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MACROD2 gene associated with autistic-like traits in a general population sample

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Abstract

There is now substantial evidence that autistic-like traits in the general population lie on a continuum, with clinical autism spectrum disorders (ASD) representing the extreme end of this distribution. In this study, we sought to evaluate five independently identified genetic associations with ASD with autistic-like traits in the general population. In the study cohort, clinical phenotype and genomewide association genotype data were obtained from the Western Australian Pregnancy Cohort (Raine) Study. The outcome measure used was the Autism Spectrum Quotient (AQ), a quantitative measure of autistic-like traits of individuals in the cohort. Total AQ scores were calculated for each individual, as well as scores for three subscales. Five candidate single nucleotide polymorphism (SNP) associations with ASD, reported in previously published genomewide association studies, were selected using a nominal cutoff value of P less than 1.0×10^{-5}. We tested whether these five SNPs were associated with total AQ and the subscales, after adjustment for possible confounders. SNP rs4141463 located in the macro domain containing 2 (*MACROD2*) gene was significantly associated with the Communication/Mindreading subscale. No other SNP was significantly associated with total AQ or the subscales. The *MACROD2* gene is a strong positional candidate risk factor for autistic-like traits in the general population.

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Conflicts of interest

There are no conflicts of interest.

Keywords

association; autistic-like traits; *MACROD2*; Raine study; single nucleotide polymorphism

Introduction

Autism spectrum disorders (ASD) represent a group of neurodevelopmental conditions characterized by impairments in social interaction and communication, and repetitive interests and behaviors. A recent review estimated the median global prevalence of ASD at 62 cases per 10 000 children (Elsabbagh *et al.*, 2012). The overall heritability of ASD is estimated at 90%, which is among the highest for any neuropsychiatric disorder (Hollander *et al.*, 2003; Skuse *et al.*, 2005). Evidence suggests that ASD have a complex inheritance in which different, likely overlapping, groups of genetic variants may cause susceptibility to disease (Veenstra-Vanderweele *et al.*, 2004). Increasing numbers of epidemiological and genetic studies have deepened the understanding of the genetic contribution to ASD, and have shown that a variety of genetic mechanisms may be involved in the etiology (Li *et al.*, 2012).

Autistic-like traits are subthreshold deficits in socialization and communication as well as restricted interests that do not meet the formal criteria for ASD (Constantino and Todd, 2003). Several authors have suggested that ASD can be conceptualized as the conditions arising in individuals found at the extreme end of a normal distribution of autistic-like traits (Gillberg, 1992; Constantino and Todd, 2003; Ronald *et al.*, 2005). Population-based studies have supported this by finding that in addition to individuals with ASD, many others exhibit subthreshold autistic or autistic-like traits (Constantino and Todd, 2003; Posserud *et al.*, 2006; Lundström *et al.*, 2012). Subthreshold autistic-like traits have been examined in general population twin studies, with heritability estimates ranging from 36 to 87% in these studies (Ronald and Hoekstra, 2011).

With the discovery of common genetic variants associated with ASD, the question is raised whether common risk loci also contribute to variation in phenotypes such as autistic-like traits (St Pourcain *et al.*, 2010). One methodological approach that is gaining influence in ASD research is the examination of the quantitative distribution of autistic-like traits in the broader community, rather than in a dichotomy of ASD patients and controls. Many authors have postulated that common genetic variants that are present in a significant proportion of the general population may play a role in the etiology of ASD (Campbell *et al.*, 2006; Alarcón *et al.*, 2008; Chakrabarti *et al.*, 2009; Wang *et al.*, 2009; Anney *et al.*, 2010; Ronald *et al.*, 2010). Further, it is thought that understanding the etiology of individual differences in autistic-like traits in the general population will help understand the causes of ASD (Ronald and Hoekstra, 2011). Between 1995 and 2008, nearly 200 genes were investigated for associations with ASD, and more than 80 of these were reported with nominally positive results (Holt *et al.*, 2010). The Simons Foundation Autism Research Initiative (SFARI) has developed a publicly available database for ASD research, SFARI Gene, centered on genes implicated in ASD susceptibility (Banerjee-Basu and Packer, 2010). SFARI Gene serves as

a comprehensive reference for all known human genes associated with ASD, currently listing 423 genes from 584 studies, as of September 2012 (Banerjee-Basu and Packer, 2010).

Despite numerous studies and reported associations, no gene has been unambiguously shown to contribute to ASD susceptibility, and independent groups have not been able to consistently replicate any associations (Yang and Gill, 2007; Abrahams and Geschwind, 2008; Holt *et al.*, 2010).

The aim of the current study was to investigate associations between autistic-like traits in a Western Australian population sample and five SNPs previously reported to be associated with ASD.

Materials and methods

Study sample population

Data were obtained from the Western Australian Pregnancy Cohort (Raine) Study, a longitudinal investigation of pregnant women and their offspring, who were recruited from King Edward Memorial Hospital, Perth, Western Australia, or nearby private practices between 1989 and 1991 (Newnham *et al.*, 1993). Of the 2900 pregnancies recruited into the Raine Study, 2868 live-born children have been under follow-up since the commencement of the study. The final sample consisted of 965 individuals from the Raine Study with both genotype and outcome measures. Participant recruitment from the study families was approved by the Human Ethics Committee at King Edward Memorial Hospital. Ethical approval for the 20-year follow-up was received from the Human Research Ethics Committee at the University of Western Australia. Participants provided written informed consent for data collection on autistic-like trait outcomes at ~ 20 years of age.

