Genes Related to Sex Steroids, Neural Growth, and Social–Emotional Behavior are Associated with Autistic Traits, Empathy, and Asperger Syndrome

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Genetic studies of autism spectrum conditions (ASC) have mostly focused on the ''low functioning'' severe clinical subgroup, treating it as a rare disorder. However, ASC is now thought to be relatively common (~1%), and representing one end of a quasi-normal distribution of autistic traits in the general population. Here we report a study of common genetic variation in candidate genes associated with autistic traits and Asperger syndrome (AS). We tested single nucleotide polymorphisms in 68 candidate genes in three functional groups (sex steroid synthesis/transport, neural connectivity, and social–emotional responsivity) in two experiments. These were (a) an association study of relevant behavioral traits (the Empathy Quotient (EQ), the Autism Spectrum Quotient (AQ)) in a population sample ($n = 349$); and (b) a case–control association study on a sample of people with AS, a ''high-functioning'' subgroup of ASC ($n = 174$). 27 genes showed a nominally significant association with autistic traits and/or ASC diagnosis. Of these, 19 genes showed nominally significant association with AQ/EQ. In the sex steroid group, this included *ESR2* and *CYP11B1*. In the neural connectivity group, this included *HOXA1*, *NTRK1*, and *NLGN4X*. In the socio-responsivity behavior group, this included *MAOB*, *AVPR1B*, and *WFS1*. Fourteen genes showed nominally significant association with AS. In the sex steroid group, this included *CYP17A1* and *CYP19A1*. In the socio-emotional behavior group, this included *OXT*. Six genes were nominally associated in both experiments, providing a partial replication. Eleven genes survived family wise error rate (FWER) correction using permutations across both experiments, which is greater than would be expected by chance. *CYP11B1* and *NTRK1* emerged as significantly associated genes in both experiments, after FWER correction ($P < 0.05$). This is the first candidate-gene association study of AS and of autistic traits. The most promising candidate genes require independent replication and fine mapping.

Keywords: genetics; Asperger syndrome; autism; empathy; autistic traits; visual search; emotion recognition; SNP; broader autism phenotype

Introduction

Autism spectrum conditions (ASC) entail a disability in social and communication development, alongside unusually narrow interests (''obsessions'') and repetitive behavior [APA, 1994; ICD-10, 1994]. ASC have a genetic basis, indicated by concordance rates from MZ and DZ twins [Bailey et al., 1995], and with heritability estimates of over 90% [Bailey et al., 1995; LaBuda, Gottesman, & Pauls, 1993]. Multiple common susceptibility alleles are implicated, along with environmental and epigenetic factors. Mixed evidence from genome-wide linkage studies of samples that do not differentiate between classic (low-functioning) autism and autism spectrum disorder have implicated nearly all chromosomes [Abrahams & Geschwind, 2008]. This could be due to genetic heterogeneity, or potential confounds from comorbid conditions (e.g. epilepsy, language delay, below average IQ, or hyperactivity).

While most neuroimaging and behavioral studies of ASC focus on the higher-functioning end of the autism spectrum (high-functioning autism and/or Asperger syndrome (AS)), the large-scale genetic studies have primarily investigated the lower-functioning end, focusing on classic autism. In this study, we address this important gap in literature, by reporting two genetic association studies. The first is of AS, and the second is of autistic traits in the general population. AS is marked by social and behavioral impairments, but is not associated with language delay during development. We chose 68 candidate genes for these two experiments, derived from

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three functional categories: (A) sex hormone-related genes; (B) genes involved in neural development and connectivity; and (C) genes involved in social and emotional responsivity (Table I). We searched for common genetic variants (single nucleotide polymorphisms (SNPs)) on the assumption that autistic traits are continuously distributed in the general population [Constantino & Todd, 2005; Sung et al., 2005]. Each of the three functional categories derives from a clear neurocognitive theory of ASC. The fetal androgen theory [Baron-Cohen, Lutchmaya, & Knickmeyer, 2004] suggests that genes involved in sex steroid synthesis and transport might be related to ASC. The neural connectivity theory [Belmonte et al., 2004], based on evidence from rat and human brains suggests that the key abnormality in autism might be related to neural growth and connectivity. Therefore, genes involved in neural growth, synaptogenesis, and synapse stabilization were included in our set of candidates. Finally, the social–emotional responsivity theory [Chakrabarti, Kent, Suckling, Bullmore, & Baron-Cohen, 2006; Dawson et al., 2002] suggests that the aberrant social behavior patterns noticed in ASC might be related, in part, to genes that are known to modulate social behavior in animals. The rationale of choice for all genes, together with relevant gene function, is described in detail in the online Supplementary material (S1). Some of these genes have been associated with autism in previous genetic studies, and these are indicated in bold in Table I. These 68 candidate genes were tested in two experiments.

In Experiment 1, we measured autistic traits in a population-based sample of volunteers without any psychiatric diagnoses, to test whether any of these genes

Table I. List of All Genes Included in the Association Study, Along with Brief Functional Roles Where Known

Neural development and connectivity	
NGF, BDNF, NTF3, NTF5, NGFR, NTRK1, NTRK2, NTRK3, TAC1, IGF1, IGF2	Neuronal survival, differentiation and growth.
RAPGEF4	Growth and differentiation of neurons. Mutations associated with classic autism.
VGF	Upregulated directly by NGF and expressed in neuroendocrine cells.
VEGF	Promotes cell growth and migration, especially during angiogenesis and vasculogenesis, often observed during hypoxia. Modulated directly by PTEN.
ARNT2	Neural response to hypoxia.
NLGN1, ***NLGN4X***, *AGRIN*	Synapse formation and maintenance in CNS neurons. *NLGN4X* mutaions have been linked to autism.
NRCAM	Neuronal adhesion and directional signalling during axonal cone growth.
EN-2(AUTS1)	Neuronal migration and cerebellar development. *EN-2* has been previously linked to ASCs in several studies.
HOXA1	Hindbrain patterning. Mixed evidence suggests a link with ASCs.
Social and emotional responsivity	
OXT, ***OXTR, AVPR1A,*** *AVPR1B*	Linked to social attachment behavior in humans and other mammals. *AVPR1A* and *OXTR* have previously been associated with ASCs.
CNR1, OPRM1, TRPV1	Mediate endogenous reward circuits, in tandem with dopaminergic pathways. Implicated in underlying rewarding features of social interactions.
MAOB	Synaptic breakdown of dopamine and serotonin. Suggested links with social cognition.
WFS1	Mutations linked to affective disorders. Overexpressed in amygdala during fear response, though exact functional role is not known.
GABRB3, GABRG3, *GABRA6*, ***ABAT***	Mediate inhibitory (GABA-ergic) neurotransmission as well as play a role in early cortical development. *GABRA6* is expressed strongly in the cerebellum; *GABRB3*, *GABRG3*, *ABAT* have all been associated with ASCs.
VIPR1	Suggested involvement in neural pathway underlying pheromone processing. Mutations associated with social behavioral abnormalities in mice. Its endogenous ligand (VIP) shows an overexpression in neonatal children with autism.
Sex hormone biosynthesis, metabolism and transport	
DHCR7	Metabolism of cholesterol: precursor for sex hormones (mutations associated with near-universal presence of ASC).
CYP1A1, CYP1B1, CYP3A, CYP7A1, CYP11A, CYP11B1, CYP17A1, CYP19A1, CYP21A2, POR	Synthesis of sex hormones such as progesterone, estrogen, cortisol, aldosterone and testosterone. *CYP21A2* and *POR* mutations associated with CAH.
HSD11B1, HSD17B2, HSD17B3, HSD17B4	Local regulation of sex steroids.
STS, SULT2A1, SRD5A1, SRD5A2	Steroid hormone metabolism.
SHBG, SCP2, TSPO, SLC25A12, SLC25A13	Intracellular transport of sex steroids as well as their important precursors and/or metabolites. Mixed evidence suggests an association of *SLC25A12* with classic autism.
AR	Intracellular receptor for testosterone.
ESR1, ESR2	Receptors for estrogen.
CGA, CGRPR, LHB, LHRHR, LHCGR, FSHB	Regulation of reproductive functions.

Genes marked in bold indicate those previously linked to ASC through genetic linkage/association studies. For list of SNPs chosen from each gene, see Table II. Yellow [dark grey] = Neural growth genes; Light grey = Social responsivity genes; Pink [medium grey] = Sex steroid genes. [Color table can be viewed online at www.interscience.wiley.com]

were associated with autistic traits. Our primary measure of autistic traits is the Autism Spectrum Quotient (AQ) [Baron-Cohen, Wheelwright, Skinner, Martin, & Clubley, 2001b], a 50-point self-report scale with a quasi-normal distribution in the general population. The AQ has good reliability and validity, with 80% of people with AS scoring above 32/50 compared to 1.5% of controls, and males scoring higher than females [Baron-Cohen et al., 2001b]. AQ results have been replicated cross-culturally, and it is independent of IQ, age, education, major personality traits [Wakabayashi, Baron-Cohen, & Wheelwright, 2006], and scores above 32 is an excellent predictor of AS diagnosis [Woodbury-Smith, Robinson, Wheelwright, & Baron-Cohen, 2005]. AQ shows heritability of ~57% in twins [Hoekstra, Bartels, Verweij, & Boomsma, 2007], and in parents of children with ASC [Bishop et al., 2004].

