTCTGAAGCTAATG
99	rs12900354	25319205	388		NA			GCTGCTTCTTACCAATTGAATGAAG[A/G]TACTATGCTTTGATGTTAAAAATTG
100	rs11631444	25341819	22614		39:38	G	0.464	TTTATAAAGGGCTGGCCACAGAGAC[A/G]CATCTTACTATATGAACGCCAGGG
101	rs11074283	25419174	77355	<i>GABRG3</i>	51:40	T	0.485	CCACCGCACTCCACCCGCACTTC[C/T]TTAGCCATGCATGGGGAGCTGCAG
102	rs1871019	25429555	10381	<i>GABRG3</i>	48:38	G	0.453	TTCTAAAAACTATCCGTTACTTTAC[A/G]TGTAAACAGGATTACGGGCATGAAG
103	rs11631143	25437997	8442	<i>GABRG3</i>	52:39	A	0.471	TGGAGATGCTTTGGGGAGAAAAATG[T/A]GJCAATGTAATTAAAGCATGCACCCCTTT
104	rs140679	25446271	8274	<i>GABRG3</i>	52:39	C	0.477	TGACCGCCATGGACCTTTTGTGAC[C/T]GTGTGCTTCTGTTGTCTTCGCCG
105	rs1382056	25509241	62970		49:27	A	0.468	AGTACCATCTACTCTAAAGGGCAGAG[A/G]TAAATGCCCTTAAACCCCTAAGATGAG
106	rs1871017	25564086	54845		45:43	T	0.512	GGGTGGATGTGCTCAGTGTGAAGCT[G/T]ATCGTTGTGTAAATTTGTCTCAAAGG
107	rs884073	25599091	35005		NA			AAGGAGCCACTGGCCTCTGTCTGGAG[A/T]GAAAGCTGCCAAAATGCGGTGTGTCTG
108	rs11074304	25674612	75521	<i>OCA2</i>	41:33	C	0.582	AGATTTCCAGAGATCCAGCTTCC[A/C]GCAGGCTCCTGAAGTCCCTCACACT
109	rs12591640	25712846	38234	<i>OCA2</i>	41:28	C	0.418	CTGCAAAACAGGAGGAAGATAAGG[C/T]GTGCCAAAAGCTCCAAAGAAATGTGTG
110	rs9806708	25746674	33828	<i>OCA2</i>	38:29	G	0.476	CGAAGACACTTGAATAGGTGCTAC[T/A]GATGAAGACGATCTATAGCACAAGGC
111	rs11074309	25779732	33058	<i>OCA2</i>	44:32	C	0.646	CAAGCAGGGAGCCCTCTGTGGACCA[C/T]AGGCTCTCAAGGAGCAGGGCAAGAGT
112	rs12439756	25822303	42571	<i>OCA2</i>	47:32	C	0.643	CACTTTCTATTACTGAGAAGAAAAAC[C/G]CTGGAAAAGTCCCGGGCTTCCCCCAG
113	rs2594935	25858633	36330	<i>OCA2</i>	NA			CTTGGATCTTCTTGTGACAAAGTAA[C/A/G]TTTATCTCCTCAATTGCAAGTTTCTCT

No	dbSNP#	Position ¹	Distance ²	Gene ³	T:U ⁴	OA ⁵	OAF ⁶	Context Sequence ⁷
114	rs11638265	25876168	17535	<i>OCA2</i>	37:36	A	0.383	TAAACTCGGGCTGTGTACCCCTGCA[A/G]AGCTCAGTGAGGGTTAGATAAAATG
115	rs12910433	25902239	26071	<i>OCA2</i>	36:35	A	0.385	CCTCACACAACCTGTCTACAAATGGA[A/G]GAAAAATGAAAGTAGTCCCACTATAC
116	rs746861	25939830	37591	<i>OCA2</i>	NA			TAAGTAGACTAAAGAAAAAACACT[C/T]GCCATTTAGCTAACTGAAATTAAATTC

Markers with nominally significant TDT are shown in bold face; 23 markers were dropped from further analyses due to Mendelian errors and they are shown in italic face. Context sequences are shown because some of the SNP assays were designed based on reverse strand.