Measure of autistic-like traits

At the 20-year follow-up, Raine Study participants who did not have a diagnosis of intellectual disability or ASD were asked to complete the Autism Spectrum Quotient (AQ; Baron-Cohen *et al.*, 2001). The AQ is a self-report questionnaire that provides a quantitative measure of autistic-like traits in the general population (Baron-Cohen *et al.*, 2001). Individuals are provided with 50 statements and asked to indicate on a 4-point scale how well each statement applies to them (strongly agree, agree, disagree, strongly disagree). The items were scored on a scale ranging from 1 to 4, on the basis of previous research, according to which this scoring method retains more information about responses than does dichotomous scoring that was first proposed for this instrument (Baron-Cohen *et al.*, 2001; Austin, 2005; Stewart and Austin, 2009; Russell-Smith *et al.*, 2011). Scores for each item are summed to provide the total AQ, with higher scores indicating greater autistic-like traits. The total AQ is known to have good test–retest reliability ($r = 0.7$), and validation studies have found that scores in the general population follow a normal quantitative distribution (Baron-Cohen *et al.*, 2001; Whitehouse *et al.*, 2011). Factor analyses of the AQ in several countries have consistently identified three clear factors, related to social ability, attention to detail/patterns, and the understanding of others. In the current study, we divided items into the subscales identified in a study of Western Australian adults (Russell-Smith *et al.*, 2011)

who were highly similar to the sample under investigation here: Social Skills, Details/Patterns, and Communication/Mindreading. There is minimal difference between the items in these subscales and those reported in other factor analyses. For the current data set, internal reliability of the scales ranged from moderate (Communication/Mindreading: α = 0.63) to good (Details/Patterns: α = 0.78) and excellent (Social Skills: α = 0.85). In this study, total AQ scores and the scores of the three subscales were used as four continuous outcome measures.

Selection of candidate single nucleotide polymorphisms

Positional candidate SNPs were identified by conducting a search in the catalog of published genomewide association (GWA) studies by the National Human Genome Research Institute (2012). The selection was based on a nominated cutoff *P*-value of less than 1.0×10^{-5}, which has been used by the National Human Genome Research Institute for the identification and archiving of putative associations (National Human Genome Research Institute, 2012). The search keywords included 'autism' as the disease/trait, and a *P*-value threshold less than 1.0×10^{-5}. These search terms together yielded five SNPs: rs4141463 in macro domain containing 2 (*MACROD2*), rs7142002 in protein phosphatase 2, regulatory subunit B' gamma (*PPP2R5C*), rs10513025 flanking sema domain, seven thrombospondin repeats (*SEMA5A*), and taste receptor, type 2, member 1 (*TAS2R1*), and rs10038113 and rs307059, both flanking cadherin 10, type 2 (*CDH10*) and cadherin 9, type 2 (*CDH9*). A summary of the candidate SNPs selected for the current study and their previous association details are presented in Table 1.

Study sample

In the Raine Study, DNA was collected from blood samples from 74% of the adolescents who attended the 14-year follow-up, and a further 5% who attended the 16-year follow-up, using standardized procedures. Candidate SNP data for this study were obtained from genomewide genotype data described previously (Jones *et al.*, 2013). Briefly, genotyping was performed on the Illumina Human 660W Quad Array (Illumina, San Diego, California, USA) and individuals were excluded due to low genotyping success (>3% missing), excessive heterozygosity, relation with another sample (identity by descent > 0.1875), ambiguous sex, and mislabeling. There were 1494 individuals whose DNA samples passed the quality control criteria and were eligible for genetic analyses, and 965 of them had completed the AQ.

Statistical methods

Generalized linear models were used to model the association of genotype and multiple covariates with autistic-like trait outcomes. Individuals included in the final analyses were those who had both genotype and autistic-like trait outcome measures, although not all participants had data for all covariates.

Covariate selection

Potential covariates that may plausibly influence AQ scores were selected. These included sex, season of birth (summer, autumn, winter, or spring), family income (family earning > or

< $24 000 at the time of pregnancy), maternal education (mother completed secondary school or did not complete secondary school), gestational age (gestation < 32 weeks, 32–37 weeks, 38–40 weeks, or > 40 weeks), father's BMI before pregnancy, mother's BMI before pregnancy, father's age at conception, mother's age at conception, and age at AQ completion.

Final models for total AQ scores and each subscale were constructed using a stepwise variable selection procedure, to identify independent predictors of each outcome from potential covariates. A *P*-value of less than 0.05 was used to select covariates to be retained for multiple linear regression modeling. Each model started with all potential covariates, and then the least significant variables were removed one at a time. Once a model containing only significant variables was reached, previously omitted variables were added back in to check for changes in significance.