Our second measure of autistic traits focused on individual differences in empathy. Empathy is a core deficit in ASC [Baron-Cohen, 1995]. The Empathy Quotient (EQ) [Baron-Cohen & Wheelwright, 2004] is a valid and reliable measure of empathy [Lawrence, Shaw, Baker, Baron-Cohen, & David, 2004], females scoring higher than males, and 81% of people with AS scoring less than 30/80 compared to 12% of controls [Baron-Cohen & Wheelwright, 2004]. Twin studies have established a genetic basis for empathy in humans [Zahn-Waxler, Robinson, & Emde, 1992]. Specific genes have also been implicated in empathy-related behavior in humans and other animals [Champagne et al., 2006]. The AQ and EQ allow a wider net to be cast in capturing genes underlying autistic traits.

In addition to the questionnaire measures, we used two performance measures related to autistic traits: the ''Reading the Mind in the Eyes'' (Eyes) Test of empathy [Baron-Cohen, Joliffe, Mortimore, & Robertson, 1997; Baron-Cohen, Wheelwright, Hill, Raste, & Plumb, 2001a] on which people with ASC score below average; and the Embedded Figures Test (EFT) of attention to detail [Jolliffe & Baron-Cohen, 1997] on which people with ASC score above average. Both are normally distributed in the population and are candidate endophenotypes, since parents and siblings of children with ASC show mild deficits on the Eyes test and above average performance on the EFT [Baron-Cohen & Hammer, 1997; Dorris, Espie, Knott, & Salt, 2004; Losh & Piven, 2007]. The EFT and Eyes tests tap highly specific components within autistic traits (emotion–recognition, and attention to detail), strengthening measures of these within the AQ and EQ.

In Experiment 2, we tested the same 68 genes for association in a sample of people with clinically diagnosed AS, the ''high-functioning'' subgroup on the autistic spectrum, in a case–control design. Almost all previous genetic studies have been conducted on samples that included both people with classic (Kanner's) autism as well as those with a diagnosis of Autism Spectrum Disorder, an approach that dates back to when ASC were regarded as rare. Today ASC is thought of as relatively common (~1%) [Baird et al., 2006; Baron-Cohen et al., 2009]. If autism is the extreme of normally distributed autistic traits, then AS is the more logical subgroup to investigate, since restricting the case sample to AS removes a suite of comorbid features. This increases the power to detect genes underlying autistic traits, independent of genes underlying learning difficulties or language delay. Family pedigrees of AS suggest heritability [Gillberg, 1991] and one full genome scan of AS has been conducted [Ylisaukko-oja et al., 2004], revealing strong linkage peaks at 1q21–22, 3p14–24, and 13q31–33.

We predicted that genes from each of the three functional categories would show significant association with caseness (certain alleles being more common in the AS group relative to population controls) and/or with individual differences on the phenotypic measures (AQ, EQ, the Eyes, and the EFT), in the population sample.

Materials and Methods

Samples

Individuals ($n = 349$; 143 males and 206 females, mean age = 22.5 years, SD = 2.6 years) free of any neurological/psychiatric diagnoses were recruited by advertisement from a student population. A student population inevitably means IQ distribution is not representative, but this is unlikely to introduce confounds since scores on the AQ [Billington, Baron-Cohen, & Wheelwright, 2007], EQ [Lawrence et al., 2004], Eyes Test [Baron-Cohen et al., 2001a] and EFT (unpublished data) are all independent of IQ. Participants were included only if they reported Caucasian ancestry for three generations. They filled in the AQ and EQ online, and the results were comparable to those reported previously by our and other groups. The mean EQ score was 44.1 (range: 9–75, mean score for males: 36.1, mean score for females: 49.6), and the mean AQ score was 16.43 (range: 3–36, mean score for males: 18.01, mean score for females: 15.33). AQ and EQ scores showed a modest negative correlation (Spearman's rho = −0.55, $P \leq 0.01$). A subset of this sample ($n = 96$) completed an online version of the Eyes Task and the EFT.

In addition, $n = 174$ cases (140 males and 34 females, mean age = 23.2 years, SD = 14.6 years) with a formal diagnosis of AS (based on DSM-IV or ICD-10) from independent clinicians were recruited through our online database. Cases were excluded if they had comorbid major psychiatric conditions (psychosis, schizophrenia, or bipolar disorder), or if they reported incompatible diagnostic features (such as a history of language delay or learning difficulties), or if they were self-diagnosed, or if

the clinician making the diagnosis was not affiliated to a recognized specialist psychiatric clinic. DSM-IV and ICD-10 criteria were used rather than ADI-R/ADOS, as the latter were designed to diagnose classic autism and their accuracy in diagnosing AS has not been confirmed. To our knowledge, this is the largest reported sample for a genetic association study of AS. As a check on diagnosis, approximately half of the cases of AS (91 of 174) also filled in the AQ. One would expect 80% of cases with AS to score equal to or more than 32 [Baron-Cohen et al., 2001b]. Out of the 91 cases, 73 (80.2%) scored at this level, confirming this sample was comparable to other published samples. As a check on IQ of the AS sample, approximately 10% of the AS group ($n = 19$) were randomly selected and administered the WASI full scale IQ test. This revealed a mean full-scale IQ score of 119.5 (SD = 21.1), which is comparable to that of a larger pool of typical student participants that these volunteers were drawn from. Ethnicity information was available for 106/174 (60.9%) of the cases, all of who were Caucasian for at least three generations. To control for possible confounds from missing ethnicity data among cases, χ^2 analyses were performed for each SNP from Table II, comparing cases with and without ethnicity information. This revealed no differences in allele frequencies between these two groups for all but two SNPs, consistent with genetic homogeneity of the cases.

SNP Selection

SNPs (216) with a minor allele frequency (MAF) ≥ 0.2 in the Caucasian population were chosen, to ensure adequate power given our sample size, which was fixed by external constraints prior to the study. This approach of selecting multiple common SNPs per gene has the advantage of checking for informative associations both directly and indirectly [Collins, Guyer, & Chakravarti, 1997]. SNPs (from dbSNP build 123) were chosen randomly from across the whole gene, including UTRs and introns. The number of SNPs per gene varied with the gene size and number of commercially available ABI assays, and is detailed in Table II. The median SNP density across all genes was one SNP per 14.1 kb; 125 of these SNPs have been genotyped in one or more populations in the HapMap database (Release 23a). We used TAGGER to estimate the coverage, which revealed that 40 SNPs were in strong LD with SNPs genotyped in the HapMap database. These SNPs covered 7.3% of HapMap variation at $r^2 > 0.8$ and 13.26% at $r^2 > 0.5$, for SNPs with MAF > 0.001. The remaining 176 of our SNPs did not exhibit a strong LD with the genotyped SNPs in the HapMap database, and hence the true coverage is considerably higher than our estimate. It should be noted that in absence of complete polymorphism data on the same sample, it is not possible to estimate the actual gene coverage.

All volunteers contributed mouth swabs for DNA extraction. These were anonymized and DNA was genotyped for the SNPs (see Table II) using standard PCR-based assays (TaqMan® SNP genotyping assays, Applied Biosystems Inc., CA). The genotyping call rate was 93.35% across all samples. Concordance for duplicate samples was 99.8%. No SNP showed a significant deviation from Hardy–Weinberg Equilibrium at $P < 0.001$. The following experiments were performed:

1. *An association study for AQ and EQ* was conducted on the population sample ($n = 349$) using nonparametric (Kruskal–Wallis) analysis of variance for each SNP, since neither the AQ nor the EQ were normally distributed in our sample (Anderson–Darling statistic = 3.26). χ^2 statistics and asymptotic *P*-values (two-tailed) were generated from this test. A sex-specific analysis was conducted for all X-linked genes. A similar analysis for the EFT and Eyes tasks was undertaken in a subset of this sample ($n = 96$), using univariate ANOVA for each SNP, since neither EFT and Eyes task scores deviated significantly from normality.
2. *A case–control association study* was conducted on all cases of AS ($n = 174$) and a subset of the population sample ($n = 155$). The controls were selected to be sex-matched with the cases, while having an AQ score < 25. An AQ < 25 cut-off was employed to exclude a small number of individuals who scored high on AQ even though they did not have a formal diagnosis. For each SNP, a Cochrane–Armitage χ^2 statistic (1 d.f.) was calculated to test the null hypothesis that the different alleles have the same distribution in cases and controls. Asymptotic P-values (two-tailed) were calculated. In addition, a Pearson's χ^2 (2 d.f., ''codominant'' test) was calculated for each SNP (see Supplementary Table S2).

