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In search of genes associated with risk for psychopathic tendencies in children: a two-stage genome-wide association study of pooled DNA

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Background: Quantitative genetic data from our group indicates that antisocial behaviour (AB) is strongly heritable when coupled with psychopathic, callous-unemotional (CU) personality traits. We have also demonstrated that the genetic influences for AB and CU overlap considerably. We conducted a genome-wide association scan that capitalises on these findings in an attempt to identify quantitative trait loci (QTLs) that may increase risk for psychopathic tendencies (AB+/CU+). **Methods:** Teacher ratings at age 7 were used to screen 8374 twins with available DNA samples for individuals that were high vs. low on both AB and CU. In Stage 1, we screened for allele frequency differences in 642,432 autosomal single-nucleotide polymorphisms (SNPs) using the Affymetrix 6.0 GeneChip with pooled DNA for high-scoring (AB+/CU+) versus low-scoring children (N = ~300/group). In Stage 2, we tested the 3000 most strongly associated SNPs from Stage 1 for association in the same direction in a second sample of high- versus low-scoring children from the same twin study (18% co-twins). **Results:** Using allele frequencies estimated from pooled DNA, we found suggestive evidence for enrichment of association in the second stage of our two-stage genome-wide association design and focus on reporting the 30 top-ranking SNPs nominally associated with psychopathic tendencies. These SNPs include neurodevelopmental genes such as ROBO2. **Conclusions:** Although none of the SNPs reached genome-wide statistical significance we have generated a list of SNPs that are potentially associated with psychopathic tendencies, which we believe warrant verification and replication in large independent and clinical samples. **Keywords:** Antisocial behaviour, psychopathy, callous-unemotional traits, genome-wide, genetics, behavioural genetics, twins. **Abbreviations:** AB: antisocial behaviour; CU: callous-unemotional; SNP: single nucleotide polymorphism; DNA: deoxyribonucleic acid; QTLs: quantitative trait loci.

Children who show antisocial behaviour (AB) from early childhood are at great risk for showing AB in adulthood, a pattern known as life-course-persistent AB (Moffitt, 2003). Callous-unemotional traits (CU) traits may predispose children to life-course-persistent AB of a particularly serious nature, namely psychopathy syndrome (Frick & Viding, 2009; Lynam, Loeber, & Stoutham-Loeber, 2008). Children with psychopathic tendencies (AB+/CU+)[1] lack empathy, are insensitive to punishment and have deficits in recognising distress in other people (Frick & Viding, 2009). It has been proposed that these children are biologically predisposed to AB (Frick & Viding, 2009). Recent data from our group have demonstrated that AB is strongly heritable for AB+/CU+ children (Viding, Blair, Moffitt, & Plomin, 2005 [h2 = .81]; 2008 [h2 = .75]). In contrast, children with non-callous AB (AB+/CU–) show mainly environmental influences for their antisocial behaviour. It is worth noting that the profile of emotional reactivity differs dramatically for these two groups, with AB+/CU+ children showing low emotional reactivity to punishment and distress, as well as poor ability to empathise with others (Frick & Viding, 2009). In contrast, AB+/CU– children appear to be emotionally reactive to threat, which is in line with the notion of environmental risk influences (such as harsh parenting) driving the genesis of AB for this group (Frick & Viding, 2009). Recent studies have also found that AB+/CU+ in early adolescence is associated with larger grey matter concentration in several frontal and temporal sites (as compared with typically developing children), indexing possible aberrant brain maturation in AB+/CU+ (De Brito et al., 2009). These findings appear opposite to those typically observed for AB (e.g., Huebner et al. 2008). Furthermore, lower amygdala reactivity to fear has been reported for AB+/CU+ (Jones, Laurens, Herba, Barker, & Viding, 2009; Marsh et al., 2008), but it may be that AB+/CU– show increased amygdala

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[1] The term psychopathic tendencies and acronym AB+/CU+ are used interchangeably in this article to denote a group of children who have elevated rates of antisocial behaviour and core psychopathic features.

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reactivity to emotional stimuli (Sterzer & Stadler, 2009). In the light of differential heritability, as well as distinctive affective processing and neural profiles associated with these different subtypes of AB, it seems reasonable to suggest that combining these two subtypes in molecular genetic analyses of AB would introduce considerable noise to the data. Furthermore, the high heritability of AB+/CU+ encourages the search for risk genes associated with this subtype. It would represent a considerable advance in the field of developmental psychopathology to identify the specific genes responsible for the heritability of AB+/CU+.