Genetic analysis

Imputed dose scores (imputed to HapMap Phase II CEU) were used for analyses. Dose scores represent the posterior probability of the minor allele and can take any value between 0 and 2. Hardy–Weinberg equilibrium (HWE) was calculated using Fisher's exact test. Genetic association tests were performed using multiple linear regression, with each SNP added separately to the full models and the SNP *P*-value considered for significance. Directional hypotheses were used for each SNP, with an association defined as significant when the direction of the effect of the SNP was in the same direction as in previous studies. Confidence intervals (95% CIs) were calculated for the effect of any significantly associated SNP. Adjustments for multiple testing were made using a Bonferroni correction, with SNP association results considered significant if the *P*-value was below 0.01.

Results

Power

Power was calculated using Quanto (Gauderman and Morrison, 2007). For a common variant with a minor allele frequency (MAF) of 0.3, we had greater than 80% power to detect a 1 U change in the three subscales: Social Skills, Details/Patterns and Communication Mindreading. For a variant with an MAF of 0.3, we had 40% power to detect a 1 U change in the total AQ.

Descriptive statistics

Characteristics of the current study sample are listed in Table 2. Just over half (51.3%) of the sample were female, and the mean age at AQ completion was 19.68 years (SD = 0.70). The mean total AQ score was 103.2 (SD = 12.60). The total AQ and each subscale were normally distributed.

Consistency of genotype distributions with HWE were examined for each candidate SNP using Fisher's exact test. Table 3 lists the genotypic distributions, MAFs, and HWE *P*-values. Results indicate that all SNPs were consistent with HWE (all *P*'s > 0.05).

Candidate single nucleotide polymorphism analysis

Covariate modeling—Models for all potential covariates were constructed. The final models for total AQ, Social Skills, Details/Patterns, and Communication/Mindreading are presented in Table 4.

Male sex, increased age at AQ completion, higher mother's BMI, and greater family income were associated with a higher total AQ score. Maternal education was significantly inversely associated with total AQ scores.

Male sex was associated with higher Social Skill scores, indicating poorer social skills, as was mother's BMI and greater family income. A higher maternal age at conception was also associated with higher Social Skill scores.

Male sex and mother's BMI were significantly associated with Details/Patterns. Father's age at conception and maternal education were both inversely associated with Detail/Pattern scores.

Higher Communication/Mindreading scores were associated with increased age at AQ completion and mother's BMI. Increased father's age at conception was associated with lower Communication/Mindreading scores. Season of birth was also a significant covariate, spring and autumn births being associated with increased Communication/Mindreading scores compared with winter birth.

Single nucleotide polymorphism analysis—Genetic association tests were performed with multiple linear regression. Each SNP was added to the covariate model for total AQ, Social Skills, Details/Patterns, and Communication/Mindreading. Tables 5-8 contain results from the analysis of candidate SNPs, after adjusting for covariates. The results in Table 8 show a significant association between SNP rs4141463 (*MACROD2* gene) and Communication/Mindreading ($P = 0.006$). Each additional copy of the effect allele, T, decreased an individual's Communication/Mindreading by 0.475 U (95% CI = −0.840 to −0.110). The effect allele T is in the same direction as in the initial report (Anney *et al.*, 2010).

Discussion

In this study, we report an association between the rs4141463 SNP that resides within intron 5 of the *MACROD2* gene on chromosome 20p12 and autistic-like traits in a Western Australian population. This SNP was previously reported to be associated with ASD by the Autism Genome Project Consortium (Anney *et al.*, 2010). In both studies, the minor allele T was associated with a decrease in autistic traits.

The candidate SNP rs4141463 is located on chromosome 20p12.1. This SNP resides within an intron of the *MACROD2* gene. The function of the MACROD2 protein is largely unknown, but it contains a macro domain, which is a high-affinity ADP-ribose-binding domain, and is important for multiple biological processes (Mouren *et al.*, 2012). Copy number variants in *MACROD2* have been reported in schizophrenia (Xu *et al.*, 2009). There is also evidence that suggests that this SNP, as well as others in the region, could act to

regulate the expression of the phospholipase D2 (*PLD2*) gene (Anney *et al.*, 2010). Phospholipase proteins could play an important role in risk for ASD. The protein derived from *PLD2* has been shown to regulate axonal growth (Kanaho *et al.*, 2009) and metabotropic receptor signaling (Dhami and Ferguson, 2006).

No significant associations were found between autistic-like traits and the remaining four SNPs. SNP rs7142002 is located within an intron of the protein phosphatase 2, regulatory subunit B', gamma (*PPP2R5C*) gene at 14q32.31, and its association with autism was reported by the Autism Genome Project Consortium (Anney *et al.*, 2010), with the C allele protective for ASD.

SNP rs10513025 is located between the genes sema domain, seven thrombospondin repeats (*SEMA5A*), and taste receptor, type 2, member 1 (*TAS2R1*) on chromosome 5p15.2 in a region of high linkage disequilibrium. In an earlier linkage and genomewide association study, the C allele was associated with a decreased risk for autism (Weiss *et al.*, 2009).

The SNP rs4307059, located in the 5p14.1 region, is in a linkage disequilibrium block in an intergenic region between the cadherin 10, type 2 (*CDH10*) and cadherin 9, type 2 (*CDH9*) genes. The GWA study in 2009 found an association between this SNP and ASD, with the C allele reducing the risk for ASD (Wang *et al.*, 2009). A study in 2010 successfully replicated the association between rs4307059 and social communication spectrum phenotypes in the general population (St Pourcain *et al.*, 2010).