To control for multiple testing of SNPs within genes as well as for multiple phenotypes, permutation testing was conducted using UNPHASED [Dudbridge, 2008] for Experiment 1, and using PLINK [Purcell et al., 2007] for Experiment 2. Since each candidate gene was individually selected on the basis of *a priori* hypothesis, independent of other genes, permutation tests were performed separately for each gene. In each permutation, the phenotypes were randomly reassigned among participants, keeping the genotypes fixed to preserve

Table II. The List of All SNPs Genotyped, Grouped by Gene

	Experiment 1						Experiment 2		
Gene variations	EQ (χ^2 statistic)	EQ (*P*-value)	AQ (χ^2 statistic)	AQ (*P*-value)	Additional data	Permutation-corrected *P*-value	C-A trend test χ^2 statistic	C-A trend test *P*-value	Permutation-corrected *P*-value
AGRN									
rs2275813	2.168	0.338	4.431	0.109	Eyes		0.071	0.790	
rs8014	0.289	0.865	2.194	0.334			1.77	0.180	
NRCAM									
rs445372	2.299	0.317	2.024	0.363			0.31	0.580	
rs1269621	0.721	0.697	0.28	0.869			1.45	0.230	
rs1269655	0.638	0.727	0.792	0.673			1.096	0.290	
NTRK1						*0.034*			*0.018*
rs6334	0.007	0.935	1.591	0.207			2.67	0.100	
rs6337	1.367	0.505	2.237	0.327			7.54*	*0.010*	
rs1007211	0.085	0.958	1.988	0.370			1.01	0.320	
rs6339	9.184*	*0.010*	3.684	0.158			0.2	0.650	
NTRK2									
rs7027979	0.901	0.637	1.924	0.382			0.38	0.540	
rs11140776	0.277	0.871	0.354	0.838			0.47	0.490	
rs10780691	2.568	0.277	2.073	0.355			0.002	0.960	
rs1490404	1.93	0.381	0.652	0.722			0.24	0.620	
rs1778934	0.63	0.730	3.201	0.202			0.65	0.420	
rs2489162	0.135	0.935	2.853	0.240			0.03	0.860	
rs1619120	1.809	0.405	2.019	0.364	Eyes		0.06	0.810	
rs993315	0.528	0.768	0.881	0.644			0.77	0.370	
rs1624327	1.532	0.465	1.787	0.409			0.004	0.950	
rs1443444	0.568	0.753	1.602	0.449			0.02	0.890	
NTRK3						0.132			0.249
rs1948066	3.74	0.154	2.471	0.291			0.44	0.510	
rs7170215	3.976	0.137	1.901	0.387	Eyes		0.41	0.840	
rs920069	7.024*	*0.030*	11.984*	*0.002*			2.03	0.150	
rs1824554	0.239	0.625	5.246*	0.022			0.15	0.690	
rs7170976	0.115	0.944	0.053	0.974			0.09	0.750	
rs922231	2.424	0.298	1.032	0.597			3.12	0.080	
rs8030107	0.727	0.695	0.378	0.828			2.1	0.150	
rs2279409	1.256	0.534	6.324*	*0.042*			0.004	0.940	
rs11073762	0.594	0.743	5.747	0.057			0.011	0.910	
rs3784410	1.964	0.375	3.064	0.216			0.59	0.440	
rs7176429	6.474*	0.039	5.468	0.065			0.44	0.510	
rs1369430	3.144	0.208	3.633	0.163			4.66*	*0.030*	
rs3784441	3.189	0.203	2.637	0.268			1.36	0.240	
rs1369423	3.054	0.217	2.526	0.283			1.9	0.170	
NLGN1									
rs993298	0.482	0.786	0.056	0.972			0.6	0.440	
NLGN4X						0.167/*0.024*			
rs5916338	1.396/1.267	0.237/0.531	0.324/0.468	0.569/0.791			0.73/0.80	0.39/0.37	

Table II. Continued

	Experiment 1						Experiment 2		
Gene variations	EQ (χ^2 statistic)	EQ (*P*-value)	AQ (χ^2 statistic)	AQ (*P*-value)	Additional data	Permutation-corrected *P*-value	C-A trend test χ^2 statistic	C-A trend test *P*-value	Permutation-corrected *P*-value
rs12836764	4.919*/5.070	*0.027*/0.079	1.835/8.555*	0.176/*0.014*			0.00006/0.48	0.99/0.49	
NGFB									
rs6330	0.11	0.947	0.753	0.686			0.01	0.920	
rs910330	1.04	0.595	5.441	0.066			0.53	0.470	
NGFR									
rs575791	0.537	0.765	0.844	0.656			3.62	0.057	
EN2						0.134			
rs2361689	0.514	0.773	1.78	0.411	Eyes		0.07	0.790	
rs1861972	8.478*	*0.014*	2.964	0.227	Eyes, EFT		0.033	0.850	
rs3735653	1.107	0.575	3.121	0.210			0.12	0.730	
HOXA1						*0.029*			
rs10951154	5.71	0.058	7.563*	*0.023*			0.25	0.620	
NTF3									0.053
rs6332	2.618	0.270	2.054	0.358			0.13	0.720	
rs7958038	0.17	0.918	1.986	0.370			1.69	0.190	
rs7132127	0.139	0.933	1.939	0.379			6.5*	*0.010*	
rs4930767	4.968	0.083	0.22	0.896			1.35	0.250	
NTF5									
rs1611775	0.19	0.909	2.119	0.347			0.29	0.590	
VGF									
rs2074686	0.847	0.655	0.235	0.889			1.34	0.250	
rs10953325	1.055	0.590	1.313	0.519			0.05	0.820	
rs1859528	0.122	0.941	1.154	0.562			0.85	0.350	
VEGF									
rs833068	4.155	0.130	4.082	0.212			2.41	0.120	
rs3025020	3.815	0.157	2.774	0.099	Eyes		0.25	0.620	
RAPGEF4 (cAMP-GEFII)									
rs6754857	0.194	0.908	0.653	0.721			0.49	0.485	
rs17746510	0.116	0.944	0.562	0.755			0.21	0.650	
rs2676501	0.429	0.807	0.05	0.975			1.38	0.240	
TAC1									
rs1229434	2.269	0.322	0.153	0.926			3.8	0.052	
rs2072100	1.279	0.527	0.006	0.997			1.39	0.237	
IGF1									0.115
rs11111272	1.466	0.480	2.309	0.315			0.049	0.820	
rs972936	0.902	0.342	3.33	0.068			1.51	0.227	
rs2946834	1.072	0.585	0.664	0.718			4.31*	*0.037*	
rs10735380	1.749	0.417	2.605	0.272			0.228	0.630	
IGF2									
rs2239681	0.158	0.924	1.789	0.409			0.029	0.864	
rs11042751	0.683	0.711	1.952	0.377			1.054	0.305	
rs734351	0.617	0.735	3.019	0.221			0.081	0.776	

BDNF								
rs6265	1.117	0.572	0.837	0.658		0.989	0.320	
ARNT2					0.184			*0.018*
rs4778599	3.038	0.219	11.127*	*0.004*		2.369	0.120	
rs4778795	2.485	0.289	2.458	0.293		3.076	0.079	
rs11856273	0.297	0.862	1.855	0.395		0.003	0.953	
rs3901896	4.749	0.093	3.246	0.197		8.45*	*0.003*	
rs7403073	2.441	0.295	0.305	0.858		0.53	0.460	
OXT								*0.016*
rs2740204	1.206	0.547	1.843	0.398		1.93	0.160	
rs2770378	1.924	0.382	2.749	0.253		6.76*	*0.009*	
OXTR					0.471			
rs237880	1.822	0.402	7.092*	*0.029*		1.81	0.180	
rs237885	0.516	0.773	2.539	0.281		1.069	0.301	
rs237898	5.074	0.079	1.134	0.567		0.023	0.870	
rs2228485	0.076	0.963	3.642	0.162		0.37	0.540	
rs237902	3.063	0.216	4.218	0.121		0.69	0.400	
AVPR 1A								
rs1042615	0.116	0.944	3.704	0.157		2.177	0.140	
AVPR1B					0.058			
rs28405931	6.082*	*0.048*	4.251	0.119		1.237	0.260	
OPRM1								
rs648893	0.258	0.879	1.491	0.475		1.315	0.250	
rs495491	5.556	0.062	4.466	0.107		0.017	0.890	
rs1381376	5.015	0.081	1.133	0.568		2.304	0.130	
rs1799971	2.696	0.260	0.234	0.890		0.1459	0.702	
CNR1					0.156			
rs6454674	4.616	0.099	5.331	0.070		0.0004	0.980	
rs806380	0.247	0.884	1.041	0.594		2.01	0.150	
rs806377	4.534	0.104	6.446*	*0.040*		0.87	0.350	
rs1049353	9.422*	*0.009*	1.682	0.431		0.48	0.490	
TRPV1								
rs224534	0.441	0.802	1.143	0.565		0.31	0.570	
rs222747	0.038	0.981	2.639	0.267		0.16	0.700	
rs8065080	0.246	0.884	1.534	0.464		0.0004	0.980	
rs224547	0.046	0.978	0.054	0.973		0.023	0.880	
GABRB3					*0.005*			
rs2873027	11.564*	*0.003*	3.864	0.145		2.243	0.134	
rs11161335	2.618	0.270	1.302	0.522		0.002	0.950	
GABRG3								
rs28431127	4.971	0.083	2.331	0.312		0.331	0.570	