Both linkage and candidate-gene association studies have been carried out for diagnosed conduct disorder, other categorical definitions of AB, as well as quantitative conduct, antisocial, and externalising symptoms (e.g., Dick et al., 2008, 2004; Kendler et al., 2006; Caspi et al., 2008; Beaver et al., 2007; Manuck, Flory, Ferrell, Mann, & Muldoon, 2000). Regions on nearly half the chromosomes have been implicated in linkage analyses, but with only modest replications. For example, Kendler et al. (2006) found linkage peaks within 100–200 cM of Dick et al. (2004) peaks on chromosomes 1 and 2. Candidate-gene allelic association studies have focused on monoamine neurotransmitter systems, particularly the serotonergic and dopaminergic systems. Recent studies have implicated monoamine oxidase A (MAOA), serotonin transporter polymorphism (5HTT), serotonin receptor 1B, cathecol-o-methyl transferase, dopamine transporter 1, dopamine receptor 2 and 4 (DRD2 and DRD4) in AB, to name just a few candidates (Caspi et al., 2008; Beaver et al., 2008; Manuck et al., 2000; Sakai et al., 2006).

Although three previous studies have focused on the narrower phenotype of adult psychopathy, these studies are limited as they involved small samples of substance-abusing adults and focused on only a few candidate genes. The first of these studies found no association between psychopathy and either of the Taq1 single nucleotide polymorphisms (SNPs) located in the 3'-untranslated region of the DRD2 (Smith et al., 1993). Two recent studies focused on a small sample of adult alcoholic patients and found an association between psychopathy and specific allelic variants of cannabinoid receptor type 1, fatty acid amide hydorlase (Hoenicka et al., 2006), as well as psychopathy scores and DRD2 C957T and ANKK1 Taq1A acting epistatically (Ponce et al., 2008). An additional study on a relatively small sample of adolescents with ADHD recently reported associations between the val allele of the cathecol-o-methyltransferase gene, the low activity allele of MAOA (MAOA-L), the short allele of the 5HTT gene (5HTT s) and 'emotional dysfunction' scores of psychopathy (Fowler et al., 2009). The latter two of these associations were unexpected based on imaging genetic data suggesting that MAOA-L and 5HTT s confer the opposite pattern of amygdala reactivity to that typically seen in individuals with psychopathy (e.g., Meyer-Lindenberg et al., 2006; Munafò, Brown, & Hariri, 2008).

The previous studies have, by nature, considered only a few genes and may have missed out on important leads. Genome-wide association (GWA) studies utilising large community samples can address some of these concerns (Hirschhorn & Daly, 2005; McCarthy et al., 2008). GWA studies systematically scan the genome with hundreds of thousands of DNA markers – SNPs and copy number variants (CNV) – made possible by microarrays (Plomin & Schalkwyk, 2007). GWA studies have identified more than 300 replicated associations reported for more than 70 traits (Donnelly, 2008), although the effect sizes of these associations are small, with the largest associations accounting for only 1% of the population variance for quantitative traits (Maher, 2008). Large samples and replication strategies are needed to meet this challenge but a problem is expense: even though the price of GWA microarrays has steadily declined, they still cost several hundred dollars each, which means that a two-stage study with 500 cases and 500 controls in each stage will cost more than a million dollars.

One economical strategy for screening large samples in multiple-stage designs is to pool DNA for groups such as those low and high on quantitative traits, which averages allele frequencies biologically for the comparison groups rather than obtaining individual genotypes and averaging them statistically (e.g., Sham, Bader, Craig, O'Donovan, & Owen, 2002; Norton, Williams, O'Donovan, & Owen, 2004; Knight & Sham, 2006). We have combined the strengths of microarrays and pooled DNA in a method we call *SNP Microarrays and Pooling* (SNPMaP) as a cost-effective alternative strategy for multiple-stage GWA screening that makes it possible to genotype samples as large as possible on GWA microarrays at a small fraction of the cost of conventional GWA (e.g., Butcher et al., 2004; Meaburn, Butcher, Schalkwyk, & Plomin, 2006; Docherty, Butcher, Schalkwyk, & Plomin, 2007), as have other groups (Kirov et al., 2006; Pearson et al., 2007). We have used SNPMaP to conduct GWA studies for several quantitative traits (Butcher, Davis, Craig, & Plomin, 2008; Docherty et al., 2010; Meaburn, Harlaar, Craig, Schalkwyk, & Plomin, 2008). Although DNA pooling retains about 70% of the statistical power of individual genotyping, there is some loss of information (Barratt et al., 2002; Sham et al., 2002). This disadvantage is mitigated by the possibility of greatly increasing sample size at no extra genotyping cost, making the technique particularly applicable and cost-effective in very large samples.