SNP rs10038113 is also located within an intergenic region between the *CDH10* and *CDH9* genes on chromosome 5p14.1. A GWA study reported an association between ASD and SNP rs10038113, with the C allele conferring an increased risk for ASD (Ma *et al.*, 2009). It is likely that one or more nearby functional variants are responsible for the signal in this area, as rs10038113 does not reside within known genes or regulatory sequences (Ma *et al.*, 2009). In the surrounding region, there are numerous segments that exhibit a high degree of evolutionary conservation, suggesting potential regulatory functions that have yet to be determined (Ma *et al.*, 2009).

MACROD2 as a positional candidate susceptibility gene

The function of the protein encoded by *MACROD2* is poorly understood. The protein contains a macro domain that is evolutionarily conserved and reaches its highest expression in the adult and fetal human brain (Debette *et al.*, 2010). Macro domains act as deacetylases that bind ADP-ribose, a signaling molecule generated by the deacetylation of acetylated lysines in histones and other proteins, and have an important role in multiple biological processes including DNA repair, transcriptional activation, and repression (Debette *et al.*, 2010; Mouren *et al.*, 2012). The rs4141463 SNP is located within a hypersensitive site in a *MACROD2* intron, which may indicate an open chromatin conformation and thus may serve a regulatory role in the expression of *MACROD2*. However, this variant may not be the actual causal variant having an impact on ASD risk as GWA localizations are based on linkage disequilibrium, implying that the actual causal variant could possibly be located some distance away on the same chromosome.

Other studies have reported copy number variants at *MACROD2* in association with schizophrenia (Xu *et al.*, 2009), brain infarct (Debette *et al.*, 2010), and brain volume in multiple sclerosis (Baranzini *et al.*, 2009). A deletion in the *MACROD2* gene has also been reported in attention deficit hyperactivity disorder (ADHD; Lionel *et al.*, 2011). In addition to the copy number variants in the *MACROD2* gene, other rare variants have also been found to overlap between ADHD and ASD studies (Lionel *et al.*, 2011). ADHD is known to be comorbid in ~ 78% of children with ASD (Lee and Ousley, 2006). Furthermore, twin studies have identified that close to three-quarters of the genetic risk factors for ADHD are shared with ASD (Kendler, 2010).

The apparent overlap of copy number variants between ADHD and ASD and other neurologic and psychiatric disorders supports evidence that variants in a common set of genes could be involved in the etiology of several neuropsychiatric disorders (Carroll and Owen, 2009; Guilmatre *et al.*, 2009; Saus *et al.*, 2010). This implies that there may be specific genetic loci or alleles that increase an individual's risk for developing any of these neuropsychiatric disorders, and that these loci may represent a common biological pathway (Carroll and Owen, 2009). The results from this study add further interest to the region on chromosome 20p12.1 as a plausible candidate region for ASD etiology. These results also support evidence that a genetic variant initially detected in individuals with clinically diagnosed ASD has an impact on autistic-like traits in the general population.

The current finding needs to be interpreted in the context of two failed replications of the rs4141463 SNP in probands with ASD. Curran *et al.* (2011) attempted to replicate the association using an independent case–control design with 1170 European cases and 35 307 controls and Prandini *et al.* (2012) sought to replicate genetic markers from recent genomewide and candidate-gene studies in the Italian Autism Network cohort, consisting of 233 probands, 423 parents, and 90 siblings. In both studies, cases met the Diagnostic and Statistical Manual of Mental Disorders, 4th ed. criteria for Autism, Asperger's syndrome, or pervasive developmental disorder not otherwise specified on the basis of the Autism Diagnostic Interview-Revised and the Autism Diagnostic Observation Schedule. Both studies failed to observe a significant association between the rs4141463 SNP and ASD, with both *P*-values greater than 0.05 (Curran and colleagues: $P = 0.50$, odds ratio = 0.99, 95% CI = 0.88–1.11; Prandini and colleagues: $P > 0.05$).

In this study, we have reported an association between rs4141463 in *MACROD2* and the Communication/Mindreading subscale in a general population of young Western Australian adults; however, we did not find associations for four other SNPs also previously reported to be associated with ASD. The failure to replicate a genetic association is a commonly observed phenomenon in complex human disease genetics research, including ASD research, and there are a host of potential reasons for this, including a false-positive finding in the original study, ethnic heterogeneity between data sets, environmental interactions, age-dependent effects, epistasis, and inadequate statistical power (Chanock *et al.*, 2007; Shriner *et al.*, 2007; Greene *et al.*, 2009). Further genetic characterization of the rs4141463 SNP association is warranted, but is beyond the scope of this study, and may possibly require exhaustive DNA sequencing to identify all variants within the *MACROD2* gene

region followed by a variety of detailed molecular biological analyses, like those we have recently described (Karimi *et al.*, 2009; Kaskow *et al*., 2014).

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References

Abrahams BS, Geschwind DH. Advances in autism genetics: on the threshold of a new neurobiology. Nat Rev Genet. 2008; 9:341–355. [PubMed: 18414403]

Alarcón M, Abrahams BS, Stone JL, Duvall JA, Perederiy JV, Bomar JM, et al. Linkage, association, and gene-expression analyses identify CNTNAP2 as an autism-susceptibility gene. Am J Hum Genet. 2008; 82:150–159. [PubMed: 18179893]

Anney R, Klei L, Pinto D, Regan R, Conroy J, Magalhaes TR, et al. A genome-wide scan for common alleles affecting risk for autism. Hum Mol Genet. 2010; 19:4072–4082. [PubMed: 20663923]

Austin EJ. Personality correlates of the broader autism phenotype as assessed by the Autism Spectrum Quotient (AQ). Pers Individ Dif. 2005; 38:451–460.

