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Associations between autistic-like traits and polymorphisms in *NFKBIL1*

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Abstract

Objective: The immune system has been suggested to be associated with neuropsychiatric disorders; for example, elevated levels of cytokines and the inflammation-related transcription factor nuclear factor kappa-B (NF-κB) have been reported in individuals with autism spectrum disorder (ASD). The aim of this study was to investigate possible associations between autistic-like traits (ALTs) and single nucleotide polymorphisms (SNPs) in *NFKB1* (encoding a subunit of the NF-κB protein complex) and NF-κB inhibitor-like protein 1 (*NFKBIL1*). *Methods:* The study was conducted in a cohort from the general population: The Child and Adolescent Twin Study in Sweden (CATSS, $n = 12\ 319$, 9–12 years old). The subjects were assessed by the Autism-Tics, ADHD, and Other Comorbidities Inventory. Five SNPs within the two genes were genotyped (*NFKBIL1*: rs2857605, rs2239707, rs2230365 and rs2071592; *NFKB1*: rs4648022). *Results:* We found significant associations for two SNPs in *NFKBIL1*: rs2239707 showed a significant distribution of genotype frequencies in the case-control analysis both for all individuals combined and in boys only, and rs2230365 was significantly associated with the ALTs-module language impairment in boys only. Furthermore, we found nominal association in the case-control study for rs2230365, replicating earlier association between this SNP and ASD in an independent genome-wide association study. *Conclusion:* The shown associations between polymorphisms in *NFKBIL1* and ALTs are supporting an influence of the immune system on neuropsychiatric symptoms.

Significant outcomes

- Polymorphisms in *NFKBIL1* were shown to be associated with ALTs in the studied population of twins.
- The ALTs subdomain language impairment was significantly associated with the SNP rs2230365 in *NFKBIL1* in boys after correction for multiple testing.
- We furthermore found an association between rs2230365 and the genotype distribution in the case-control analysis, replicating associations previously seen in this SNP with autism.

Limitations

- The A-TAC scores used in this study were based on parental reports, where response styles could vary between parents.
- Associations between *NFKB1* and *NFKBIL1* polymorphisms and ALTs should be reproduced in a patient-control population.
- Due to the limited number of subjects (especially girls) presenting ALTs scores above the cut-off for a proxy for ASD diagnosis, it was not possible to perform case-control analyses in girls.

Introduction

Autism spectrum disorder (ASD) describes a range of complex neurodevelopmental disorders which are characterised by impairments in social interactions and both verbal and non-verbal communication as well as by restrictive/repetitive behaviour. Several different risk factors have been proposed to contribute to the development of ASD, such as environmental toxicants (Rossignol *et al.*, 2014), perinatal complications (Schieve *et al.*, 2014) and infections (Meltzer & Van De Water, 2017). These risk factors share a common feature, namely inflammation; a plausible underlying mechanism may be that cytokines and other inflammatory mediators

are essential for brain development (Boulanger, 2009). We have previously reported associations between immune-related genes and personality traits in different populations (Suchankova *et al.*, 2009; Suchankova *et al.*, 2011; Suchankova *et al.*, 2012; Suchankova *et al.*, 2012) showing the relevance of the immune system for mental functions. Therefore, it is reasonable to attribute the detrimental effects of inflammation due to, for example, hypoxia or infection with respect to ASD not only to an injurious inflammatory milieu but also to a direct involvement of a disturbed balance between pro- and anti-inflammatory substances in the mechanisms taking part in the developing brain regulating, for example, synaptic pruning, leading to a higher risk of neuropsychiatric disorders (Meltzer & Van De Water, 2017). Indeed, ASD has been linked to an excess of neurons in the prefrontal cortex (Courchesne *et al.*, 2011), maybe an effect of, amongst others, the abovementioned dysregulation of immune mediators in the brain. An increasing number of studies report associations between abnormalities in the immune system and ASD (Meltzer & Van De Water, 2017) such as altered T-cell response (Ashwood *et al.*, 2011a) and reduced natural killer cell cytotoxicity (Enstrom *et al.*, 2009). Elevated levels of inflammatory cytokines have been shown in both blood (Croonenberghs *et al.*, 2002; Ashwood *et al.*, 2011b) and brain tissue (Li *et al.*, 2009) of individuals with ASD when compared to controls. A recent study observing a vast number of subjects ($n = 1\,791\,520$) for up to 41 years reported significantly increased risks of autism and depression in subjects exposed to any maternal infection during pregnancy, including urinary tract infection (Al-Haddad *et al.*, 2019). Several other studies also suggest a role of maternal immune system activation and inflammation in ASD (Lee *et al.*, 2015); increased levels of C-reactive protein in maternal sera (Brown *et al.*, 2014) as well as maternal infection, such as rubella (Chess, 1971; Hutton, 2016) and influenza (Atladottir *et al.*, 2012), were shown to be significantly associated with ASD in offspring. Moreover, a link between autoimmune diseases and ASD has been reported (Gesundheit *et al.*, 2013) as well as the presence of brain reactive autoantibodies in the plasma from mothers of children with ASD (Braunschweig *et al.*, 2013). A high degree of genetic heritability in ASD has been reported in twin studies (Tick *et al.*, 2016) and candidate genes situated in nearly all of the chromosomes have been proposed (Woodbury-Smith & Scherer, 2018).

In this study, we investigate two candidate genes that are central to immune functions: *NFKB1* on chromosome 4, encoding a protein that is processed to a DNA binding subunit of the nuclear factor kappa-B (NF-κB) protein complex, and *NFKBIL1* that encodes a protein suggested to regulate NF-κB (Boodhoo *et al.*, 2004; Ramasawmy *et al.*, 2008) as well as alternative splicing of both human and viral genes (An *et al.*, 2013). NF-κB is a protein complex functioning as a transcription factor that regulates the expression of pro-inflammatory genes and is also suggested to be of relevance for the regulation of neural growth and development (Gutierrez, 2005) as well as for synaptogenesis and plasticity (Methot *et al.*, 2013). Also, NF-κB levels have been shown to be aberrantly expressed in both blood (Naik *et al.*, 2011) and brain tissues (Young *et al.*, 2011) of individuals diagnosed with ASD. *NFKBIL1* lies within the human leucocyte antigen (HLA) class III region on chromosome 6; HLA is the human version of the major histocompatibility complex and the genes in the HLA region are involved in immunological processes such as histocompatibility, the complement cascade and autoimmunity (Fernando *et al.*, 2008). Involvement of the HLA region in the pathophysiology of ASD was first suggested over 30 years ago (Stubbs & Magenis, 1980). With respect to the HLA region, linkage studies in Italian families have shown associations between certain HLA haplotypes and ASD (Guerini *et al.*, 2011), and several other HLA gene associations with ASD have been reported (Torres *et al.*, 2012; Torres *et al.*, 2016).

ASD has been suggested to represent the extreme end of a normal distribution of autistic-like traits (ALTs), which we studied in this report. ASD and ALTs have been shown to share common genetic influences, suggesting aetiological similarity between them (Robinson *et al.*, 2011; Lundström *et al.*, 2012; Bralten *et al.*, 2018). Studies showed that ASD is four times more prevalent in males than females, and the underlying reasons for this gender bias is still poorly understood (Lai *et al.*, 2014; Werling, 2016). Both male-specific risk models and female-specific protective effects have been proposed, the latter suggesting that females would have to have greater genetic and/or environmental load than males to manifest the same degree of impairment and reach diagnostic threshold. This notion is supported by a study of autistic traits conducted in dizygotic twins of two independent cohorts, where siblings of female probands displayed more autistic characteristics than siblings of male probands (Robinson *et al.*, 2013). This emphasises the importance of stratification by sex in order to elucidate the possible mechanisms behind ASD etiology.

Polymorphisms in our chosen candidate genes have previously been associated with, for example, autoimmune diseases such as Grave's disease and neonatal lupus, as well as with ASD and schizophrenia (for references, see Table 1). Of special note is a study by Anney *et al.* where a genome-wide scan to identify common risk variation for autism showed nominal association of the SNP rs2230365 in *NFKBIL1* with ASD (Anney *et al.*, 2010). Considering these previous associations, as well as the proposed role of autoimmune diseases in ASD and the known influence of NF-κB on brain development, these polymorphisms may be involved in the pathophysiology of ASD.

Aims of the study

The aim of this study was to investigate whether single nucleotide polymorphisms (SNPs) in the candidate genes *NFKB1* and *NFKBIL1* were associated with ALTs in a large cohort ($n = 12\,319$) from the general population. Boys and girls were both analysed combined and separately in order to assess possible sex-dependent differences.