Table II. Continued

Gene variations	Experiment 1						Experiment 2		
	EQ (χ^2 statistic)	EQ (*P*-value)	AQ (χ^2 statistic)	AQ (*P*-value)	Additional data	Permutation-corrected *P*-value	C-A trend test χ^2 statistic	C-A trend test *P*-value	Permutation-corrected *P*-value
rs4887536	0.372	0.830	2.237	0.327			0.49	0.480	
GABRA6						0.212			
rs13172914	1.7	0.427	1.763	0.414			0.11	0.730	
rs13183266	11.039*	*0.004*	3.532	0.171			0.39	0.530	
rs10037092	5.104	0.078	2.031	0.362	Eyes		0.56	0.460	
ABAT									
rs2302607	2.631	0.268	2.709	0.258	Eyes		0.2	0.655	
rs1731017	0.947	0.623	0.04	0.980			0.066	0.790	
rs1641010	0.692	0.707	0.522	0.770			0.793	0.373	
rs2270287	5.921	0.052	5.715	0.057			0.046	0.828	
rs1641003	0.768	0.681	3.721	0.156			3.384	0.068	
MAOB						0.551/*0.003*			
rs2283729	2.577/5.067	0.108/0.079	0.67/7.553*	0.413/*0.023*			0.00823/0.72	0.9277/0.397	
rs1799836	0.404/1.446	0.525/0.485	1.435/2.682	0.213/0.262			0.5759/0.20	0.4479/0.65	
VIPR						0.105			
rs417387	3.933	0.140	7.880*	*0.019*			1.47	0.224	
rs437876	2.006	0.157	0.321	0.571			0.25	0.620	
rs342511	3.582	0.167	1.858	0.395	EFT		1.67	0.196	
WFS1						*0.015*			
rs734312	9.905*	*0.007*	6.345*	0.042			0.006	0.930	
rs4234730	11.587*	*0.003*	7.143*	*0.028*			0.119	0.730	
rs1046322	0.314	0.855	0.253	0.881			0.69	0.400	
CGRPR									
rs35034167	0.35	0.839	0.427	0.808			1.66	0.190	
rs1983372	1.517	0.468	3.209	0.201	Eyes		1.84	0.174	
CGA									
rs981086	0.539	0.764	5.265	0.072			0.2818	0.595	
rs9444470	1.123	0.570	4.572	0.102			0.337	0.561	
rs9342103	1.591	0.451	0.332	0.847			2.413	0.120	
ESR1						0.777			0.296
rs4583998	0.959	0.619	0.442	0.802			1.696	0.192	
rs1884051	1.209	0.546	0.315	0.854			0.542	0.461	
rs827421	0.972	0.615	4.517	0.105			0.88	0.348	
rs2228480	0.364	0.834	0.203	0.904			0.2014	0.653	
rs11155819	2.987	0.225	7.178*	*0.028*			3.773*	0.052	
rs7774230	0.575	0.750	1.409	0.494			3.978*	*0.046*	
rs712221	1.405	0.495	1.354	0.508			2.75	0.090	
rs6905370	1.07	0.586	1.686	0.430			0.21	0.640	
rs1801132	0.733	0.693	0.709	0.702			1.83	0.176	
rs2077647	0.195	0.907	1.206	0.547			0.204	0.650	
ESR2						*0.006*			0.094
rs1271572	4.819	0.090	17.3*	*0.000*			3.04	0.080	

rs1256030	5.141	0.076	14.527*	*0.001*			1.676	0.195		
rs1152579	1.095	0.314	0.59	0.442			3.332	0.067		
rs1152582	4.665	0.097	12.772*	*0.002*			4.236*	*0.039*		
rs915057	4.799	0.091	14.3*	*0.001*			3.079	0.079		
rs1256049	3.622	0.164	12.021*	*0.002*			0.829	0.362		
AR1										
rs1204039	0.616/0.283	0.433/0.868	0.522/5.206	0.47/0.074	EFT(males)		0.09/2.221	0.759/0.1362		
rs5918760	1.048/0.197	0.306/0.906	0.184/5.331	0.668/0.07	EFT(males)		0.013/1.68	0.906/0.942		
rs6152	0.968/0.563	0.325/0.755	0.201/1.593	0.654/0.451	EFT(males)		0.01/1.31	0.928/0.352		
LHB										
rs753307	4.699	0.095	1.818	0.403			1.593	0.206		
LHCGR									0.138	
rs4555391	5.659	0.059	3.213	0.201			4.51*	*0.030*		
rs7584253	2.977	0.226	0.465	0.793			0.4249	0.515		
rs6545061	2.804	0.246	1.41	0.494			0.382	0.536		
rs2293275	0.294	0.863	0.388	0.824			0.033	0.850		
GNRHR										
rs2062302	0.561	0.756	4.294	0.117			1.15	0.284		
rs974483	2.729	0.255	1.126	0.569			0.252	0.615		
FSHB										
rs532667	1.332	0.514	1.261	0.532			2.119	0.146		
SULT2A1										
rs2547241	0.158	0.924	3.888	0.143			0.1072	0.743		
rs182420	1.526	0.466	1.71	0.425			0.027	0.868		
DHCR7										
rs4944957	0.639	0.726	2.31	0.315			0.8783	0.349		
rs12419334	0.573	0.751	1.22	0.543			0.7621	0.383		
rs736894	1.328	0.515	1.644	0.440			0.889	0.345		
STS										
rs2024159	1.809/1.282	0.179/0.527	2.666/2.67	0.102/0.263			0.51/1.234	0.82/0.2667		
rs7058445	1.45/1.757	0.228/0.415	2.175/3.521	0.14/0.172			0.06/0.7508	0.806/0.3862		
HSD11B1										0.054
rs4844880	1.365	0.505	0.469	0.791			2.203	0.138		
rs2884090	0.711	0.701	1.088	0.580			5.895*	*0.015*		
rs11576775	4.072	0.131	4.147	0.125			0.6053	0.437		
HSD17B2						0.390				
rs2873459	9.137*	*0.010*	4.666	0.097	Eyes		0.12	0.729		
rs4398102	6.807*	*0.033*	3.367	0.186	Eyes		1.858	0.172		
rs4445895	2.25	0.325	1.64	0.440			0.285	0.593		
rs4497679	6.129*	*0.047*	2.978	0.226	Eyes		0.094	0.758		
rs4889456	0.78	0.677	1.037	0.595			1.219	0.262		

Table II. Continued

	Experiment 1						Experiment 2		
Gene variations	EQ (χ^2 statistic)	EQ (*P*-value)	AQ (χ^2 statistic)	AQ (*P*-value)	Additional data	Permutation-corrected *P*-value	C-A trend test χ^2 statistic	C-A trend test *P*-value	Permutation-corrected *P*-value
rs6564964	0.529	0.768	0.676	0.713			0.7722	0.379	
rs8044837	2.258	0.323	0.852	0.653			1.61	0.194	
HSD17B3									
rs1807197	0.914	0.633	0.488	0.784	EFT		0.009	0.924	
rs1927883	1.233	0.540	1.327	0.515	EFT		0.006	0.934	
rs2026001	0.93	0.628	1.796	0.407			1.531	0.215	
rs2476920	3.026	0.220	0.751	0.687	EFT		0.419	0.517	
rs2476923	0.866	0.876	2.055	0.358			0.4567	0.499	
rs371119	4.057	0.132	0.975	0.614	EFT		0.524	0.469	
rs913580	1.079	0.583	0.47	0.791	EFT		0.076	0.782	
HSD17B4						0.692			
rs25640	0.593	0.744	5.237	0.073			0.063	0.801	
rs257973	0.212	0.899	5.704	0.058			0.201	0.653	
rs32651	0.939	0.625	1.904	0.386			0.2877	0.592	
rs3850201	0.557	0.757	0.42	0.811			0.6343	0.426	
rs426899	1.021	0.600	1.712	0.425			0.733	0.392	
rs7737181	0.154	0.926	6.346*	0.042	Eyes		0.1114	0.739	
CYP1A1									
rs1456432	0.118	0.943	1.997	0.368			0.081	0.776	
rs2606345	2.38	0.304	1.63	0.443			0.199	0.655	
rs4646421	0.375	0.829	0.411	0.814			2.45	0.117	
CYP1B1									
rs162556	0.078	0.962	0.017	0.992	Eyes		2.236	0.134	
rs163086	0.806	0.668	3.304	0.192			2.4	0.121	
CYP3A									
rs2242480	1.616	0.446	0.595	0.743			0.027	0.869	
CYP7A1									
rs11786580	2.188	0.335	0.198	0.906			0.657	0.417	
rs10957056	2.746	0.253	0.015	0.993			0.236	0.627	
rs1023649	0.865	0.649	0.476	0.788	Eyes		1.44	0.230	
CYP11A									
rs2279357	2.103	0.349	0.39	0.823			1.22	0.270	
CYP11B1						*0.033*			*0.010*
rs4534	1.104	0.576	1.21	0.546			5.68*	*0.017*	
rs4541	1.046	0.593	1.604	0.448			6.68*	*0.009*	
rs5288	7.28*	*0.007*	2.101	0.147	EFT		2.58	0.108	
CYP17A1									*0.047*
rs6163	3.322	0.190	0.726	0.695			5.424*	*0.019*	
rs4919685	3.696	0.158	1.465	0.481			4.16*	*0.040*	
rs619824	0.11	0.946	4.231	0.121			4.236*	*0.039*	
CYP19A1									0.084
rs10046	2.616	0.270	1.692	0.429			0.136	0.712	