Banerjee-Basu S, Packer A. SFARI Gene: an evolving database for the autism research community. Disease Models & Mechanisms. 2010; 3:133–135. [PubMed: 20212079]

Baranzini SE, Wang J, Gibson RA, Galwey N, Naegelin Y, Barkhof F, et al. Genome-wide association analysis of susceptibility and clinical phenotype in multiple sclerosis. Hum Mol Genet. 2009; 18:767–778. [PubMed: 19010793]

Baron-Cohen S, Wheelwright S, Skinner R, Martin J, Clubley E. The autism-spectrum quotient (AQ): evidence from Asperger syndrome/high-functioning autism, males and females, scientists and mathematicians. J Autism Dev Disord. 2001; 31:5–17. [PubMed: 11439754]

Basu SN, Kollu R, Banerjee-Basu S. AutDB: a gene reference resource for autism research. Nucleic Acids Res. 2009; 37:D832–D836. Database issue. Epub 2008 Nov 10. [PubMed: 19015121]

Campbell DB, Sutcliffe JS, Ebert PJ, Militerni R, Bravaccio C, Trillo S, et al. A genetic variant that disrupts MET transcription is associated with autism. Proc Natl Acad Sci USA. 2006; 103:16834–16839. [PubMed: 17053076]

Carroll LS, Owen MJ. Genetic overlap between autism, schizophrenia and bipolar disorder. Genome Med. 2009; 1:102. [PubMed: 19886976]

Chakrabarti B, Dudbridge F, Kent L, Wheelwright S, Hill-Cawthorne G, Allison C, et al. Genes related to sex steroids, neural growth, and social-emotional behavior are associated with autistic traits, empathy, and Asperger syndrome. Autism Res. 2009; 2:157–177. [PubMed: 19598235]

Chanock SJ, Manolio T, Boehnke M, Boerwinkle E, Hunter DJ, Thomas G, et al. NCI-NHGRI Working Group on Replication in Association Studies. Replicating genotype-phenotype associations. Nature. 2007; 447:655–660. [PubMed: 17554299]

Constantino JN, Todd RD. Autistic traits in the general population: a twin study. Arch Gen Psychiatry. 2003; 60:524–530. [PubMed: 12742874]

Curran S, Bolton P, Rozsnyai K, Chiocchetti A, Klauck SM, Duketis E, et al. ITAN. No association between a common single nucleotide polymorphism, rs4141463, in the MACROD2 gene and autism spectrum disorder. Am J Med Genet B Neuropsychiatr Genet. 2011; 156B:633–639. [PubMed: 21656903]

Debette S, Bis JC, Fornage M, Schmidt H, Ikram MA, Sigurdsson S, et al. Genome-wide association studies of MRI-defined brain infarcts: meta-analysis from the CHARGE Consortium. Stroke. 2010; 41:210–217. [PubMed: 20044523]

Dhami GK, Ferguson SS. Regulation of metabotropic glutamate receptor signaling, desensitization and endocytosis. Pharmacol Ther. 2006; 111:260–271. [PubMed: 16574233]

Elsabbagh M, Divan G, Koh YJ, Kim YS, Kauchali S, Marcín C, et al. Global prevalence of autism and other pervasive developmental disorders. Autism Res. 2012; 5:160–179. [PubMed: 22495912]

Gillberg CL. The Emanuel Miller Memorial Lecture 1991. Autism and autistic-like conditions: subclasses among disorders of empathy. J Child Psychol Psychiatry. 1992; 33:813–842. [PubMed: 1634591]

Greene CS, Penrod NM, Williams SM, Moore JH. Failure to replicate a genetic association may provide important clues about genetic architecture. PLoS One. 2009; 4:e5639. [PubMed: 19503614]

Guilmatre A, Dubourg C, Mosca AL, Legallic S, Goldenberg A, Drouin-Garraud V, et al. Recurrent rearrangements in synaptic and neurodevelopmental genes and shared biologic pathways in schizophrenia, autism, and mental retardation. Arch Gen Psychiatry. 2009; 66:947–956. [PubMed: 19736351]

Hollander E, King A, Delaney K, Smith CJ, Silverman JM. Obsessive-compulsive behaviors in parents of multiplex autism families. Psychiatry Res. 2003; 117:11–16. [PubMed: 12581816]

Holt R, Barnby G, Maestrini E, Bacchelli E, Brocklebank D, Sousa I, et al. EU Autism MOLGEN Consortium. Linkage and candidate gene studies of autism spectrum disorders in European populations. Eur J Hum Genet. 2010; 18:1013–1019. [PubMed: 20442744]

Jones RM, Cadby G, Melton PE, Abraham LJ, Whitehouse AJ, Moses EK. Genome-wide association study of autistic-like traits in a general population study of young adults. Front Hum Neurosci. 2013; 7:658. [PubMed: 24133439]