Material and methods

Cohort

Subjects included in this study were from the Child and Adolescent Twin Study in Sweden (CATSS), which is an ongoing study that targets all twins born in Sweden since July 1992 (Anckarsäter *et al.*, 2011). Among the 12 426 individuals (6223 males and 6203 females) 2301 were monozygotic twin pairs, 3872 dizygotic twin pairs and 80 subjects without their co-twin. Zygosity was determined by a panel of 47 SNPs (Hannelius *et al.*, 2007). Due to brain damage or known chromosomal abnormalities, such as Down syndrome and fragile X syndrome, 107 individuals were excluded from the statistical analyses, leaving a total of 12 319 individuals. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008, and the protocol has been approved by the Karolinska Institutet Ethical

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Table 1. SNP details

Gene	Chromosome	SNP	Location	MAF	Reference/associated phenotype or disease
NFKB1	4q24	rs4648022	Intron 6	0.08 (T)	Cerhan *et al.* 2008/Non-Hodgkins lymphoma
NFKBIL1	6p21.33	rs2857605	Intron 2	0.23 (G)	Wang *et al.* 2009/Non-Hodgkins lymphoma
					Aberg *et al.* 2013/Schizophrenia*
		rs2239707	Intron 2	0.36 (G)	Wang *et al.* 2009/Non-Hodgkins lymphoma
		rs2230365	Exon 3	0.16 (T)	Wang *et al.* 2009/Non-Hodgkins lymphoma
					Anney *et al.* 2010/ASD*
					Clancy *et al.* 2010/Neonatal Lupus
		rs2071592	Promoter	0.33 (T)	Castiblanco *et al.* 2008/Systemic lupus erythematosus and Sjögren's syndrome

SNP, single nucleotide polymorphism; MAF, minor allele frequency.
*Nominal association.

Review Board. Informed consent for inclusion was given by parents of all participants (Anckarsäter *et al.*, 2011).

Measurements

Parents were contacted when their twins were 9–12 years of age and asked to participate in a telephone interview containing, among other instruments, the Autism-Tics, ADHD, and Other Comorbidities Inventory (A-TAC), which is a validated tool for parental interviews screening general populations for childhood ASD and comorbid conditions (Hansson *et al.*, 2005; Larson *et al.*, 2010). The A-TAC comprises 17 questions on ALTs. They reflect the three core symptom domains of ASD, as described in the *Diagnostic and Statistical Manual of Mental Disorders*, fourth edition (DSM-IV) (American Psychiatric Association, 1994): language impairment (six questions), social interaction impairment (six questions) and restrictive/repetitive behaviour (five questions). Each item has three possible answers: 'no' (coded 0), 'yes, to some extent' (coded 0.5) and 'yes' (coded 1). The total ALTs score is the sum of the 17 questions. A cut-off of ≥8.5 was used as a validated proxy for clinical ASD diagnosis (with a sensitivity/specificity of 0.71/0.95) (Larson *et al.*, 2010; Mårland *et al.*, 2017).

DNA extraction and genotyping

DNA was extracted from saliva samples using OraGene® DNA self-collection kits (DNA Genotek, Inc., Ottawa, ON, Canada). One SNP in *NFKB1* (rs4648022) and four SNPs in *NFKBIL1* (rs2857605, rs2239707, rs2230365 and rs2071592) were genotyped using the Kompetitive Allele Specific (KASPar®) PCR SNP genotyping system (LGC Genomics, Hoddesdon, UK), as shown in Table 1 with further information on position and previous studies reporting associations.

Statistical analysis

Statistical analyses to estimate the associations between SNPs and continuous measures of ALTs were performed using a linear mixed effect model in the PROC MIXED procedure of the SAS 9.3 (SAS Institute, Inc., Cary, NC) software. The validated research proxy for a clinical ASD diagnosis was used to model a case-control analysis using the generalised linear mixed model for binary data in the PROC GLIMMIX procedure in SAS 9.3. Due to the limited number of subjects (especially girls) with high ALTs scores, it was not possible to perform case-control analyses in girls only. While monozygotic twins share 100% of their genome, dizygotic twins share only about 50%. Two different variance–covariance matrices were modelled (1) for monozygotic twins and (2) for dizygotic twins. Correlations between individuals in groups (1) and (2) were calculated using R-side random effects with an unstructured variance–covariance matrix. Thus, all statistical analyses were adjusted for kinship and the CATSS population was analysed as a representative sample of children from the general population in Sweden. Mean scores and standard deviations were calculated using the 'PROC MEANS' procedure in SAS 9.3. The linkage disequilibrium (LD) was analysed using the pairwise LD measure *D*' and a LD plot showing *D*' and r^2 was generated using Haploview software (Barrett *et al.*, 2005). To correct for multiple testing, the Bonferroni method was applied; the analyses of five SNPs and three ALT-modules as well as case-control resulted in 20 independent tests and the corrected *p*-value limit of 0.0025. The genotyping success rate was 96–98%, and all SNPs were found to be in Hardy–Weinberg equilibrium.

Results

The results from the case-control analysis using the A-TAC score as a proxy for an ASD diagnosis are shown in Table 2. Associations between total ALTs as well as its modules language impairment, social interaction impairment and restrictive/repetitive behaviour and SNPs in *NFKB1* and *NFKBIL1*, respectively, are shown in Table 3.

Associations that survived Bonferroni correction were found for two SNPs in *NFKBIL1*; case-control analysis revealed a significantly different distribution of genotypes in rs2239707 in all individuals combined ($p = 0.0016$) and in boys only ($p = 0.0006$; Table 2), and rs2230365 was significantly associated with the ALT-module language impairment in boys only ($p = 0.0009$; Table 3).

The SNP rs2239707 furthermore showed associations with the modules language impairment (in boys only) and social interaction impairment (in all individuals combined), that did, though, not remain significant after correction for multiple testing. For rs2230365 we also found nominal associations: with total ALTs (in all individuals combined and boys only), the other two ALT-modules social interaction impairment (in all individuals combined) and restrictive/repetitive behaviour (in boys only), and in the case-control study (in boys only). Nominal evidence for association was moreover found for another SNP in *NFKBIL1*, rs2857605, and the total ALTs score as well as its module language impairment (in girls only). Both rs2857605 in *NFKBIL1* and rs4648022 in *NFKB1* showed a different distribution of genotypes in the case-control analysis (in all individuals combined).

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Table 2. Distribution of genotype frequencies above and below cut-off for clinical proxy for ASD shown in number (%)

Gene/SNP *Genotype*	Below cut-off for clinical proxy for ASD			Above cut-off for clinical proxy for ASD			***p*-value**		
NFKBIL1	All	Boys	Girls	All	Boys	Girls	All	Boys	Girls
rs2857605									
	N = 11 957	*n = 5972*	*n = 5985*	*n = 84*	*n = 66*	*n = 18*			
A:A	7161 (59.9%)	3593 (60.2%)	3568 (59.6%)	53 (63.1%)	41 (62.1%)	12 (66.7%)	**0.036**	0.108	–
G:A	4194 (35.1%)	2076 (34.8%)	2118 (35.4%)	23 (27.4%)	18 (27.3%)	5 (27.8%)			
G:G	602 (5.0%)	303 (5.0%)	299 (5.0%)	8 (9.5%)	7 (10.6%)	1 (5.5%)			
rs2239707									
	n = 11 918	*n = 5941*	*n = 5977*	*n = 85*	*n = 67*	*n = 18*			
A:A	4913 (41.2%)	2476 (41.7%)	2437 (40.8%)	38 (44.7%)	32 (47.8%)	6 (33.3%)	**0.0016***	**0.0006***	–
G:A	5548 (46.6%)	2764 (46.5%)	2784 (46.6%)	27 (31.8%)	17 (25.4%)	10 (55.6%)			
G:G	1457 (12.2%)	701 (11.8%)	756 (12.6%)	20 (23.5%)	18 (26.8%)	2 (11.1%)			
rs2230365									
	n = 11 964	*n = 5972*	*n = 5992*	*n = 84*	*n = 65*	*n = 19*			
C:C	8421 (70.4%)	4253 (71.2%)	4168 (69.5%)	59 (70.2%)	49 (75.4%)	10 (52.6%)	0.057	**0.010**	–
C:T	3258 (27.2%)	1588 (26.6%)	1670 (27.9%)	20 (23.8%)	11 (16.9%)	9 (47.4%)			
T:T	285 (2.4%)	131 (2.2%)	154 (2.6%)	5 (6.0%)	5 (7.7%)	0 (0.0%)			
rs2071592									
	n = 11 727	*n = 5850*	*n = 5877*	*n = 80*	*n = 63*	*n = 17*			
A:A	5228 (44.6%)	2526 (43.2%)	2702 (46.0%)	40 (50.0%)	30 (47.6%)	10 (58.8%)	–	–	–
T:A	5164 (44.0%)	2627 (44.9%)	2537 (43.1%)	27 (33.8%)	22 (34.9%)	5 (29.4%)			
T:T	1335 (11.4%)	697 (11.9%)	638 (10.9%)	13 (16.2%)	11 (17.5%)	2 (11.8%)			
NFKB1									
rs4648022									
	n = 11 962	*n = 5960*	*n = 6002*	*n = 86*	*n = 68*	*n = 18*			
C:C	10 067 (84.2%)	5008 (84.0%)	5059 (84.3%)	69 (80.2%)	53 (77.9%)	16 (88.9%)	**0.045**	0.058	–
T:C	1785 (14.9%)	893 (15.0%)	892 (14.9%)	14 (16.3%)	12 (17.7%)	2 (11.1%)			
T:T	110 (0.9%)	59 (1.0%)	51 (0.8%)	3 (3.5%)	3 (4.4%)	0 (0%)			

Significant *p*-values after correction for multiple testing are indicated by an asterisk (*). Missing *p*-values indicate that statistical analyses could not be performed (-).