rs767199	1.009	0.604	0.404	0.817		0.016	0.898		
rs1902585	1.8	0.407	0.964	0.617		4.678*	*0.030*		
rs11636639	0.019	0.990	0.077	0.962		0.85	0.356		
CYP21A2									
rs6467	0.234	0.890	1.529	0.466		0.337	0.562		
SRD5A1									
rs12418164	1.767	0.413	2.57	0.277		1.739	0.187		
SRD5A2									
rs12467911	2.854	0.240	0.554	0.758		0.5671	0.451		
rs12470143	0.002	0.999	1.014	0.602		0.36	0.840		
POR									
rs3898649	1.058	0.589	1.232	0.540		0.4219	0.516		
rs7804806	2.629	0.269	1.675	0.433		1.868	0.172		
rs2286821	3.146	0.207	3.411	0.182		0.3584	0.549		
SHBG									
rs6257	3.227	0.199	3.616	0.164	Eyes	1.556	0.212		
rs6259	1.828	0.401	3.984	0.136	EFT	0.92	0.337		
SCP2									0.095
rs7552139	0.517	0.772	1.356	0.508		0.4819	0.488		
rs7548389	0.069	0.966	0.121	0.941		0.007	0.930		
rs12747412	0.984	0.612	1.193	0.551		4.356*	*0.036*		
rs1288362	0.874	0.646	2.079	0.354		1.141	0.285		
TSPO (PBR)									
rs13056026	0.41	0.815	4.2	0.122		2.901	0.080		
rs3937387	0.36	0.835	3.243	0.198		0.5256	0.469		
rs138922	1.383	0.501	3.127	0.209		0.8165	0.366		
SLC25A12									
rs3821095	3.961	0.138	2.321	0.313		3.551	0.059		
rs6433317	0.467	0.792	5.042	0.080		2.117	0.146		
rs10497374	1.447	0.485	1.066	0.587		0.9971	0.318		
SLC25A13									
rs11773446	3.696	0.158	1.048	0.592		0.814	0.367		
rs10278888	2.291	0.318	0.405	0.817		0.4002	0.527		
rs2301629	0.454	0.797	2.935	0.231		1.17	0.279		

χ^2 statistics and corresponding *P*-values are reported for all analyses on AQ, EQ, and case–control data (Experiments 1 and 2), and values ≤ 0.05 have been italicized and asterisked. Only significant associations (at two-tailed $P \leq 0.05$) with performance measures (Eyes Test, EFT) are indicated in the column marked "Additional data." For X-linked genes, test statistics are reported separately for males and females, in that order. Corrected *P*-values after family wise error rate correction using 1,000 permutations are indicated for genes with nominally significant SNPs, and values ≤ 0.05 have been italicized. [Color table can be viewed online at www.interscience.wiley.com]

their correlation structure. The multiple phenotypes for each subject were permuted together so as to preserve the correlation structure among phenotypes. Each SNP was then tested for association to each permuted phenotype and the minimum *P*-value recorded. The permutation was repeated 1,000 times and the corrected *P*-value was the estimated proportion of permutations in which the minimum *P*-value was less than or equal to the minimum *P*-value seen in the original data. When the family wise error rate (FWER)-corrected *P*-value is significant, we may infer that at least one SNP in the gene is associated and that there is gene-wise significance. This gene-wise *P*-value thus reflects the *P*-value of the most significant SNP after FWER correction.

Results

In Experiment 1, autistic traits (measured on AQ and/or EQ) were nominally associated at $P \leq 0.05$ with SNPs from 19 genes. In Experiment 2, SNPs from 14 genes were nominally associated at $P \leq 0.05$ with AS. The results of the codominant test (2 d.f.) for Experiment 2 were very similar to the ones obtained by the Cochrane-Armitage trend test (1 d.f. χ^2), and are reported in Supplementary Table S2. Across both experiments, six genes showed nominal significance at $P \leq 0.05$. (see Fig. 1 for a summary of all nominally significant genes across the two experiments). A complete list of genotyped SNPs (grouped by gene), with their corresponding test statistics and nominal and family wise error rate (FWER)-corrected *P*-values, is reported in Table II.

In Experiment 1, 8 of the 68 genes showed gene-wise significance after 1,000 permutations, across all four phenotypes (AQ, EQ, Eyes Test, EFT). In Experiment 2, five genes showed gene-wise significance after 1,000 permutations. Two genes (*CYP11B1* and *NTRK1*) survived FWER correction in both the experiments (see Tables II and III). In Experiment 1, the probability of at least 8 out of 71 tests (65 autosomal+3 X-linked genes which were analyzed separately for males and females) being nominally significant atp ≤ 0.05, if all null hypotheses are true, is given by the binomial distribution as $P = 0.025$. Thus, while variation in this set of candidate genes could have no impact on the quantitative traits, our results suggest the choice of candidate genes is not random with respect to the quantitative traits. We report all associations that reach the nominal $P \leq 0.05$ (Table II), with the understanding that the family-wise null hypothesis is rejected and the nominally significant genes are the strongest candidates for further replication. Genes that have nominally significant SNPs in both experiments are indicated in Table III, in order to show the direction of association for each SNP. This reveals a largely consistent direction of association for nominally associated SNPs across both experiments, and provides evidence for a partial replication. SNPs in 17 genes showed nominally significant association with cognitive performance measures (Eyes Test and/or EFT), and these are indicated in Table II.

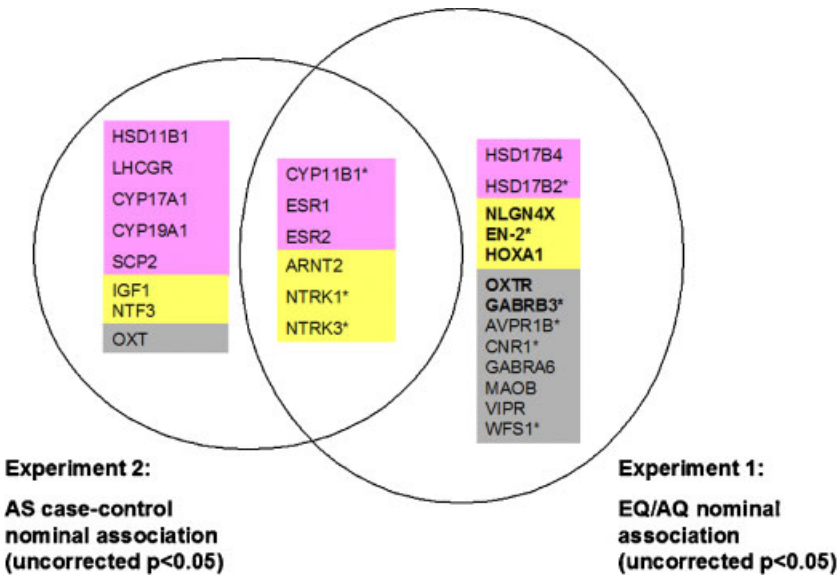

Figure 1. 27 genes showing nominal association with either (1) AS case-control analysis, and/or (2) autistic trait measures (AQ, EQ) in the population sample. The interaction summarizes genes that show a nominal association in both experiments. Gene functional groups are color coded: Pink [medium grey] (sex hormone related), Yellow [dark grey] (neural connectivity related), and Light grey (social–emotional responsivity related). Genes in bold indicate replications of associations reported in earlier studies. * indicates a nominally significant association with EQ. [Color figure can be viewed online at www.interscience.wiley.com]

Table III. List of the Six Genes that Show Nominal Significance at $P \leq 0.05$ in Both Experiments