Kanaho Y, Funakoshi Y, Hasegawa H. Phospholipase D signalling and its involvement in neurite outgrowth. Biochim Biophys Acta. 2009; 1791:898–904. [PubMed: 19341813]

Karimi M, Goldie LC, Cruickshank MN, Moses EK, Abraham LJ. A critical assessment of the factors affecting reporter gene assays for promoter SNP function: a reassessment of -308 TNF polymorphism function using a novel integrated reporter system. Eur J Hum Genet. 2009; 17:1454–1462. [PubMed: 19471307]

Kaskow BJ, Diepeveen LA, Proffitt JM, Rea AJ, Ulgiati D, Blangero J, et al. Molecular prioritization strategies to identify functional genetic variants in the cardiovascular disease-associated expression QTL Vanin-1. Eur J Hum Genet. 2014; 22:688–695. [PubMed: 24045843]

Kendler KS. Advances in our understanding of genetic risk factors for autism spectrum disorders. Am J Psychiatry. 2010; 167:1291–1293. [PubMed: 21041249]

Lee DO, Ousley OY. Attention-deficit hyperactivity disorder symptoms in a clinic sample of children and adolescents with pervasive developmental disorders. J Child Adolesc Psychopharmacol. 2006; 16:737–746. [PubMed: 17201617]

Li X, Zou H, Brown WT. Genes associated with autism spectrum disorder. Brain Res Bull. 2012; 88:543–552. [PubMed: 22688012]

Lionel AC, Crosbie J, Barbosa N, Goodale T, Thiruvahindrapuram B, Rickaby J, et al. Rare copy number variation discovery and cross-disorder comparisons identify risk genes for ADHD. Sci Transl Med. 2011; 3:95ra75.

Lundström S, Chang Z, Råstam M, Gillberg C, Larsson H, Anckarsäter H, Lichtenstein P. Autism spectrum disorders and autistic like traits: similar etiology in the extreme end and the normal variation. Arch Gen Psychiatry. 2012; 69:46–52. [PubMed: 22213788]

Ma D, Salyakina D, Jaworski JM, Konidari I, Whitehead PL, Andersen AN, et al. A genome-wide association study of autism reveals a common novel risk locus at 5p14.1. Ann Hum Genet. 2009; 73:263–273. [PubMed: 19456320]

Mouren, MC.; Bourgeron, T.; Delorme, R. Common variants as risk factors for ASD. In: Garralda, ME.; Raynaud, J-P., editors. Brain, mind and developmental psychopathology in childhood. Jason Aronson; United Kingdom: 2012.

National Human Genome Research Institute. A catalog of genome-wide association studies [Online]. National Human Genome Research Institute; Bethesda, MD: 2012. 2012; Available at: http://www.genome.gov/26525384 [accessed April 2012]

Newnham JP, Evans SF, Michael CA, Stanley FJ, Landau LI. Effects of frequent ultrasound during pregnancy: a randomised controlled trial. Lancet. 1993; 342:887–891. [PubMed: 8105165]

Posserud MB, Lundervold AJ, Gillberg C. Autistic features in a total population of 7-9-year-old children assessed by the ASSQ (Autism Spectrum Screening Questionnaire). J Child Psychol Psychiatry. 2006; 47:167–175. [PubMed: 16423148]

Prandini P, Pasquali A, Malerba G, Marostica A, Zusi C, Xumerle L, et al. Italian Autism Network (ITAN). The association of rs4307059 and rs35678 markers with autism spectrum disorders is replicated in Italian families. Psychiatr Genet. 2012; 22:177–181. [PubMed: 22739633]

Ronald A, Hoekstra RA. Autism spectrum disorders and autistic traits: a decade of new twin studies. Am J Med Genet B Neuropsychiatr Genet. 2011; 156B:255–274. [PubMed: 21438136]

Ronald A, Happé F, Plomin R. The genetic relationship between individual differences in social and nonsocial behaviours characteristic of autism. Dev Sci. 2005; 8:444–458. [PubMed: 16048517]

Ronald A, Butcher LM, Docherty S, Davis OS, Schalkwyk LC, Craig IW, Plomin R. A genome-wide association study of social and non-social autistic-like traits in the general population using pooled DNA, 500 K SNP microarrays and both community and diagnosed autism replication samples. Behav Genet. 2010; 40:31–45. [PubMed: 20012890]

Russell-Smith SN, Maybery MT, Bayliss DM. Relationships between autistic-like and schizotypy traits: an analysis using the Autism Spectrum Quotient and Oxford-Liverpool Inventory of Feelings and Experiences. Pers Individ Dif. 2011; 51:128–132.

Saus E, Brunet A, Armengol L, Alonso P, Crespo JM, Fernández-Aranda F, et al. Comprehensive copy number variant (CNV) analysis of neuronal pathways genes in psychiatric disorders identifies rare variants within patients. J Psychiatr Res. 2010; 44:971–978. [PubMed: 20398908]

Shriner D, Vaughan LK, Padilla MA, Tiwari HK. Problems with genome-wide association studies. Science. 2007; 316:1840–1842. [PubMed: 17600199]

Skuse DH, Mandy WP, Scourfield J. Measuring autistic traits: heritability, reliability and validity of the Social and Communication Disorders Checklist. Br J Psychiatry. 2005; 187:568–572. [PubMed: 16319410]

St Pourcain B, Wang K, Glessner JT, Golding J, Steer C, Ring SM, et al. Association between a high-risk autism locus on 5p14 and social communication spectrum phenotypes in the general population. Am J Psychiatry. 2010; 167:1364–1372. [PubMed: 20634369]

Stewart ME, Austin EJ. The structure of the Autism-Spectrum Quotient (AQ): evidence from a student sample in Scotland. Pers Individ Dif. 2009; 47:224–228.