However, none of these remained significant after applying Bonferroni correction. We found no associations with ALTs for rs2071592 in *NFKBIL1*.

Fig. 1 shows the LD plot of the four SNPs in *NFKBIL1* (rs2230365, rs2239707, rs2857605 and rs2071592).

Discussion

Two of the four investigated SNPs in *NFKBIL1* showed significant associations in the case-control analysis and with the ALT-module language impairment, respectively. Furthermore, the association between rs2230365 and ALTs replicates earlier findings by Anney *et al.* showing nominal association of this SNP with ASD (Anney *et al.*, 2010).

The amino acid sequence of the *NFKBIL1* protein has previously been shown to contain protein–protein interaction domains that most closely resemble those of the well-studied pro-inflammatory transcription factor NF-κB (Albertella & Campbell, 1994), and thus *NFKBIL1* has been suggested to be a member of the inhibitor of NF-κB family of proteins. However, *NFKBIL1* isoforms have been proposed to be incapable of binding to NF-κB but instead associate with mRNA and proteins that are involved in the translation of mRNA into protein, suggesting that it may be involved in mRNA processing or the regulation of translation (Greetham *et al.*, 2007). A study by An *et al.* demonstrated a role of *NFKBIL1* in the regulation of alternative splicing in viral influenza genes and human immune-related genes (An *et al.*, 2013). Whether or not *NFKBIL1* can directly interact with NF-κB, these functional studies support the idea of *NFKBIL1* as a regulator of gene expression and protein translation, which affects genes and pathways within the immune system. Furthermore, Chen *et al.* found *NFKBIL1* levels prior to infection to be significantly associated with hepatitis C virus clearance (Chen *et al.*, 2016), supporting a role of *NFKBIL1* in the innate immune response to viral infections. This is of interest, because a connection has been proposed between viral infections and ASD (Libbey *et al.*, 2005).

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Table 3. Association analysis between polymorphisms and ALTs, as well as the modules language impairment, social interaction impairment and restrictive/repetitive behaviour shown in means (SD)

Gene/SNP																
NFKBIL1		ALTs				Language impairment				**Social interaction impairment**				**Restrictive/repetitive behaviour**			
		A:A	**G:A**	**G:G**		**A:A**	**G:A**	**G:G**		**A:A**	**G:A**	**G:G**		**A:A**	**G:A**	**G:G**	
		(n = 7214)	*(n = 4217)*	*(n = 610)*	*p*-value	*(n = 7216)*	*(n = 4217)*	*(n = 609)*	*p*-value	*(n = 7208)*	*(n = 4214)*	*(n = 607)*	*p*-value	*(n = 7216)*	*(n = 4217)*	*(n = 610)*	*p*-value
rs2857605	*All*	0.71 (1.41)	0.67 (1.36)	0.73 (1.63)	0.311	0.24 (0.56)	0.22 (0.54)	0.24 (0.64)	0.246	0.25 (0.58)	0.23 (0.55)	0.25 (0.64)	0.181	0.22 (0.56)	0.22 (0.56)	0.24 (0.60)	0.778
	Boys	0.87 (1.64)	0.86 (1.59)	1.01 (2.02)	0.340	0.29 (0.62)	0.28 (0.63)	0.36 (0.79)	0.106	0.30 (0.66)	0.28 (0.62)	0.32 (0.77)	0.495	0.29 (0.65)	0.31 (0.67)	0.32 (0.73)	0.519
	Girls	0.55 (1.10)	0.49 (1.04)	0.44 (1.02)	**0.050**	0.19 (0.47)	0.16 (0.43)	0.11 (0.39)	**0.016**	0.21 (0.49)	0.18 (0.46)	0.18 (0.47)	0.094	0.16 (0.44)	0.14 (0.41)	0.15 (0.42)	0.343
		A:A	**G:A**	**G:G**		**A:A**	**G:A**	**G:G**		**A:A**	**G:A**	**G:G**		**A:A**	**G:A**	**G:G**	
		(n = 4951)	*(n = 5575)*	*(n = 1477)*		*(n = 4954)*	*(n = 5574)*	*(n = 1476)*		*(n = 4949)*	*(n = 5569)*	*(n = 1473)*		*(n = 4953)*	*(n = 5575)*	*(n = 1477)*	
rs2239707	*All*	0.71 (1.42)	0.68 (1.32)	0.75 (1.63)	0.148	0.23 (0.55)	0.23 (0.53)	0.25 (0.65)	0.410	0.26 (0.60)	0.23 (0.53)	0.26 (0.65)	**0.037**	0.23 (0.57)	0.22 (0.55)	0.24 (0.60)	0.595
	Boys	0.90 (1.69)	0.84 (1.50)	1.01 (2.03)	0.113	0.28 (0.63)	0.28 (0.58)	0.36 (0.81)	**0.013**	0.31 (0.69)	0.28 (0.58)	0.32 (0.78)	0.141	0.30 (0.67)	0.29 (0.64)	0.32 (0.72)	0.549
	Girls	0.53 (1.05)	0.52 (1.10)	0.51 (1.08)	0.422	0.18 (0.45)	0.18 (0.47)	0.15 (0.41)	0.208	0.20 (0.48)	0.19 (0.47)	0.21 (0.49)	0.271	0.15 (0.43)	0.15 (0.43)	0.16 (0.44)	0.873
		C:C	**C:T**	**T:T**		**C:C**	**C:T**	**T:T**		**C:C**	**C:T**	**T:T**		**C:C**	**C:T**	**T:T**	
		(n = 8480)	*(n = 3278)*	*(n = 290)*		*(n = 8482)*	*(n = 3277)*	*(n = 290)*		*(n = 8474)*	*(n = 3272)*	*(n = 290)*		*(n = 8482)*	*(n = 3278)*	*(n = 290)*	
rs2230365	*All*	0.70 (1.40)	0.69 (1.38)	0.94 (1.73)	**0.028**	0.23 (0.55)	0.23 (0.56)	0.33 (0.71)	**0.034**	0.25 (0.58)	0.24 (0.56)	0.33 (0.68)	**0.026**	0.23 (0.57)	0.21 (0.54)	0.27 (0.67)	0.170
	Boys	0.90 (1.66)	0.80 (1.53)	1.29 (2.13)	**0.009**	0.29 (0.63)	0.27 (0.62)	0.51 (0.90)	**0.0009***	0.30 (0.66)	0.26 (0.60)	0.40 (0.80)	0.057	0.31 (0.68)	0.26 (0.60)	0.38 (0.78)	**0.030**
	Girls	0.50 (1.03)	0.58 (1.21)	0.63 (1.20)	0.377	0.17 (0.44)	0.19 (0.50)	0.17 (0.41)	0.276	0.19 (0.47)	0.21 (0.52)	0.28 (0.56)	0.143	0.14 (0.42)	0.17 (0.47)	0.19 (0.54)	0.332
		A:A	**T:A**	**T:T**		**A:A**	**T:A**	**T:T**		**A:A**	**T:A**	**T:T**		**A:A**	**T:A**	**T:T**	
		(n = 5268)	*(n = 5191)*	*(n = 1348)*		*(n = 5269)*	*(n = 5190)*	*(n = 1349)*		*(n = 5260)*	*(n = 5187)*	*(n = 1348)*		*(n = 5270)*	*(n = 5191)*	*(n = 1348)*	
rs2071592	*All*	0.70 (1.40)	0.68 (1.34)	0.73 (1.50)	0.650	0.23 (0.55)	0.23 (0.54)	0.24 (0.56)	0.629	0.25 (0.57)	0.23 (0.54)	0.27 (0.63)	0.183	0.23 (0.57)	0.22 (0.55)	0.21 (0.57)	0.960
	Boys	0.88 (1.64)	0.85 (1.57)	0.92 (1.78)	0.757	0.29 (0.64)	0.28 (0.61)	0.30 (0.66)	0.618	0.29 (0.65)	0.28 (0.62)	0.33 (0.74)	0.364	0.30 (0.68)	0.29 (0.64)	0.28 (0.67)	0.913
	Girls	0.53 (1.11)	0.50 (1.02)	0.52 (1.06)	0.617	0.17 (0.44)	0.17 (0.46)	0.18 (0.43)	0.924	0.20 (0.49)	0.18 (0.45)	0.21 (0.49)	0.343	0.15 (0.44)	0.14 (0.42)	0.14 (0.42)	0.855
NFKB1																	
		C:C	**T:C**	**T:T**		**C:C**	**T:C**	**T:T**		**C:C**	**T:C**	**T:T**		**C:C**	**T:C**	**T:T**	
		(n = 10 136)	*(n = 1799)*	*(n = 113)*		*(n = 10 136)*	*(n = 1800)*	*(n = 113)*		*(n = 10 127)*	*(n = 1796)*	*(n = 113)*		*(n = 10137)*	*(n = 1800)*	*(n = 113)*	
rs4648022	*All*	0.70 (1.40)	0.71 (1.42)	0.96 (1.90)	0.213	0.23 (0.55)	0.24 (0.58)	0.30 (0.75)	0.590	0.25 (0.58)	0.24 (0.57)	0.33 (0.67)	0.324	0.22 (0.56)	0.22 (0.57)	0.33 (0.69)	0.112
	Boys	0.88 (1.64)	0.89 (1.70)	1.15 (2.15)	0.567	0.29 (0.63)	0.30 (0.70)	0.31 (0.78)	0.768	0.30 (0.66)	0.28 (0.65)	0.40 (0.81)	0.409	0.29 (0.66)	0.30 (0.68)	0.44 (0.77)	0.328
	Girls	0.53 (1.09)	0.53 (1.05)	0.73 (1.53)	0.322	0.17 (0.46)	0.18 (0.43)	0.29 (0.73)	0.301	0.20 (0.48)	0.20 (0.48)	0.24 (0.44)	0.480	0.15 (0.43)	0.14 (0.41)	0.20 (0.55)	0.324

Significant *p*-values after correction for multiple testing are indicated by an asterisk (*).