To our knowledge, no GWA study of any phenotype related to psychopathy has been published to date, let alone a GWA study of the highly heritable AB+/

CU+ in children. We undertook the first GWA study of AB+/CU+ in the general population, using dimensional measures of AB and CU. In an attempt to identify associations of small effect size we used a two-stage design in which the first stage was used to screen for possible associations and the second stage was used to test a reduced set of associations. The Affymetrix 6.0 GeneChip microarray was employed; this includes nearly a million SNP markers and nearly a million non-polymorphic probes designed to assess structural variants such as insertions and deletions, called copy number variants (CNVs). In this paper we focus on the traditional GWA analysis of SNPs and will address CNVs in a later paper, because the analysis of CNVs is not straightforward and most CNVs are rare, which creates problems of statistical power (Henrichsen, Chaignat, & Reymond, 2009).

Although we have found that AB is highly heritable in children with CU, that CU itself is highly heritable, and that there is considerable genetic overlap between AB and CU, we did not expect to find any genes of large effect size on AB+/CU+. Well-powered GWA studies of heritable traits rarely find 'hits' that account for over 1% of variance on a complex trait. Based on our power calculations, we were, however, cautiously optimistic that in our current study we could detect SNP associations for AB+/CU+ that account for 1% of the variance. Moreover, despite the rarity of SNP associations that account for more than 1% of the variance, it is worth screening the genome to determine whether the highly heritable AB+/CU+ trait is an exception to this emerging rule of small effect sizes. In GWA research it is increasingly recognised that the small effect sizes of individual SNPs hinder attempts at replication and that it is useful to focus on aggregate sets of SNPs (Plomin, Haworth, & Davis, 2009). In the present GWA study, we present results for the top set of SNPs as well as for the individual SNPs.

Method

Participants

The general population sample came from the Twins Early Development Study (TEDS), a UK-based sample born in England and Wales in 1994–96 (Oliver & Plomin, 2007). Children who did not have ethnicity information or DNA available were excluded. Other exclusion criteria were extreme medical conditions (e.g., autism spectrum disorders), severe perinatal difficulties, or non-Caucasian ethnicity. The TEDS sample is reasonably representative of the UK population (Oliver & Plomin, 2007). Comparing the TEDS sample that provided data when the twins were age 7 to the General Household Survey (ONS, 2002), 94% vs. 93% were white, 48% vs. 50% were male, and 37% vs. 32% of mothers had one or more A-level (UK advanced educational qualification). Four percent of children in the TEDS 7-year sample had a statement of special educational needs vs. 3% of children in England (Department for Education and Skills, 2002).

Informed written consent was obtained from all of the families at each assessment. The consent procedure was approved by the Institute of Psychiatry and Maudsley Ethics Committee.

Sample selection

Stage 1: high and low AB+/CU+ groups. Online Appendix 1 contains the bivariate scatterplot for the AB and CU trait measures and the high group cutoff criteria according to standardised scores. For Stage 1, children were selected at the low and high extremes of both AB and CU quantitative trait distributions – see *Measures* section. Children were selected for the AB+/CU+ high-scoring group if they scored in the most severe 16% of the sample for both AB and CU and had contributed DNA for the TEDS study. This cut-off enabled us to select 300 high-scoring children (209M and 91F). The low-scoring group were selected from the lowest 35% for both AB and CU to provide a gender-matched comparison group for AB+/CU+ (N = 300; 205M, 95F). The choice of cut-off was guided by quantitative genetic research in TEDS showing that heritability of both AB and CU is high in this age group, regardless of cut-off (Viding et al., 2005; Viding, Frick, & Plomin, 2007) and by statistical genetic simulations that show that such cut-offs balance the power obtained in DNA pooling studies from using extreme cut-offs and from using large samples (Sham et al., 2002). The cut-offs are less extreme for the low groups because of the restricted variation at the low end of the distributions, as well as the need to balance the gender ratio.