Veenstra-Vanderweele J, Christian SL, Cook EH Jr. Autism as a paradigmatic complex genetic disorder. Annu Rev Genomics Hum Genet. 2004; 5:379–405. [PubMed: 15485354]

Wang K, Zhang H, Ma D, Bucan M, Glessner JT, Abrahams BS, et al. Common genetic variants on 5p14.1 associate with autism spectrum disorders. Nature. 2009; 459:528–533. [PubMed: 19404256]

Weiss LA, Arking DE, Daly MJ, Chakravarti A, Gene Discovery Project of Johns Hopkins the Autism Consortium. A genome-wide linkage and association scan reveals novel loci for autism. Nature. 2009; 461:802–808. [PubMed: 19812673]

Whitehouse AJ, Hickey M, Ronald A. Are autistic traits in the general population stable across development? PLoS One. 2011; 6:e23029. [PubMed: 21829684]

Xu B, Woodroffe A, Rodriguez-Murillo L, Roos JL, van Rensburg EJ, Abecasis GR, et al. Elucidating the genetic architecture of familial schizophrenia using rare copy number variant and linkage scans. Proc Natl Acad Sci USA. 2009; 106:16746–16751. [PubMed: 19805367]

Yang MS, Gill M. A review of gene linkage, association and expression studies in autism and an assessment of convergent evidence. Int J Dev Neurosci. 2007; 25:69–85. [PubMed: 17236739]

Table 1

Summary table of candidate SNPs (genomewide association studies; search conducted April 2012)

References	Initial sample size (source)	Replication sample size (source)	SNP (gene)[a]	Effect allele	*P*-value	Odds ratio (95% CI; where included)	Platform (No. of SNPs)
Wang *et al.* (2009)	3101 family members, 1204 cases, 6491 controls (AGRE, ACC)	1390 family members, 108 cases, 540 controls (CAP, CART)	rs4307059 (*CDH10/CDH9*)	C	2×10^{-10}	0.84	Illumina HumanHap550 (474 019)
Ma *et al*. (2009)	1390 family members (CAP)	2390 family members (AGRE)	rs100038113 (*CHD10/CHD9*)	C	3×10^{-6}	1.33 (1.11-1.43)	Illumina 1M Infinium (775 311)
Weiss and Arking (2009)	1031 families with 1553 affected offspring (AGRE, NIMH)	2073 trios (NIMH, Montreal, EDSP, MGH-Finnish, CHB, HMCA, AGP, FAFS, ITS)	rs10513025 (*SEMA5A/TAS2R1*)	C	1.7×10^{-6}	0.55	Affymetrix 5.0 (365 000)
Anney *et al*. (2010)	1385 affected children from 1369 families (AGRE, SAGE control cohort)	1086 affected children from 595 families. 1965 European, African American and other controls	rs4141463 (*MACROD2*)	T	3.7×10^{-8}	0.73 (0.66-0.82)	Illumina 1M Infinium (842 348)
			rs7142002 (*PPP2R5C*)	C	3×10^{-6}	0.64 (0.53-0.78)	

ACC, Autism Case–Control Cohort; AGP, Autism Genome Project; AGRE, Autism Genetic Resource Exchange; CAP, Collaborative Autism Project; CART, Center for Autism Research and Treatment; CHB, Children's Hospital Boston; EDSP, Santangelo Early Development Stages of Psychopathology Study; FAFS, Finnish Autism Family Samples; HMCA, Homozygosity Mapping Collaborative for Autism; ITS, Iranian Trio Samples; MGH-Finnish, Massachusetts General Hospital and Finnish Planmed Oy collaboration; NIMH, National Institute of Mental Health, Autism Genetics Initiative; SAGE, Study on Addiction: Genetics and Environment; SNP, single nucleotide polymorphism; TEDS, Twins Early Development Study.

[a]For intergenic SNPs, 5′/3′ flanking genes listed.

Table 2

Characteristics of Raine study participants with AQ and GWA data available (*n* = 965)

Continuous	***n***	**Mean (SD)**
Maternal age at conception (years)	965	28.66 (5.65)
Paternal age at conception (years)	646	31.68 (6.18)
Maternal BMI at conception	956	22.25 (4.00)
Paternal BMI at conception	827	24.44 (3.28)
Age at AQ completion (years)	965	19.68 (0.70)
Total AQ score	965	103.2 (12.60)

Categorical	***n***	***n*(%)**
Season of birth	965	
Summer		301 (31.2)
Autumn		222 (23.0)
Winter		196 (20.3)
Spring		246 (25.5)
Sex	965	
Male		470 (48.7)
Female		495 (51.3)
Gestational age	955	
< 32 weeks		13 (1.4)
32-37 weeks		157 (16.4)
38-40 weeks		624 (65.3)
> 40 weeks		161 (16.9)
Family income	937	
Income < $24 000		293 (31.3)
Income > $24 000		644 (68.7)
Maternal education	956	
Secondary school not completed		501 (52.4)
Secondary school completed		455 (47.6)

AQ, Autism Spectrum Quotient; GWA, genomewide association.