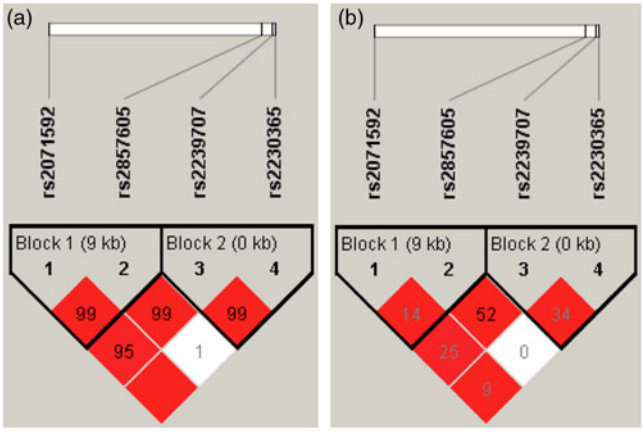


Fig. 1. The linkage disequilibrium between the four SNPs in *NFKBIL1*. Both *D'* (a) measure and r^2 (b) measure are shown.

Our results concerning rs2230365 and ALTs, replicating earlier findings in an independent cohort (Anney *et al.*, 2010), suggest a possible role of rs2230365 in ASD. However, a more recent meta-analysis including further cohorts did not replicate this association (The Autism Spectrum Disorders Working Group of the Psychiatric Genomics Consortium, 2017). Apart from the obvious advantages of meta-analysis, there are also limitations to this method; considering the heterogeneity of pooled data, selection biases and the wide array of methods that can be used, meta-analysis data require careful interpretation and should be used with caution (Zeggini & Ioannidis, 2009). Also, one should take into consideration that the development of ASD is a multifactorial process with genetic factors causing a vulnerability for ASD and environmental factors such as infection increasing the incidence of this disorder, via for example gene–environment interactions (Nardone & Elliott, 2016). It is therefore possible that individuals with the same genetic background develop differently due to divergent activation of both their own and their mothers' immune systems, leading to not only inter-individual but also inter-population variation in the severity of phenotype. Nevertheless, another very recent study conducted in a large sample of individuals with ASD ($n = 18\ 381$) and controls ($n = 27\ 969$) also saw no genome-wide significant associations between SNPs in *NFKBIL1* and ASD (Grove *et al.*, 2019), and our findings should be interpreted with caution.

Case-control analysis revealed a nominally significant association for rs2230365 in boys only, but not when including girls. Applying a mixed model analysis on the continuous measures of ALTs found the association between rs2230365 and ALTs to be nominally significant in boys and, albeit to a lesser extent, in all individuals, but not in girls, suggesting that this variant might contribute less to a phenotype in girls. A study investigating the effects of copy number variation and SNPs in probands with neurodevelopmental disorders as well as in an ASD cohort found that females carried significantly more neurodevelopmental-related genetic mutations than males with the same symptoms (Jacquemont *et al.*, 2014), suggesting an increased mutational burden and more resilience to genetic insults in females. Furthermore, a study investigating cytokine levels in children with ASD found significantly different cytokine responses between boys and girls (Masi *et al.*, 2017), further supporting the notion of a potential sex-specific biology, including immune functions, in the etiology of autism. These findings illustrate the importance of gender-stratification in autism research, and studies including larger female cohorts are required to better understand the influence of sex on neurodevelopmental disorders.

Several studies have shown associations between genetic variations in *NFKBIL1* and susceptibility to inflammatory or autoimmune diseases such as neonatal lupus (Clancy *et al.*, 2010) and systemic lupus erythematosus (Castiblanco & Anaya, 2008) (Table 1). A haplotype analysis of the SNPs rs2857605-rs2239707-rs2230365 in *NFKBIL1* furthermore showed associations with non-Hodgkins lymphoma (Wang *et al.*, 2009). The HLA region, where *NFKBIL1* is located, has not only been implicated in autoimmune diseases (Gough & Simmonds, 2007) but also in ASD (Anney *et al.*, 2010; Gesundheit *et al.*, 2013). Interestingly, autoimmune diseases have also been found to be more frequent in families with autistic children (Sweeten *et al.*, 2003), especially in mothers of autistic children (Comi *et al.*, 1999). Furthermore, the *NFKBIL1* SNP rs2857605 has been nominally associated with an increased risk for schizophrenia in a family-based replication study (Aberg *et al.*, 2013). Variations in *NFKBIL1* could be an early factor in the cascade of events leading from an underlying dysregulation of the immune system to neuropsychiatric disorders, via for example autoimmune mechanisms or response to infections.

Despite studies showing associations between rs2071592 in the promoter region of *NFKBIL1* and increased disease risk for, amongst others, systemic lupus erythematosus (Castiblanco & Anaya, 2008) suggesting functionality of this SNP, we saw no associations between rs2071592 and ALTs.

Different genetic influences may be of importance in various autistic endophenotypes and only a modest genetic overlap with respect to social and non-social ALTs have been reported (Ronald *et al.*, 2010). Considering autistic traits separately may be fruitful in order to elucidate how neuropsychiatric symptoms develop. Both SNPs that were associated with total ALTs, rs2857605 and rs2230365 in *NFKBIL1*, were found to be associated with the core symptom language impairment. This is of interest, as language impairment has been associated with HLA alleles previously in a case-control study (Nudel *et al.*, 2014), suggesting a role of HLA loci in language disorders. Furthermore, the HLA region has been implicated with dyslexia (Cardon *et al.*, 1994).

With respect to the other gene investigated in this study, coding for a subunit in NF-κB, our case-control analysis revealed a different distribution of genotypes in the *NFKB1* SNP rs4648022 that did, however, not survive correction for multiple testing.

Apart from its role in immunity, NF-κB is suggested to also regulate apoptosis and neurogenesis and to be involved in synaptic plasticity and memory (Malik *et al.*, 2011; Crampton & O'Keeffe, 2013; Snow *et al.*, 2014), making it a regulatory molecule not only for immune functions but also for neurodevelopment. The NF-κB family consists of several structurally related proteins that form homo- and heterodimers with each other. One of the subunits is the DNA-binding p50, originating from *NFKB1*. Associations have been reported between the *NFKB1* SNP rs4648022 and non-Hodgkins lymphoma, a blood cancer closely associated with immune function (Cerhan *et al.*, 2008). Although only nominally significant, our finding regarding rs4648022 supports previous findings on a potential role of NF-κB in autism; one of these studies reported elevated NF-κB levels in blood immune cells and significantly increased DNA binding activity of NF-κB in autistic children (Naik *et al.*, 2011). Aberrant levels and functionality of a key transcription factor such as NF-κB, as seen in individuals diagnosed with ASD, may cause changes in innate immunity, possibly leading to a dysfunctional neuroimmune environment affecting

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neurodevelopment. Even though one study found no differences in cerebellar expression and cerebellar and frontal cortex activity of NF-κB in autistic individuals (Malik *et al.*, 2011), the lack of significant results in that study might be due to low expression levels of NF-κB, not detectable with the used method (Western blot). Another study from the same group regarding frontal cortex tissue of autistic subjects showed altered expression of genes that are regulated by NF-κB (Sheikh *et al.*, 2010). An expression profiling study of the superior temporal gyrus of autistic subjects revealed an increase in transcription levels of several immune system-related genes as well as genes that are involved in cell communication, differentiation and apoptosis, including 22 genes within the NF-κB pathway (Garbett *et al.*, 2008). Moreover, a whole genome transcriptomic analysis of brains of individuals with ASD found significant differences in candidate pathways of immune regulation and alternative splicing regulation, suggesting that the observed immune upregulation may be related to abnormal ongoing plasticity in the brain of ASD patients (Voineagu *et al.*, 2013). Associations between polymorphisms in *NFKB1* and bipolar disorder have been reported in a susceptibility loci replication study (Gonzalez *et al.*, 2016), further supporting involvement of NF-κB pathways in neuropsychiatric disorders.