		Experiment 1					Experiment 2								
Gene		χ^2	*P*-value (uncorr)				χ^2	*P*-value (uncorr)	Odds ratio	Cases			Controls		
NTRK1	EQ			CC	CT	TT				CC	CT	TT	CC	CT	TT
rs6337		1.37	0.505	*0.104*	*0.3425*	*0.555*	7.54	0.010	1.696	*0.09*	*0.32*	*0.60*	*0.16*	*0.40*	*0.45*
				50.03	40.88	44.5									
				TT	GT	GG				TT	TG	GG	TT	TG	GG
rs6339		9.184	0.010	*0.01*	*0.12*	*0.87*	0.2	0.650	1.155	*0.01*	*0.14*	*0.86*	*0.01*	*0.12*	*0.88*
				48	43.9	43.93									
NTRK3	AQ														
				GG	AG	AA				GG	AG	AA	GG	AG	AA
rs920069		11.984	0.002	*0.5455*	*0.39*	*0.06*	2.03	0.150	0.777	*0.52*	*0.39*	*0.08*	*0.45*	*0.43*	*0.12*
				16.99	16	13.95									
				AA	AG	GG				AA	AG	GG	AA	AG	GG
rs1369430		3.63	0.163	*0.1754*	*0.4892*	*0.3354*	4.66	0.030	0.698	*0.46*	*0.46*	*0.08*	*0.36*	*0.48*	*0.15*
				14.19	16.75	17.18									
ARNT2	AQ														
				AA	AG	GG				AA	AG	GG	AA	AG	GG
rs4778599		11.127	0.004	*0.1*	*0.45*	*0.44*	2.37	0.120	0.754	*0.11*	*0.32*	*0.57*	*0.11*	*0.44*	*0.44*
				12.3	16.7	16.8									
				TT	TC	CC				TT	TC	CC	TT	TC	CC
rs3901896		3.25	0.197	58	140	115	8.45	0.003	0.600	*0.12*	*0.41*	*0.47*	*0.24*	*0.42*	*0.35*
				14.88	16.87	16.43									
ESR1	AQ														
				CC	CT	TT				CC	CT	TT	CC	CT	TT
rs11155819		7.178	0.028	*0.11*	*0.42*	*0.47*	3.773	0.052	1.433	*0.15*	*0.41*	*0.44*	*0.05*	*0.45*	*0.50*
				17.4	16.8	16.2									
				AA	AG	GG				AA	AG	GG	AA	AG	GG
rs7774230		1.41	0.494	*0.104*	*0.4128*	*0.4832*	3.978	0.046	1.386	*0.29*	*0.48*	*0.23*	*0.21*	*0.49*	*0.31*
				16.06	16.63	16.34									
ESR2	AQ														
				AA	AC	CC				AA	AC	CC	AA	AC	CC
rs1271572		17.3	0.0001	0.2	0.4367	0.3608	3.04	0.080	0.745	*0.16*	*0.46*	*0.39*	*0.22*	*0.47*	*0.31*
				15.28	15.15	18.72									
				GG	GC	CC				GG	GC	CC	GG	GC	CC
rs1152582		14.3	0.001	*0.1824*	*0.465*	*0.3526*	4.236	0.039	0.717	*0.15*	*0.47*	*0.38*	*0.21*	*0.51*	*0.28*
				15.18	15.39	18.45									
CYP11B1	EQ														
				TT	TC	CC				TT	TC	CC	TT	TC	CC
rs4541		1.05	0.593	0.003	0.036	0.96	6.68	0.009	0.091	*0.00*	*0.01*	*0.99*	*0.01*	*0.05*	*0.94*
				29	44	43.69									
				GG	GT	TT				GG	GT	TT	GG	GT	TT
rs5288		7.28	0.007	*0*	*0.1*	*0.99*	2.58	0.108		*0.00*	*0.02*	*0.98*	*0.00*	*0.00*	*1.00*
				0.00	63.30	43.80									

Genotype frequencies for each nominally associated SNP is shown in italics. χ^2 statistics and associated *P*-values are reported for both experiments, and odds ratios are reported for Experiment 2. For genes with multiple nominally associated SNPs with the same quantitative trait, the SNP with the strongest association is shown. For Experiment 1, mean trait scores for each genotypic group is reported. For Experiment 2, proportion of participants in the AS group ($N = 174$) and the control group ($N = 155$) are indicated next to each genotype.

Haplotype analysis was attempted for all the genes with multiple nominally significant SNPs, using UNPHASED. This did not reveal any significantly associations, either with the case–control data or with the quantitative trait data. This is like to be due to the insufficient coverage of each gene by the multiple SNPs.

Discussion

This is the first hypothesis-driven study to test for genes associated with autistic traits in a population sample, and in a case–control sample for AS. Nominally significant SNPs in 19 genes were found to be associated with one or both of AQ and EQ (measures of autistic traits) in a typical adult sample. SNPs in 14 genes showed a nominally significant difference in allele frequency in the AS case–control analysis. Six of these genes were associated with both autistic traits in Experiment 1 as well as with AS in Experiment 2, suggesting a degree of internal replication. After correcting for multiple SNPs and phenotypes, SNPs in eleven genes remained significant across both experiments. This is more than would be expected from a random selection of candidate genes.

CYP11B1 and *NTRK1* were found to be significant, after FWER correction, in both the experiments, making these the strongest candidates for further replication. Of the 27 nominally associated genes, five of them (*NLGN4X*, *OXTR*, *GABRB3*, *HOXA1*, *EN2*) have been reported in earlier association studies of classic autism. Seventeen genes also showed significant association with performance measures of autistic traits (Eyes Test or EFT), seven of which (*EN2*, *HSD17B4*, *CYP11B1*, *VIPR1*, *NTRK3*, *HSD17B2*, *GABRA6*) overlapped with the genes associated with AQ and/or EQ. Table IV shows a summary of the most significant genes in the two experiments, that survive FWER correction, along with mouse phenotypes and relevant human data, where available. In Table IV and the following section, we discuss these genes in more detail.

Sex Hormones-Related Genes

ASC are associated with strong sex differences, with males and females receiving a diagnosis of classic autism in a ratio of 4:1, and a diagnosis of AS in a ratio of 9:1 [Wing, 1988]. Our study found that SNPs from ten genes related to sex hormone synthesis and metabolism were nominally significant in the case–control and/or the quantitative trait association analysis. Three of these (*CYP11B1*, *CYP17A1*, and *ESR2*) survived FWER correction.

In the *ESR2* gene, the C allele in rs1271572 and rs1152582 were associated with higher AQ in the typical population, and were also found to be more frequent in cases than in controls (Table III and Fig. 2). A similar pattern of results was seen for the nominally significant SNP rs11155819 in the *ESR1* gene. *ESR1* and *ESR2* code for the two main estrogen receptors. In the fetal brain testosterone is aromatized to estradiol and exerts its effects on neural development through acting on these receptors, and mediating selective cell survival. It promotes the defeminization of the developing male brain in mice [Kudwa, Bodo, Gustafsson, & Rissman, 2005]. Estrogen is thought to mediate social interaction in rodents, and this is supported by the presence of estrogen receptors in areas of the brain involved in emotion and affective behavior, such as the amygdala and the hippocampus. In addition, estradiol, acting through ER-β receptors (homologous to ESR2 in humans), is crucial for dendritic development in cerebellar Purkinje cells in mice [Sakamoto, Mezaki, Shikimi, Ukena, & Tsutsui, 2003]. Given that cerebellar Purkinje cell abnormalities have consistently been reported in autism, this finding represents a convergent genetic lead.

To the best of our knowledge, specific studies looking at estrogen in ASC have not yet been carried out. However, testosterone is converted to estradiol and acts through estrogen receptors in the developing rodent brain [Kudwa et al., 2005], and variations in the estrogen receptors can affect testosterone action.

Longitudinal studies from our laboratory over the last 10 years have found levels of fetal testosterone in typically developing children are negatively correlated with eye contact, vocabulary development [Lutchmaya, Baron-Cohen, Raggatt, & Manning, 2004], empathy [Knickmeyer, Baron-Cohen, Raggatt, & Taylor, 2005; Chapman et al., 2006]. Fetal testosterone levels are also positively correlated with narrow interests [Knickmeyer et al., 2005], systemizing [Auyeung et al., 2006], and autistic traits as measured using the AQ [Auyeung et al., 2009]. The ratio of testosterone to estrogen is also thought to affect the 2^{nd} to 4^{th} digit ratio (2D:4D), which is masculinized in ASC.