Table 3

Genotype and minor allele frequencies

		Major homozygote		Heterozygote		Minor homozygote			
SNP	**Gene**[a]	**Gen**	***n* (%)**	**Gen**	***n* (%)**	**Gen**	***n* (%)**	**HWE *P*-value**	**MAF**
rs10513025	*SEMA5A/TASR1*	TT	872 (94)	CT	55 (6)	CC	0 (0)	1.00	2.97
rs10038113	*CDH10/CDH9*	TT	344 (36)	CT	455 (48)	CC	154 (16)	0.89	40.03
rs4307059	*CDH10/CDH9*	TT	384 (40)	CT	427 (44)	CC	154 (16)	0.06	38.62
rs7142002	*PPP2R5C*	TT	841 (88)	CT	115 (12)	CC	3 (< 1)	1.00	6.31
rs4141463	*MACROD2*	CC	325 (34)	TC	459 (49)	TT	160 (17)	0.95	41.26

Gen, genotype; HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency; SNP, single nucleotide polymorphism.

[a]For intergenic SNPs, 5′/3′ flanking genes listed.

Table 4

Models for potential covariates

	Total AQ			Social skills			Details/Patterns			Communication/Mindreading		
Variable	**Coefficient**	**SE**	***P*-value**	**Coefficient**	**SE**	***P*-value**	**Coefficient**	**SE**	***P*-value**	**Coefficient**	**SE**	***P*-value**
Sex[a]	51.539	22.961	0.025	0.997	0.395	0.012	1.503	0.366	< 0.001			
Age at AQ completion	2.706	0.803	< 0.001							0.430	0.190	0.024
Season of birth[b]												
Summer										0.528	0.365	0.149
Autumn										0.831	0.387	0.032
Spring										1.008	0.374	0.007
Mother's BMI	0.263	0.100	0.009	0.106	0.049	0.031	0.108	0.047	0.024	0.096	0.034	0.004
Father's age at conception							−0.066	0.030	0.027	−0.052	0.021	0.014
Mother's age at conception				0.083	0.037	0.026						
Family income[c]	2.541	0.889	0.004	1.129	0.453	0.007						
Maternal education[d]	−2.685	0.826	0.001				−0.794	0.367	0.031			
Age × Sex	−2.446	1.166	0.036									

AQ, Autism Spectrum Quotient.

[a]Reference level set to female.

[b]Reference season set to winter.

[c]Reference level set to income greater than $24 000.

[d]Reference level set to mother did not complete secondary school.

Table 5

Multiple linear regression analysis for total AQ*

SNP	Gene	Effect allele	Coefficient	SE	*P*-value
rs10513025	*SEMA5A, TAS2R1*	C	1.931	1.710	0.87
rs10038113	Intergenic	C	−0.070	0.580	0.548
rs4307059	*CDH10, CDH9*	C	1.085	0.565	0.9725
rs7142002	*PPP2R5C*	C	0.354	1.164	0.6195
rs4141463	*MACROD2*	T	−0.948	0.583	0.052

AQ, Autism Spectrum Quotient.

*Adjusted for sex, age at AQ completion, mother's BMI, family income, and maternal education.

Table 6

Multiple linear regression analysis for social skills*

SNP	Gene	Effect allele	Coefficient	SE	*P*-value
rs10513025	*SEMA5A, TAS2R1*	C	0.930	0.838	0.866
rs10038113	Intergenic	C	0.011	0.285	0.485
rs4307059	*CDH10, CDH9*	C	0.436	0.277	0.942
rs7142002	*PPP2R5C*	C	0.348	0.572	0.728
rs4141463	*MACROD2*	T	−0.179	0.286	0.267

*Adjusted for sex, mother's BMI, mother's age at conception and family income.

Table 7

Multiple linear regression analysis for details/patterns*

SNP	Gene	Effect allele	Coefficient	SE	*P*-value
rs10513025	*SEMA5A, TAS2R1*	C	0.314	0.787	0.655
rs10038113	Intergenic	C	−0.044	0.267	0.665
rs4307059	*CDH10, CDH9*	C	0.243	0.258	0.826
rs7142002	*PPP2R5C*	C	−0.332	0.536	0.268
rs4141463	*MACROD2*	T	−0.256	0.264	0.166

*Adjusted for sex, mother's BMI, father's age at conception and maternal education.

Table 8

Multiple linear regression analysis for communication/mindreading*

SNP	Gene	Effect allele	Coefficient	SE	*P*-value
rs10513025	*SEMA5A, TAS2R1*	C	0.571	0.558	0.846
rs10038113	Intergenic	C	0.065	0.189	0.365
rs4307059	*CDH10, CDH9*	C	−0.224	0.183	0.111
rs7142002	*PPP2R5C*	C	−0.337	0.380	0.188
rs4141463	*MACROD2*	T	−0.475	0.186	0.006

AQ, Autism Spectrum Quotient.

*Adjusted for age at AQ completion, season of birth, mother's BMI, and father's age at conception.