The ALTs-associated SNP rs2230365 in *NFKBIL1* is synonymous and rs4648022 in *NFKB1* as well as rs2857605 and rs2239707 in *NFKBIL1* are located in intronic regions, which makes their functional significance uncertain. However, a meta-analysis of 14 independent ASD cohorts identified an intronic marker to be the only genome-wide significant risk locus (The Autism Spectrum Disorders Working Group of the Psychiatric Genomics Consortium, 2017). Although intronic, this marker lies in a region that overlaps several genes, including a transcription factor playing a role in neuronal differentiation. Furthermore, another meta-analysis study surveying over 2000 publications found that synonymous SNPs, which cause no change to the encoded amino acid, are as likely to be involved in disease mechanisms. Indeed, synonymous and non-synonymous SNPs share similar likelihood and effect size for disease association (Chen *et al.*, 2010). Studies have furthermore demonstrated that synonymous SNPs can affect mRNA splicing, stability and structure (Hunt *et al.*, 2009) as well as protein folding and subsequent changes in substrate specificity (Kimchi-Sarfaty *et al.*, 2007). Regarding LD and the high *D*' values in the LD plot of the four SNPs in *NFKBIL1* (Fig. 1), the moderate r^2 values motivated investigation of all SNPs. It is furthermore unclear whether our findings reflect indirect association; the SNPs may be in LD with another, unidentified functional variant in the vicinity. However, this does not exclude effects of these SNPs on the expression of *NFKBIL1* via mechanisms such as alternative splicing or binding of non-coding RNAs (Cooper, 2010).

The limitations of the present study concern the data collection by means of parental reports, the non-clinical diagnostics applied and the limited number of subjects, especially girls, presenting ALTs scores above the cut-off for a proxy for ASD diagnosis. Although all statistical analyses were adjusted for twins, problem behaviour may be under-reported in the co-twin of individuals perceived by the parent as having more severe emotional–behavioural problems, and response style could vary between parents where some admit a larger amount of problems, and others deny them throughout. However, this should be compensated for considering the size of this study. Since our study was not conducted in a patient versus control cohort, but a sample representing the general population with ALTs measured using a continuous scale, the groups with high ALTs scores were often very small, especially when looking at girls separately. It was, thus, not possible to perform case-control analyses for girls. Further studies of *NFKB1* and *NFKBIL1* in relation to ASD in a patient-control population are therefore highly warranted. It should furthermore be noted that the general utility of candidate gene studies has been questioned, as it has been difficult to replicate findings from most studies; a recent study investigating the effect of commonly published candidate genes in depression found no significant association between these genes and depression phenotypes (Border *et al.*, 2019), questioning the validity of findings from candidate genes studies. However, one should take into account that although both autism and depression are very heterogenous in phenotypes and in their genetic architecture, autism has one of the highest heritability estimates among neuropsychiatric disorders (Sullivan & Geschwind, 2019), and while depression certainly has a strong genetic component, epigenetic mechanisms influenced by, for example, stress and childhood adversity seem to play a highly important role (Shadrina *et al.*, 2018). Furthermore, while genome-wide association studies examine associations at thousands of common variants across the genome, with no underlying hypothesis as to what kind of genes or pathways might be involved, candidate studies allow for a hypothesis-driven investigation of endogenous molecules. Considering the intricate and complex relationship between the immune system and brain functions, it is not always evident if immune system dysregulation is causing a phenotype or caused by it, and the advantage of the candidate gene approach is that possible associations between the genetic code and a phenotype can be established. Another frequent point of criticism regarding candidate gene studies is the small sample sizes frequently employed, and that many of these studies have likely been severely underpowered, contributing to a false positive discovery rate, for example as discussed by Border *et al.* (2019). However, this concern seems unfounded in the light of the size of this study.

In conclusion, we have shown that genetic variation in *NFKBIL1* may possibly contribute to ASD susceptibility, perhaps via for example dysfunctional effects on brain development directly, an altered inflammatory response to external stimuli and/or autoimmune mechanisms leading to neurodevelopmental impairments. However, although some of our results in *NFKBIL1* replicate earlier findings, other studies showed no associations between variants in this gene and ASD, and the present genetic data needs to be confirmed in future studies.

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References

Aberg, KA, **Liu, Y**, **Bukszár, J**, **McClay, JL**, **Khachane, AN**, **Andreassen, OA**, **Blackwood, D**, **Corvin, A**, **Djurovic, S**, **Gurling, H**, **Ophoff, R**, **Pato, CN**, **Pato, MT**, **Riley, B**, **Webb, T**, **Kendler, K**, **O'Donovan, M**, **Craddock, N**, **Kirov, G**, **Owen, M**, **Rujescu, D**, **St Clair, D**, **Werge, T**, **Hultman, CM**, **Delisi, LE**, **Sullivan, P** and **Van Den Oord, EJ** (2013) A comprehensive family-based replication study of schizophrenia genes. *JAMA Psychiatry* 70(6), 573–581.

Albertella MR and **Campbell RD** (1994) Characterization of a novel gene in the human major histocompatibility complex that encodes a potential new member of the I kappa B family of proteins. *Human Molecular Genetics* 3(5), 793–799.

Al-Haddad, BJS, **Jacobsson, B**, **Chabra, S**, **Modzelewska, D**, **Olson, EM**, **Bernier, R**, **Enquobahrie, DA**, **Hagberg, H**, **Östling, S**, **Rajagopal, L**, **Adams Waldorf, KM** and **Sengpiel, V** (2019) Long-term risk of neuropsychiatric disease after exposure to infection in utero. *JAMA Psychiatry* 91985, 1–9.

American Psychiatric Association. Diagnostic and statistical manual of mental disorders 4th Edn (DSM-IV). Washington, DC: American Psychiatric Association; 1994.

An J, **Nakajima T**, **Shibata H**, **Arimura T**, **Yasunami M** and **Kimura A** (2013) A novel link of HLA locus to the regulation of immunity and infection: NFKBIL1 regulates alternative splicing of human immune-related genes and influenza virus M gene. *Journal of Autoimmunity* 47, 25–33.

Anckarsäter, H, **Lundström, S**, **Kollberg, L**, **Kerekes, N**, **Palm, C**, **Carlström, E**, **Långström, N**, **Magnusson, PKE**, **Halldner, L**, **Bölte, S**, **Gillberg, C**, **Gumpert, C**, **Råstam, M** and **Lichtenstein, P** (2011) The child and adolescent twin study in Sweden (CATSS). *Twin Research and Human Genetics* 14(6), 495–508.

Anney, R, **Klei, L**, **Pinto, D**, **Regan, R**, **Conroy, J**, **Magalhaes, TR**, **Correia, C**, **Abrahams, BS**, **Sykes, N**, **Pagnamenta, AT**, **Almeida, J**, **Bacchelli, E**, **Bailey, AJ**, **Baird, G**, **Battaglia, A**, **Berney, T**, **Bolshakova, N**, **Bölte, S**, **Bolton, PF**, **Bourgeron, T**, **Brennan, S**, **Brian, J**, **Carson, AR**, **Casallo, G**, **Casey, J**, **Chu, SH**, **Cochrane, L**, **Corsello, C**, **Crawford, EL**, **Crossett, A**, **Dawson, G**, **de Jonge, M**, **Delorme, R**, **Drmic, I**, **Duketis, E**, **Duque, F**, **Estes, A**, **Farrar, P**, **Fernandez, BA**, **Folstein, SE**, **Fombonne, E**, **Freitag, CM**, **Gilbert, J**, **Gillberg, C**, **Glessner, JT**, **Goldberg, J**, **Green, J**, **Guter, SJ**, **Hakonarson, H**, **Heron, EA**, **Hill, M**, **Holt, R**, **Howe, JL**, **Hughes, G**, **Hus, V**, **Igliozzi, R**, **Kim, C**, **Klauck, SM**, **Kolevzon, A**, **Korvatska, O**, **Kustanovich, V**, **Lajonchere, CM**, **Lamb, JA**, **Laskawiec, M**, **Leboyer, M**, **Le Couteur, A**, **Leventhal, BL**, **Lionel, AC**, **Liu, XQ**, **Lord, C**, **Lotspeich, L**, **Lund, SC**, **Maestrini, E**, **Mahoney, W**, **Mantoulan, C**, **Marshall, CR**, **McConachie, H**, **McDougle, CJ**, **McGrath, J**, **McMahon, WM**, **Melhem, NM**, **Merikangas, A**, **Migita, O**, **Minshew, NJ**, **Mirza, GK**, **Munson, J**, **Nelson, SF**, **Noakes, C**, **Noor, A**, **Nygren, G**, **Oliveira, G**, **Papanikolaou, K**, **Parr, JR**, **Parrini, B**, **Paton, T**, **Pickles, A**, **Piven, J**, **Posey, DJ**, **Poustka, A**, **Poustka, F**, **Prasad, A**, **Ragoussis, J**, **Renshaw, K**, **Rickaby, J**, **Roberts, W**, **Roeder, K**, **Roge, B**, **Rutter, ML**, **Bierut, LJ**, **Rice, JP**, **Salt, J**, **Sansom, K**, **Sato, D**, **Segurado, R**, **Senman, L**, **Shah, N**, **Sheffield, VC**, **Soorya, L**, **Sousa, I**, **Stoppioni, V**, **Strawbridge, C**, **Tancredi, R**, **Tansey, K**, **Thiruvahindrapduram, B**, **Thompson, AP**, **Thomson, S**, **Tryfon, A**, **Tsiantis, J**, **van Engeland, H**, **Vincent, JB**, **Volkmar, F**, **Wallace, S**, **Wang, K**, **Wang, Z**, **Wassink, TH**, **Wing, K**, **Wittemeyer, K**, **Wood, S**, **Yaspan, BL**, **Zurawiecki, D**, **Zwaigenbaum, L**, **Betancur, C**, **Buxbaum, JD**, **Cantor, RM**, **Cook, EH**, **Coon, H**, **Cuccaro, ML**, **Gallagher, L**, **Geschwind, DH**, **Gill, M**, **Haines, JL**, **Miller, J**, **Monaco, AP**, **Nurnberger, JI**, **Paterson, AD**, **Pericak-Vance, MA**, **Schellenberg, GD**, **Scherer, SW**, **Sutcliffe, JS**, **Szatmari, P**, **Vicente, AM**, **Vieland, VJ**, **Wijsman, EM**, **Devlin, B**, **Ennis, S** and **Hallmayer, J** (2010) A genome-wide scan for common alleles affecting risk for autism. *Human Molecular Genetics* 19(20), 4072–4082.