Footnotes:

- ¹ base-pair position in March 2006 assembly on the UCSD genome browser (<http://genome.ucsc.edu/>);
- ² distance to next SNP;
- ³ known genes that contain the SNPs;
- ⁴ Transmitted vs. not-transmitted;
- ⁵ Over-transmitted allele;
- ⁶ Over-transmitted allele frequency;
- ⁷ context sequence surrounding SNPs typed in this study. Please note some SNP assays were designed on reverse strands and others were on forward strands.

Table 3

Variants nominally associated with autism by TDT

Marker	Gene	Allele	T	NT	χ^2	p-value (86 trios)	p-value ² (Core 72 trios)
5-HTTLPR	SLC6A4	S	57	27	10.714	0.0011	0.0140
		L	27	57			
rs8037745	SNRPN	G	50	29	5.582	0.0181	0.0115
		A	29	50			
rs3212337	GABRB3	T	52	32	4.762	0.0291	0.0593
		C	32	52			
rs2075716	GABRA5	C	51	31	4.878	0.0272	0.0041
		T	31	51			
rs7180500	GABRG3	A	45	25	5.714	0.0168	0.0072
		C	25	45			
rs1382056	GABRG3/OCA2 ¹	A	49	28	5.727	0.0167	0.0548
		G	28	49			

¹Intergenic between *GABRG3* and *OCA2* gene.²P-value calculated from 72 Core Sample.

Table 4

Odds ratios and 95% confidence intervals for additive genetic effects models of 5-HTTLPR and the associated 15q11-q13 SNPs.

Effect	N/I	df	Estimate	SE	Wald χ^2	Odds Ratio	Lower Bound	Upper Bound	P-value
5-HTTLPR	82	1	0.8242	0.2399	11.8043	2.280	1.425	3.649	0.0006
rs8037745	82	1	0.5390	0.2378	5.1376	1.714	1.076	2.732	0.0234
$G \times G^2$	82	1	-0.1893	0.2816	0.4516	0.828	0.477	1.437	0.5016
5-HTTLPR	81	1	0.8242	0.2399	11.8043	2.280	1.425	3.649	0.0006
rs3212337	81	1	0.5306	0.2301	5.3185	1.700	1.083	2.669	0.0211
$G \times G^2$	81	1	0.3802	0.2718	1.9562	1.463	0.858	2.492	0.1619
5-HTTLPR	83	1	0.7295	0.2343	9.6949	2.074	1.310	3.283	0.0018
rs2075716	83	1	0.4578	0.2295	3.9800	1.581	1.008	2.478	0.0460
$G \times G^2$	83	1	-0.1636	0.2742	0.3559	0.849	0.496	1.453	0.5508
5-HTTLPR	74	1	0.8602	0.2543	11.4392	2.364	1.436	3.891	0.0007
rs7180500	74	1	0.5188	0.2526	4.2180	1.680	1.024	2.756	0.0400
$G \times G^2$	74	1	0	0.2747	0	1.000	0.584	1.713	1.0000
5-HTTLPR	84	1	0.7472	0.2336	10.2294	2.111	1.336	3.337	0.0014
rs1382056	84	1	0.6131	0.2435	6.3395	1.846	1.146	2.975	0.0118
$G \times G^2$	84	1	0.4239	0.2811	2.2742	1.528	0.881	2.651	0.1315

¹Trios with incomplete data were dropped for each analysis.

²The effect of the interaction term, $G \times G$, comes from the additive model *with* the interaction term. Since the interaction term was not significant, the more parsimonious model was chosen and the main effects are presented here for the additive model *without* the interaction term (all main effects remain $p < .05$ with or without the interaction term).