CYP17 catalyses the production of dehydroepiandrosterone (DHEA, a precursor of testosterone), as well as androstenedione (a precursor of estradiol). Polymorphisms of this gene have been associated with Polycystic Ovary Syndrome (PCOS) in women [Park et al., 2008]. We have previously reported an increased rate of PCOS and other testosterone-related medical conditions in women with ASC [Ingudomnukul, Baron-Cohen, Wheelwright, & Knickmeyer, 2007]. Hence this too represents a convergent finding. The products of *CYP17A1* are also known to be involved in neocortical organization in the developing rodent brain [Compagnone & Mellon, 1998]. *CYP11B1* is cellularly localized in the mitochondria and converts 11-deoxycortisol to cortisol. Polymorphisms in this gene and the *CYP11A* gene are associated with congenital adrenal hyperplasia (CAH) [Kuribayashi et al., 2005] in which FT is elevated. CAH is associated with higher AQ than in the general population [Knickmeyer et al., 2005]. rs4541 and rs5288 are nonsynonymous coding polymorphisms in *CYP11B1*, which were significant in both

Table IV. Summary of All Significant Genes that Survive FWER Correction, Grouped by Functional Category

GENE_NAME	Gene symbol	Chromosomal position	Observations in human relevant to ASC	Behavioral phenotype in animal models
HOMEOBOX A1	**HOXA1**	7p15.3	Homozygous truncating mutations in HOXA1 interferes with brain development and results in a myriad of phenotypes including mental retardation and autism [Tischfield, 2005].	KO mice show disrupted hindbrain patterning [Rossel et al., 1999].
NEUROTROPHIC TYROSINE KINASE, RECEPTOR, TYPE 1	NTRK1	1q21–q22	Mutations associated with congenital pain insensitivity in humans [Indo, 2001].	KO mice show severe sensory and sympathetic neuropathy, especially for nociceptive neurons [Smeyne et al., 1994; Patel et al., 2000].
ARYL-HYDROCARBON RECEPTOR NUCLEAR TRANSLOCATOR 2	ARNT2	15q24	Expression of Arnt2 is known to be limited to the neural tissues in mice (No human data available).	Arnt2 KO mice die shortly after birth and exhibit hypocellular supraoptic nuclei (SON) and the paraventricular nuclei (PVN) in the hypothalamus. Secretory neurones expressing arginine vasopressin, oxytocin, corticotrophin-releasing hormone and somatostatin are completely absent in SON and PVN [Hosoya, 2001].
NEUROLIGIN 4, X-LINKED	**NLGN4X**	Xp22.32–p22.31	NLGN mutations associated with autism [Ylisaukko-oja et al., 2004; Jamain et al., 2003].	KO mice show drastically reduced inhibitory neurotransmission in brainstem [Varoqueaux et al., 2006].
WOLFRAM SYNDROME 1 (WOLFRAMIN)	WFS1	4p16	Mutations associated with Wolfram Syndrome, marked by progressive neurodegeneration [Inoue et al., 1998]. Haplotypes associated with affective disorders [Koido et al., 2004].	Mice exposed to cat odour show overexpression in amygdala [Koks et al., 2002].
GAMMA-AMINOBUTYRIC ACID (GABA) A RECEPTOR, BETA 3	**GABRB3**	15q11.2–q12	A chromosomal deletion in this region is associated with Angelman syndrome, marked by hypersocialization [Knoll et al. 1989; DeLorey et al., 2007].	KO mice show spontaneous seizures and abnormal background EEG patterns [DeLorey et al., 2007] and cochlear neuropathy [Maison et al., 2006].
OXYTOCIN, PREPRO- (NEUROPHYSIN I)	OXT	20p13	OT infusion reduces repetitive behaviour in people with autism [Hollander et al., 2003], and increases trust and empathy in control humans [Kosfeld et al., 2005].	KO mice show abnormal social recognition [Ferguson et al., 2001].
MONOAMINE OXIDASE B	MAOB	Xp11.23	Partial deletions of locus is associated with social behavioural deficits in females [Good et al., 2003].	An increased response to stress in Maob-deficient mice [Grimsby et al., 1997].
CYTOCHROME P450, FAMILY 11, SUBFAMILY B, POLYPEPTIDE 1	CYP11B1	8q21	This enzyme has 11-β-hydroxylase activity and mutations in this gene are associated with CAH. [Helmberg et al., 1992; Kuribayashi et al., 2005].	Cyp11b1 mRNA is expressed in the rat amygdala and cerebral cortex and is associated with sex differences [Mellon et al., 1993].
ESTROGEN RECEPTOR 2 (ER BETA)	ESR2	14q23.2	Abundunt expression of Esr2 mRNA in the hippocampal formation (primarily the subiculum), claustrum, and cerebral cortex; expression also in the subthalamic nucleus and thalamus (ventral lateral nucleus) [Osterlund et al., 2000].	Esr2ko male mice show increased female-typical behaviour. Esr2 KO female mice exhibit enhanced anxiety and significantly lower serotonin (5-HT) content in the bed nucleus of the stria terminalis, preoptic area, and hippocampus [Imwalle, 2005].
CYTOCHROME P450, FAMILY 17, SUBFAMILY A, POLYPEPTIDE 1	CYP17A1	10q24.3	Catalyses the formation of the precursors of both testosterone and oestradiol in the adrenal gland. Polymorphisms have been associated with Polycystic Ovary Syndrome in women [Park et al., 2008] which is more frequent in women with ASC (see text).	A gene knockout causes embryonic lethality in mice [Blair & Mellon, 2004]. Haploinsufficiency of this gene is associated with dysregulated steroidigenesis and infertility in male mice [Liu et al., 2005].

Mouse phenotypes and relevant human data are included, where available. Gene functional groups are colour coded in the online version of the table: Pink [medium grey] (Sex hormone related), Yellow [dark grey] (Neural connectivity related) and Light grey (Social–emotional responsivity related). Full references are provided in S3 (Supplementary material). Gene names in bold indicate replication of previously reported associations with classic autism. [Color table can be viewed online at www.interscience.wiley.com]

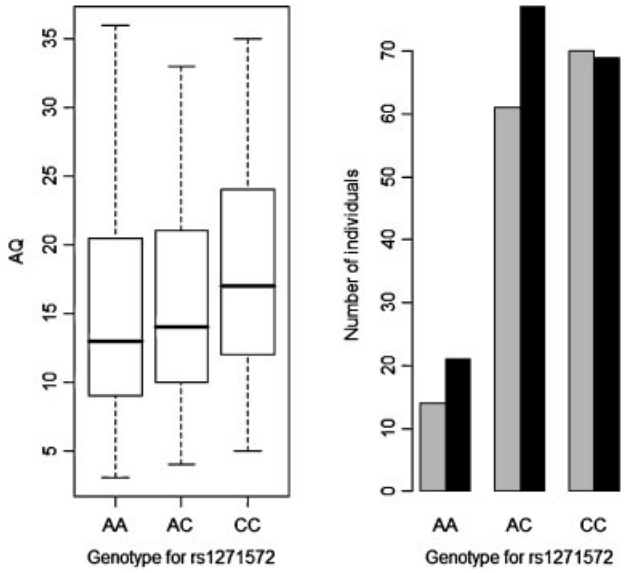

Figure 2. rs1271572 in the *ESR2* gene is one of several SNPs that show a convergent effect across the two experiments (for a list of these SNPs, see Table III). The left panel displays the AQ scores of each genotype in the typical sample (Experiment 1). Boxes denote the 1st to 3rd quartiles, and the central line denotes the median AQ score. Box width is proportional to the number of individuals in each genotype. The right panel is a bar chart showing the case–control distribution for each genotype in Experiment 2 (grey [blue], ASC participants; black [red], control participants).

the case-control analysis and associated with EQ and the Eyes Task in the general population.

It should be noted though that different SNPs were significant in the two experiments. rs5288 was significant in Experiment 1, but showed no significant differences in allelic frequency between cases and controls in Experiment 2. rs4541 showed a significant difference in allele frequency between cases and controls, but was not associated with a significant difference in mean EQ scores between the different genotypic groups in Experiment 1. This could be due to a variety of factors including limited gene coverage, stochastic variation in LD between the two samples or sampling variations. Additionally in the case–control analysis, a nominally significant association was seen for rs1902585 in *CYP19A1*. This gene codes for aromatase, the enzyme that converts testosterone to estradiol. Together, these results implicate genes involved in the synthesis and metabolism of sex steroids in the etiology of both ASC and autistic traits, possibly pointing to an abnormal balance of testosterone in estrogen signalling. At the time of this article going to press, a study by Hu et al. [2009] has just been published, which reports increased expression of genes related to androgen signalling in autistic children compared to their non-autistic siblings. Together, this provides some of the first genetic evidence in support of the role of sex-steroids in the etiology of ASC [Baron-Cohen, Knickmeyer, & Belmonte, 2005].

Genes Involved in Neural Development and Connectivity

Abnormal neural connectivity has been proposed to underlie ASC [Belmonte et al., 2004]. Our study found that SNPs from eight genes involved in neural development and connectivity were nominally associated with the case–control and/or the quantitative trait analysis. Four of these (*HOXA1*, *NLGN4X*, *NTRK1*, and *ARNT2*) survived FWER correction.

rs10951154 in *HOXA1* has been previously associated with head size in ASC [Conciatori et al., 2004] as well as with head growth rate [Muscarella et al., 2006]. Our result shows that G-allele carriers are associated with a higher AQ than the AA homozygotes. This is consistent with the finding that the G-allele has been found to be associated with larger head size and greater head growth rate [Muscarella et al., 2006]. rs12836764 in the *NLGN4X* UTR was significantly associated with both EQ and AQ in females. This supports earlier findings implicating this gene in autism [Jamain et al., 2003; Yan et al., 2005]. A large-scale association study of autism found a significant association with neurexins [AGP, 2007] that interact with neuroligins in mediating glutamatergic synaptogenesis. rs1861972 in the *EN-2* gene was associated with EQ in our population sample, although this did not survive a FWER correction. This provides partial support for an earlier report of this SNP being associated with autism [Benayed et al., 2005]. In addition to replicating these earlier findings, we found five hitherto unreported genes in this functional group that were associated with AS or autistic traits. These include three members of the neurotrophin family, particularly *NTRK1*, *NTRK3*, and *NTF3*. SNPs in *NTRK1* showed a significant association in both the experiments, which survived FWER correction at $P<0.05$. *NTRK1* is situated within a peak (1q21–22) reported in the first ever linkage study of AS [Ylisaukko-oja et al., 2004] and thus provides an independent validation. Nerve growth factor (NGF), signalling through TrkA (the protein product of *NTRK1*), mediates most neurotrophic action of NGF [Sofroniew, Howe, & Mobley, 2001]. A primary role of the TrkA in the developing brain is in determining the fate and growth of neurites, in whether they become axons or dendrites [Da Silva, Hasegawa, Miyagi, Dotti, & Abad-Rodriguez, 2005]. Given the known abnormalities in structural and functional connectivity in the autistic brain, *NTRK1* provides an interesting candidate for future research.