Ashwood P, **Krakowiak P**, **Hertz-Picciotto I**, **Hansen R**, **Pessah IN** and **Van de Water J** (2011a) Altered T cell responses in children with autism. *Brain, Behavior, and Immunity* 25(5), 840–849.

Ashwood P, **Krakowiak P**, **Hertz-Picciotto I**, **Hansen R**, **Pessah IN** and **Van de Water J** (2011b) Associations of impaired behaviors with elevated plasma chemokines in autism spectrum disorders. *Journal of Neuroimmunology* 232(1–2), 196–199.

Atladottir HO, **Henriksen TB**, **Schendel DE** and **Parner ET** (2012) Autism after infection, febrile episodes, and antibiotic use during pregnancy: an exploratory study. *Pediatrics* 130(6), e1447–e1454.

Barrett JC, **Fry B**, **Maller J** and **Daly MJ** (2005) Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21(2), 263–265.

Boodhoo, A, **Wong, AM-L**, **Williamson, D**, **Voon, D**, **Lee, S**, **Allcock, RJN** and **Price, P** (2004) A promotor polymorphism in the central MHC gene, IKBL, influences the binding of transcription factors USF1 and E47 on disease-associated haplotypes. *Gene Expression* 12, 1–11.

Border, R, **Johnson, EC**, **Evans, LM**, **Smolen, A**, **Berley, N**, **Sullivan, PF** and **Keller, MC** (2019) No support for historical candidate gene or candidate gene-by-interaction hypotheses for major depression across multiple large samples. *The American Journal of Psychiatry* (July): 1–12. doi: 10.1176/appi.ajp.2018.18070881

Boulanger LM (2009) Immune proteins in brain development and synaptic plasticity. *Neuron* 64(1), 93–109.

Bralten, J, **Van Hulzen, KJ**, **Martens, MB**, **Galesloot, TE**, **Arias Vasquez, A**, **Kiemeney, LA**, **Buitelaar, JK**, **Muntjewerff, JW**, **Franke, B** and **Poelmans, G** (2018) Autism spectrum disorders and autistic traits share genetics and biology. *Molecular Psychiatry* 23(5), 1205–1212.

Braunschweig, D, **Krakowiak, P**, **Duncanson, P**, **Boyce, R**, **Hansen, RL**, **Ashwood, P**, **Hertz-Picciotto, I**, **Pessah, IN** and **Van De Water, J** (2013) Autism-specific maternal autoantibodies recognize critical proteins in developing brain. *Translational Psychiatry*. 3(7), e277–e279.

Brown AS, **Sourander A**, **Hinkka-Yli-Salomäki S**, **McKeague IW**, **Sundvall J** and **Surcel HM** (2014) Elevated maternal C-reactive protein and autism in a national birth cohort. *Molecular Psychiatry* 19(2), 259–264.

Cardon LR, **Smith SD**, **Fulker DW**, **Kimberling WJ**, **Pennington BF** and **DeFries JC** (1994) Quantitative trait locus for reading disability on chromosome 6. *Science* 266(5183), 276–279.

Castiblanco J and **Anaya J-M** (2008) The IkappaBL gene polymorphism influences risk of acquiring systemic lupus erythematosus and Sjögren's syndrome. *Human Immunology* 69(1), 45–51.

Cerhan, JR, **Liu-Mares, W**, **Fredericksen, ZS**, **Novak, AJ**, **Cunningham, JM**, **Kay, NE**, **Dogan, A**, **Liebow, M**, **Wang, AH**, **Call, TG**, **Habermann, TM**, **Ansell, SM** and **Slager, SL** (2008) Genetic variation in tumor necrosis factor and the nuclear factor-κB canonical pathway and risk of non-Hodgkin's lymphoma. *Cancer Epidemiology, Biomarkers & Prevention* 17(11), 3161–3169.

Chen R, **Davydov EV**, **Sirsota M** and **Butte AJ** (2010) Non-synonymous and synonymous coding SNPS show similar likelihood and effect size of human disease association. *PLoS One* 5(10), 1–6.

Chen, Y, **Shen, C**, **Guha, D**, **Ding, M**, **Kulich, S**, **Ashimkhanova, A**, **Rinaldo, C**, **Seaberg, E**, **Margolick, JB**, **Stosor, V**, **Martï¿½nez-Maza, O** and **Gupta, P** (2016) Identification of the transcripts associated with spontaneous HCV clearance in individuals co-infected with HIV and HCV. *BMC Infectious Diseases* 16(1), 1–12.

Chess S (1971) Autism in children with congenital rubella. *Journal of Autism and Childhood Schizophrenia* 1(1), 33–47.

Clancy, RM, **Marion, MC**, **Kaufman, KM**, **Ramos, PS**, **Adler, A**, **Harley, JB**, **Langefeld, CD** and **Buyon, JP** (2010) Identification of candidate loci at 6p21 and 21q22 in a genome-wide association study of cardiac manifestations of neonatal lupus. *Arthritis & Rheumatology* 62(11), 3415–3424.

Comi AM, **Zimmerman AW**, **Frye VH**, **Law PA** and **Peeden JN** (1999) Familial clustering of autoimmune disorders and evaluation of medical risk factors in autism. *Journal of Child Neurology* 14(6), 388–394.

Cooper DN (2010) Functional intronic polymorphisms: buried treasure awaiting discovery within our genes. *Human Genomics* 4(5), 284–288.

Courchesne, E, **Mouton, PR**, **Calhoun, ME**, **Semendeferi, K**, **Ahrens-Barbeau, C**, **Hallet, MJ**, **Barnes, CC** and **Pierce, K** (2011) Neuron number and size in prefrontal cortex of children with autism. *The Journal of the American Medical Association* 306(18), 2001–2010.

Crampton SJ and **O'Keeffe GW** (2013) NF-κB: emerging roles in hippocampal development and function. *The International Journal of Biochemistry & Cell Biology* 45(8), 1821–1824.

Croonenberghs J, **Deboutte D** and **Maes M** (2002) Activation of the inflammatory response system in autism. *Neuropsychobiology* 45, 1–6.

https://doi.org/10.1017/neu.2019.18 Published online by Cambridge University Press

Enstrom, AM, **Lit, L**, **Onore, CE**, **Gregg, JP**, **Hansen, RL**, **Pessah, IN**, **Hertz-Picciotto, I**, **Van de Water, JA**, **Sharp, FR** and **Ashwood, P** (2009) Altered gene expression and function of peripheral blood natural killer cells in children with autism. *Brain, Behavior, and Immunity* 23(1), 124–133.

Fernando, MMA, **Stevens, CR**, **Walsh, EC**, **De Jager, PL**, **Goyette, P**, **Plenge, RM**, **Vyse, TJ** and **Rioux, JD** (2008) Defining the role of the MHC in autoimmunity: a review and pooled analysis. *PLoS Genetics* 4(4), e1000024. doi: 10.1371/journal.pgen.1000024

Garbett, K, **Ebert, PJ**, **Mitchell, A**, **Lintas, C**, **Manzi, B**, **Mirnics, K** and **Persico, AM** (2008) Immune transcriptome alterations in the temporal cortex of subjects with autism. *Neurobiology of Disease* 30(3), 303–311.

Gesundheit, B, **Rosenzweig, JP**, **Naor, D**, **Lerer, B**, **Zachor, DA**, **Procházka, V**, **Melamed, M**, **Kristt, DA**, **Steinberg, A**, **Shulman, C**, **Hwang, P**, **Koren, G**, **Walfisch, A**, **Passweg, JR**, **Snowden, JA**, **Tamouza, R**, **Leboyer, M**, **Farge-Bancel, D** and **Ashwood, P** (2013) Immunological and autoimmune considerations of autism spectrum disorders. *Journal of Autoimmunity* 44, 1–7.

Gonzalez, S, **Gupta, J**, **Villa, E**, **Mallawaarachchi, I**, **Rodriguez, M**, **Ramirez, M**, **Zavala, J**, **Armas, R**, **Dassori, A**, **Contreras, J**, **Flores, D**, **Jerez, A**, **Ontiveros, A**, **Nicolini, H** and **Escamilla, M** (2016) Replication of genome-wide association study (GWAS) susceptibility loci in a Latino bipolar disorder cohort. *Bipolar Disorder* 18(6), 520–527.

Gough SCL and **Simmonds MJ** (2007) The HLA region and autoimmune disease: associations and mechanisms of action. *Current Genomics* 8(7), 453–465.

Greetham, D, **Ellis, CD**, **Mewar, D**, **Fearon, U**, **an Ultaigh, SN**, **Veale, DJ**, **Guesdon, F** and **Wilson, AG** (2007) Functional characterization of NF-kappaB inhibitor-like protein 1 (NFkappaBIL1), a candidate susceptibility gene for rheumatoid arthritis. *Human Molecular Genetics* 16(24), 3027–3036.

Grove, J, **Ripke, S**, **Als, TD**, **Mattheisen, M**, **Walters, RK**, **Won, H**, **Pallesen, J**, **Agerbo, E**, **Andreassen, OA**, **Anney, R**, **Awashti, S**, **Belliveau, R**, **Bettella, F**, **Buxbaum, JD**, **Bybjerg-Grauholm, J**, **Bækvad-Hansen, M**, **Cerrato, F**, **Dellenvall, K**, **Chambert, K**, **Christensen, JH**, **Churchhouse, C**, **Demontis, D**, **De Rubeis, S**, **Devlin, B**, **Djurovic, S**, **Dumont, AL**, **Goldstein, JI**, **Hansen, CS**, **Hauberg, ME**, **Hollegaard, MV**, **Hope, S**, **Howrigan, DP**, **Huang, H**, **Hultman, CM**, **Klei, L**, **Maller, J**, **Martin, J**, **Martin, AR**, **Moran, JL**, **Nyegaard, M**, **Nærland, T**, **Palmer, DS**, **Palotie, A**, **Pedersen, CB**, **Pedersen, MG**, **DPoterba, T**, **Poulsen, JB**, **Pourcain, BS**, **Reichert, J**, **Qvist, P**, **Rehnström, K**, **Reichenberg, A**, **Robinson, EB**, **Roeder, K**, **Roussos, P**, **Saemundsen, E**, **Sandin, S**, **Satterstrom, FK**, **Smith, GD**, **Stefansson, H**, **Steinberg, S**, **Stevens6, CR**, **Sullivan, PF**, **Turley, P**, **Walters, GB**, **Xu, X**, Consortium ASDW.G. of the P.G., BUPGEN, Consortium MDDW.G. of the P.G., Team MR, **Stefansson, K**, **Geschwind, DH**, **Nordentoft, M**, **Hougaard, DM**, **Werge, T**, **Mors, O**, **Mortensen, PB**, **Neale, BM**, **Daly, MJ** and **Børglum, AD** (2019) Identification of common genetic risk variants for autism spectrum disorder. *Nature Genetics* 51, 431–444.

Guerini, FR, **Bolognesi, E**, **Chiappedi, M**, **De Silvestri, A**, **Ghezzo, A**, **Zanette, M**, **Rusconi, B**, **Manca, S**, **Sotgiu, S**, **Agliardi, C** and **Clerici, M** (2011) HLA polymorphisms in Italian children with autism spectrum disorders: results of a family based linkage study. *Journal of Neuroimmunology* 230(1–2), 135–142.

Gutierrez H (2005) NF- B signalling regulates the growth of neural processes in the developing PNS and CNS. *Development* 132(7), 1713–1726.

Hannelius, U, **Gherman, L**, **Makela, VV**, **Lindstedt, A**, **Zucchelli, M**, **Lagerberg, C**, **Tybring, G**, **Kere, J** and **Lindgren, CM** (2007) Large-scale zygosity testing using single nucleotide polymorphisms. *Twin Research and Human Genetics* 10(4), 604–625.

Hansson SL, **Röjvall AS**, **Rastam M**, **Gillberg C**, **Gillberg C** and **Anckarsäter H** (2005) Psychiatric telephone interview with parents for screening of childhood autism - Tics, attention-deficit hyperactivity disorder and other comorbidities (A-TAC): preliminary reliability and validity. *British Journal of Psychiatry* 187(September), 262–267.

Hunt R, **Sauna ZE**, **Ambudkar SV**, **Gottesman MM** and **Kimchi-Sarfaty C** (2009) Silent (Synonymous) SNPs: should we care about them? *Methods in Molecular Biology* 578(1), 23–39.

Hutton J (2016) Does rubella cause autism: a 2015 reappraisal? *Frontiers in Human Neuroscience* 10(February), 1–15.

Jacquemont, S, **Coe, BP**, **Hersch, M**, **Duyzend, MH**, **Krumm, N**, **Bergmann, S**, **Beckmann, JS**, **Rosenfeld, JA** and **Eichler, EE** (2014) A higher mutational burden in females supports a "female protective model" in neurodevelopmental disorders. *American Journal of Human Genetics* 94(3), 415–425.

Kimchi-Sarfaty, C, **Oh, JM**, **Kim, IW**, **Sauna, ZE**, **Calcagno, AM**, **Ambudkar, SV** and **Gottesman, MM** (2007) A "silent" polymorphism in the MDR1 gene changes substrate specificity. *Science* 315(5811), 525–528.

Lai MC, **Lombardo MV** and **Baron-Cohen S** (2014) Autism. *Lancet* 383, 896–910.

Larson, T, **Anckarsäter, H**, **Gillberg, C**, **Ståhlberg, O**, **Carlström, E**, **Kadesjö, B**, **Råstam, M**, **Lichtenstein, P** and **Gillberg, C** (2010) The autism-tics, AD/HD and other comorbidities inventory (A-TAC): further validation of a telephone interview for epidemiological research. *BMC Psychiatry* 10, 1.

Lee, BK, **Magnusson, C**, **Gardner, RM**, **Blomström, Å**, **Newschaffer, CJ**, **Burstyn, I**, **Karlsson, H** and **Dalman, C** (2015) Maternal hospitalization with infection during pregnancy and risk of autism spectrum disorders. *Brain, Behavior, and Immunity* 44, 100–105.

Li, X, **Chauhan, A**, **Sheikh, AM**, **Patil, S**, **Chauhan, V**, **Li, XM**, **Ji, L**, **Brown, T** and **Malik, M** (2009) Elevated immune response in the brain of autistic patients. *Journal of Neuroimmunology* 207(1–2), 111–116.

Libbey JE, **Sweeten TL**, **McMahon WM** and **Fujinami RS** (2005) Autistic disorder and viral infections. *Journal of NeuroVirology* 11(1), 1–10.

Bralten, J, **Van Hulzen, KJ**, **Martens, MB**, **Galesloot, TE**, **Arias Vasquez, A**, **Kiemeney, LA**, **Buitelaar, JK**, **Muntjewerff, JW**, **Franke, B** and **Poelmans, G** (2012) Autism spectrum disorders and autisticlike traits: similar etiology in the extreme end and the normal variation. *Archives of General Psychiatry* 69(1), 46–52.

Malik, M, **Tauqeer, Z**, **Sheikh, AM**, **Wen, G**, **Nagori, A**, **Yang, K**, **Brown, WT** and **Li, X** (2011) NF-κB signaling in the brain of autistic subjects. *Mediators of Inflammation*, 2011, Article ID 785265, 10. doi: 10.1155/2011/785265.

Mårland, C, **Lichtenstein, P**, **Degl'Innocenti, A**, **Larson, T**, **Råstam, M**, **Anckarsäter, H**, **Gillberg, C**, **Nilsson, T** and **Lundström, S** (2017) The Autism-Tics, ADHD and other Comorbidities inventory (A-TAC): previous and predictive validity. *BMC Psychiatry* 17(1), 1–8.

Masi, A, **Breen, EJ**, **Alvares, GA**, **Glozier, N**, **Hickie, IB**, **Hunt, A**, **Hui, J**, **Beilby, J**, **Ravine, D**, **Wray, J**, **Whitehouse, AJO** and **Guastella, AJ** (2017) Cytokine levels and associations with symptom severity in male and female children with autism spectrum disorder. *Molecular Autism* 8(1), 1–11.

Meltzer A and **Van De Water J** (2017) The role of the immune system in autism spectrum disorder. *Neuropsychopharmacology* 42(1), 284–298.

Methot, L, **Hermann, R**, **Tang, Y**, **Lo, R**, **Al-Jehani, H**, **Jhas, S**, **Svoboda, D**, **Slack, RS**, **Barker, PA** and **Stifani, S** (2013) Interaction and antagonistic roles of NF-κB and Hes6 in the regulation of cortical neurogenesis. *Molecular and Cellular Biology* 33(14), 2797–2808.