It should be noted that different SNPs were significant in the two experiments, which again maybe due to a range of factors such as limited gene coverage, stochastic variation in LD between the two samples, or sampling variations. NTRK3 and NTF3 is a ligand–receptor pair in the neurotrophin family of molecules that are expressed from

very early in development, and is involved in the formation of the neural tube. Two SNPs in *NTRK3* were found to be nominally associated in both experiments, but neither survived a FWER correction. Two SNPs in the *ARNT2* gene were found to be associated in both the experiments, and survived FWER correction in Experiment 2. rs4778599 in this gene showed a consistent effect across both the experiments, in that the C-allele was associated with a higher AQ score (Experiment 1) and a trend toward a greater proportion of participants with an ASC diagnosis (Experiment 2). This gene is involved both in the development of the neuroendocrine cells in the hypothalamus [Michaud, DeRossi, May, Holdener, & Fan, 2000] as well as in the neural response to hypoxia [Maltepe, Keith, Arsham, Brorson, & Simon, 2000].

These findings point to a key role played by these neurodevelopmental genes in the development of autistic traits.

Genes Involved in Social–Emotional Responsivity

Social–emotional responsivity is one of the core cognitive and behavioral domains marked by impairments in ASC. We found SNPs from nine genes linked to social–emotional responsivity (largely from animal models) to be nominally associated with ASC and/or autistic traits. Four of these (*MAOB*, *GABRB3*, *WFS1*, *OXT*) survived FWER correction.

MAOB was significantly associated in females only, and this is consistent with the earlier studies showing the importance of this locus in social cognition, both in humans and mouse models [Good et al., 2003; Grimsby et al., 1997]. MAOB knockout mice are also known to demonstrate a heightened response to novelty and a lack of habituation [Lee, Chen, Shih, & Hiroi, 2004]. These features resemble those seen in ASC. The rationale for testing GABA-related genes came from the fact that social behavior has been linked to GABA-ergic activity in the CNS [File & Seth, 2003], and that GABA receptors play a crucial role early in cortical development through their effect on neuronal migration as well as on the development of excitatory and inhibitory synapses [Di Cristo, 2007]. In this sense, GABA-related genes could have been placed in both the neurodevelopmental group of candidate genes too. We found *GABRB3* was significantly associated with EQ in the typical sample, thus corroborating a role of this locus (15q11–q13) in autism [Ashley-Koch et al., 2006; Buxbaum et al., 2002]. Gabrb3 knockout mice have been shown to demonstrate low social and exploratory behavior as well as smaller cerebellar vermal volumes, pointing to a potential animal model for autism [DeLorey, Sahbaie, Hashemi, Homanics, & Clark, 2007].

Another significant association in this functional class of genes was the Wolframin (*WFS1*) gene. Wolframin is strongly expressed in the amygdala, especially in response to fear-inducing stimuli. The amygdala is one of the key brain regions where functional and structural abnormalities have been consistently found in ASC [Baron-Cohen et al., 2000]. Two SNPs in *WFS1* showed a strong association with both AQ and EQ. One of these, rs734312, is a nonsynonymous coding SNP and belongs to a haplotype that shows an increased risk for affective disorders [Koido et al., 2004]. This result supports a role for this gene in emotional responsivity.

Three genes from the oxytocin-vasopressin system (*OXTR*, *OXT*, and *AVPR1B*) were found to be nominally associated with ASC and/or with AQ and EQ. These genes have suggestive links with autism [Insel, O'Brien, & Leckman, 1999; Wu et al., 2005] and/or social behavior in animal models. Of these, *OXT* survived a FWER correction in Experiment 2. Oxytocin is of particular interest, given the recent reports of oxytocin levels being low in autism, and treatment effects of both intranasal and intravenous administration of oxytocin [Hollander et al., 2003]. Oxytocin levels are also correlated with empathy and prosocial measures, such as the Eyes Test [Domes, Heinrichs, Michel, Berger, & Herpertz, 2007] and trust in neuroeconomic studies [Kosfeld, Heinrichs, Zak, Fischbacher, & Fehr, 2005]. These provide partial support for the involvement of the oxytocin–vasopressin system in autistic traits. Together, these results support the idea that genes implicated in social and emotional responsivity contribute to individual differences in traits related to ASC.

Functional Overlap Between Gene Groups

A detailed pathway analysis will be reported elsewhere, but we briefly mention some interactions between the three functional categories of genes associated with ASC and/or autistic traits. Sexual dimorphism begins before gonadal sex has been determined [De Vries et al., 2002]. For example, *ARNT2* shows a sexually dimorphic pattern of expression in the brain before gonadal differentiation has occurred [Dewing, Shi, Horvath, & Vilian, 2003]. Chromosomal sex is thus the first step in the development of sexual dimorphism in the brain. A second step involves patterns of sex steroid synthesis and metabolism, from around week 12 in utero. The role of testosterone in affecting neural development by averting programmed cell death, influencing neural connectivity, and altering neurochemical profiles is well established [Baron-Cohen et al., 2005]. Estradiol is known to upregulate TrkA in the developing brain, which suggests a neuroprotective function for it [Sohrabji, Miranda, & Toran-Allenrand, 1994]. The sex steroids also modulate neurotransmitter systems involved in social–emotional responsivity. For example, testosterone and estradiol modulate GABAergic and serotoninergic transmission [Robichaud & Debonnel, 2005] and vasopressin expression [Han & De Vries, 2003]. The neurotrophins and GABA-related genes also interact during cortical development [Lujan, Shigemoto, & Lopez-Bendito, 2005]. This is

to emphasize that although the three functional gene groups were selected independently, there are extensive interactions between them in nature—a detailed treatment of these is beyond the scope of this study.

This is the first candidate gene association study of AS, and of autistic traits in the general population. However, our study also has some limitations. First, our sample sizes were moderate and we deliberately restricted the minor allele frequency of our genotyped SNPs to those $>20\%$. None of our results would survive an experiment-wide Bonferroni correction for the total number of genes tested. However, it should be noted that the unlike a hypothesis-free whole genome association study using $\sim$1 million probes, the multiple comparison problem is several orders of magnitude lower (216 SNPs), so a Bonferroni correction would be too conservative for our analysis [Edwin, 2008; Moran, 2003]. Second, the controls in the case–control analysis were drawn from the general population sample used for the AQ/EQ association analysis, so these two analyses are not completely independent. As an initial exploratory study, our results have generated promising leads and these await independent replication. Finally, we have relied on self-reported ethnicity for all participants, which might be susceptible to subtle confounds due to population stratification.

Despite these limitations, we have succeeded in demonstrating association in the set of candidate genes chosen, significantly higher than would be expected from a random selection of genes. This is important because this study was designed to test multiple strong candidates, rather than being an exploratory whole-genome scan. Our prior belief in each candidate was fairly strong and certainly higher than it would be in a scan. It is therefore reasonable to report genes with gene-wise significance. Because this is based on the number of genes reaching nominal significance, we suggest all such genes are plausible targets for further replication. A caveat is that the genotyping density was variable across genes, owing to the constraints of allele frequency and extent of linkage disequilibrium, so that there is variability in power. Since we tested only a subset of the common variants within each gene, we refrain from speculating on the mechanisms of possible ''risk alleles'' in the development of autistic traits. We are following up these initial associations with fine-mapping of the causal variants, which may reveal genetic mechanisms with more precision.

Additionally, while treating AS as a milder subgroup of autism without comorbid conditions is a novel and useful approach in refining the phenotype, there is a need to conduct replication studies on this set of significant genes in a sample with classic autism. We are currently testing this in our lab.

In this study, we have identified 27 nominally significant candidate genes, some of which are associated with autistic traits in the general population and/or AS. These genes fall into the three functional categories related to sex-steroid synthesis and metabolism, neural development and connectivity, and social–emotional responsivity, providing some support for three theories of autism. Our future studies will test these genes in expression studies, as well as in classic autism to establish which combination of common SNPs (which individually are nonpathological) are common to the etiology of any ASC.

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