Naik US, **Gangadharan C**, **Abbagani K**, **Nagalla B**, **Dasari N** and **Manna SK** (2011) A study of nuclear transcription factor-kappa B in childhood autism. *PLoS One* 6(5), 1–6.

Nardone S and **Elliott E** (2016) The interaction between the immune system and epigenetics in the etiology of autism spectrum disorders. *Frontiers in Neuroscience* 10(July), 1–9.

Nudel, R, **Simpson, NH**, **Baird, G**, **O'Hare, A**, **Conti-Ramsden, G**, **Bolton, PF**, **Hennessy, ER**, **Monaco, AP**, **Knight, JC**, **Winney, B**, **Fisher, SE**, **Newbury, DF**, **Slonims, V**, **Clark, A**, **Watson, J**, **Simonoff, E**, **Pickles, A**, **Everitt, A**, **Seckl, J**, **Cowie, H**, **Cohen, W**, **Nasir, J**, **Bishop, DVM** and **Simkin, Z** (2014) Associations of HLA alleles with specific language impairment. *Journal of Neurodevelopmental Disorders* 6(1), 1–9.

Nudel, R, **Simpson, NH**, **Baird, G**, **O'Hare, A**, **Conti-Ramsden, G**, **Bolton, PF**, **Hennessy, ER**, **Monaco, AP**, **Knight, JC**, **Winney, B**, **Fisher, SE**, **Newbury, DF**, **Slonims, V**, **Clark, A**, **Watson, J**, **Simonoff, E**, **Pickles, A**, **Everitt, A**, **Seckl, J**, **Cowie, H**, **Cohen, W**, **Nasir, J**, **Bishop, DVM** and **Simkin, Z** (2008) Variants in the promoter region of IKBL/NFKBIL1 gene may mark susceptibility to the development of chronic Chagas' cardiomyopathy among Trypanosoma cruzi-infected individuals. *Molecular Immunology* 45(1), 283–288.

Robinson, E, **Koenen, KC**, **Mccormick, MC**, **Munir, K**, **Hallett, V**, **Happé, F**, **Ronald, A**, **Plomin, R** and **Ronald, A** (2011) Evidence that autistic traits show the same etiology in the general population and at the quantitative

https://doi.org/10.1017/neu.2019.18 Published online by Cambridge University Press

extremes (5%, 2.5% and 1%). *Archives of General Psychiatry* 68(11), 1113–1121.

Robinson EB, **Lichtenstein P**, **Anckarsater H**, **Happe F** and **Ronald A** (2013) Examining and interpreting the female protective effect against autistic behavior. *Proceedings of the National Academy of Sciences of the United States of America* 110(13), 5258–5262.

Ronald, A, **Butcher, LM**, **Docherty, S**, **Davis, OSP**, **Schalkwyk, LC**, **Craig, IW** and **Plomin, R** (2010) 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. *Behavior Genetics* 40(1), 31–45.

Rossignol D, **Genuis S** and **Frye R** (2014) Environmental toxicants and autism spectrum disorders: a systematic review. *Translational Psychiatry* 4, e360. doi: 10.1038/tp.2014.4

Schieve, LA, **Tian, LH**, **Baio, J**, **Rankin, K**, **Rosenberg, D**, **Wiggins, L**, **Maenner, MJ**, **Yeargin-Allsopp, M**, **Durkin, M**, **Rice, C**, **King, L**, **Kirby, RS**, **Wingate, MS** and **Devine, O** (2014) Population attributable fractions for three perinatal risk factors for autism spectrum disorders, 2002 and 2008 autism and developmental disabilities monitoring network. *Annals of Epidemiology* 24(4), 260–266.

Shadrina M, **Bondarenko EA** and **Slominsky PA** (2018) Genetics factors in major depression disease. *Frontiers in Psychiatry* 9(July), 1–18.

Sheikh, AM, **Malik, M**, **Wen, G**, **Chauhan, A**, **Chauhan, V**, **Gong, CX**, **Liu, F**, **Brown, WT** and **Li, X** (2010) BDNF-Akt-Bcl2 antiapoptotic signaling pathway is compromised in the brain of autistic subjects. *Journal of Neuroscience Research* 88(12), 2641–2647.

Snow WM, **Stoesz BM**, **Kelly DM** and **Albensi BC** (2014) Roles for NF-κB and gene targets of NF-κB in synaptic plasticity, memory, and navigation. *Molecular Neurobiology* 49(2), 757–770.

Stubbs EG and **Magenis RE** (1980) HLA and autism. *Journal of Autism and Developmental Disorders* 10(1), 15–19.

Suchankova P, **Baghaei F**, **Rosmond R**, **Holm G**, **Anckarsäter H** and **Ekman A** (2011) Genetic variability within the S100B gene influences the personality trait self-directedness. *Psychoneuroendocrinology* 36(6), 919–923.

Suchankova P, **Henningsson S**, **Baghaei F**, **Rosmond R**, **Holm G** and **Ekman A** (2009) Genetic variability within the innate immune system influences personality traits in women. *Genes, Brain and Behavior* 8(2), 212–217.

Suchankova, P, **Klang, J**, **Cavanna, C**, **Holm, G**, **Nilsson, S**, **Jönsson, EG** and **Ekman, A** (2012) Is the Gly82Ser polymorphism in the RAGE gene relevant to schizophrenia and the personality trait psychoticism? *Journal of Psychiatry & Neuroscience* 37(2), 122–128.

Suchankova P, **Pettersson R**, **Nordenström K**, **Holm G** and **Ekman A** (2012) Personality traits and the R668Q polymorphism located in the MMP-9 gene. *Behavioural Brain Research* 228(1), 232–235.

Sullivan PF and **Geschwind DH** (2019) Defining the genetic, genomic, cellular, and diagnostic architectures of psychiatric disorders. *Cell* 177, 162–183.

Sweeten TL, **Bowyer SL**, **Posey DJ**, **Halberstadt GM** and **McDougle CJ** (2003) Increased prevalence of familial autoimmunity in probands with pervasive developmental disorders. *Pediatrics* 112(5), e420–e420.

The Autism Spectrum Disorders Working Group of The Psychiatric Genomics Consortium (2017) Meta-analysis of GWAS of over 16,000 individuals with autism spectrum disorder highlights a novel locus at 10q24.32 and a significant overlap with schizophrenia. *Molecular Autism* 8(21), 1–17.

Tick B, **Bolton P**, **Happé F**, **Rutter M** and **Rijsdijk F** (2016) Heritability of autism spectrum disorders: a meta-analysis of twin studies. *The Journal of Child Psychology and Psychiatry and Allied Disciplines* 57(5), 585–595.

Torres, AR, **Sweeten, TL**, **Johnson, RC**, **Odell, D**, **Westover, JB**, **Bray-Ward, P**, **Ward, DC**, **Davies, CJ**, **Thomas, AJ**, **Croen, LA** and **Benson, M** (2016) Common genetic variants found in HLA and KIR immune genes in autism spectrum disorder. *Frontiers in Neuroscience* 10(OCT), 1–13.

Torres AR, **Westover JB** and **Rosenspire AJ** (2012) HLA immune function genes in autism. *Autism Research and Treatment* 2012, 1–13.

Voineagu, I, **Wang, X**, **Johnston, P**, **Lowe, JK**, **Tian, Y**, **Mill, J**, **Cantor, R**, **Blencowe, BJ**, **Daniel, H**, **Horvath, S**, **Mill, J**, **Cantor, R**, **Blencowe, BJ** and **Geschwind, DH** (2013) Transcriptomic analysis of autistic brain reveals convergent molecular pathology. *Nature* 474(7351), 380–384.

Wang, SS, **Purdue, MP**, **Cerhan, JR**, **Zheng, T**, **Menashe, I**, **Armstrong, BK**, **Lan, Q**, **Hartge, P**, **Kricker, A**, **Zhang, Y**, **Morton, LM**, **Vajdic, CM**, **Holford, TR**, **Severson, RK**, **Grulich, A**, **Leaderer, BP**, **Davis, S**, **Cozen, W**, **Yeager, M**, **Chanock, SJ**, **Chatterjee, N** and **Rothman, N** (2009) Common gene variants in the Tumor Necrosis Factor (TNF) and TNF receptor superfamilies and NF-kB transcription factors and non-hodgkin lymphoma risk. *PLoS One* 4(4), e5360. doi: 10.1371/journal.pone.0005360

Werling DM (2016) The role of sex-differential biology in risk for autism spectrum disorder. *Biology of Sex Differences* 7(1), 1–18.

Woodbury-Smith M and **Scherer SW** (2018) Progress in the genetics of autism spectrum disorder. *Developmental Medicine & Child Neurology* 60(5), 445–451.

Young AMH, **Campbell E**, **Lynch S**, **Suckling J** and **Powis SJ** (2011) Aberrant NF-kappaB expression in autism spectrum condition: a mechanism for neuroinflammation. *Frontiers in Psychiatry* 2(MAY), 1–8.

Zeggini E and **Ioannidis JPA** (2009) Meta-analysis in genome-wide association studies. *Pharmacogenomics* 10(2), 191–201.

https://doi.org/10.1017/neu.2019.18 Published online by Cambridge University Press