If both members of a twin pair fell in the extreme AB+/CU+ group, the more extreme-scoring child was selected to be included in the high group. Similarly, in the low group the lowest-scoring twin was selected. All children within a group were unrelated to each other.

Stage 2. The same sample selection criteria from Stage 1 was applied to the second screening stage, yielding 293 high- (207M and 86F) and 293 low- (209M and 84F) scoring subjects to be included in this pooling stage. In order to obtain a sufficient N, some of the Study 2 twins were co-twins of some of the Study 1 twins (18% of the Study 2; co-twin N = 210 [115 MZ; 95DZ]). However, we deliberately avoided any direct overlap between the two studies (e.g., if an individual had been selected for Study 1 they could not be selected for Study 2). Although this within-family comparison group is not statistically independent of the discovery sample, the value of this replication sample is that if replication is not found in this sample tested at the same age on the same measures administered at the same time, then replication is highly unlikely to be found anywhere.

Measures

AB at 7 was assessed using teacher ratings of the Strengths and Difficulties Questionnaire (SDQ; Goodman, 1997) five-item conduct problem scale (α = .71).

Each item was rated on a three-point scale as *certainly true*, *somewhat true*, or *not true*. The SDQ is a widely used screening instrument in the UK and its reliability and validity have been demonstrated using a large, national sample. Three of the items reflected aggressive or bad-temper tendencies, whereas the remaining two assessed lying and stealing.

CU traits at 7 were assessed using teacher ratings on seven CU items available in TEDS (α = .74): three Antisocial Process Screening Device (ASPD; Frick & Hare, 2001) items, and four SDQ items. These were original ASPD items (e.g., 'Does not show feelings or emotions') or were selected to reflect CU traits (e.g., 'Considerate of other people's feelings' [reverse scored]) (see Viding et al., 2005). None of the items overlapped with any of the SDQ conduct problem scale items.

DNA pool construction: Stage 1 and Stage 2

Both screening stages followed the same design: within the high and low AB+/CU+ groups individuals were randomly allocated to one of ten pools, so that each DNA pool contained approximately 30 individuals. Genomic DNA for each individual was extracted from buccal swabs (Freeman et al., 2003), suspended in EDTA TE buffer (.01M Tris-HCl, .001M EDTA, pH 8.0) and quantified in triplicate using PicoGreen® dsDNA quantification reagent (Cambridge Bioscience, UK). Once quantified (±.5 ng/μl), an equal amount of DNA (120 ng) for each sample was used to create the DNA pool.

Generation of SNP-MaP allele frequency estimates

DNA pools were prepared for hybridisation to the Affymetrix 6.0 GeneChip® microarray in accordance with the standard protocol for individual DNA samples (see the Affymetrix Genome-wide Human SNP Nsp/Sty 6.0 user guide for full protocol). In both pooling stages, each of the 20 DNA pools was assayed on a separate microarray and scanned using the GeneChip® Scanner 3000 with High-Resolution Scanning Upgrade and GeneChip® Operating software (GCOS) v1.4. Cell intensity (.CEL) files were created using GeneChip® Genotyping Analysis Software (GTYPE v4.0) and exported for analysis. Probe intensities were derived from the CEL files, quantile normalised and combined to produce Relative Allele Signal (RAS) scores using the SNPMaP package (Davis, Plomin, & Schalkwyk, 2009) for the R statistical computing environment (R Development Core Team, 2008). RAS scores have been demonstrated as reliable and valid indices of relative allele frequency in pooled DNA (Butcher et al., 2004; Docherty et al., 2007; Kirov et al., 2006; Meaburn et al., 2005; Meaburn et al., 2006).

Test for association

SNPs on the X and Y chromosomes and SNPs with minor allele frequencies lower than 5% in the CEPH population (US residents with northern and western European ancestry; http://www.hapmap.org) were removed from the analysis at this stage because of reduced statistical power. This left 642,432 autosomal SNPs.

While the use of pooled DNA does not allow us to assess population substructure by conventional methods, we did test for the presence of stratification by looking for any association of SNPs falling in the 12 autosomal ancestry-informative regions identified by the Wellcome Trust Case Control Consortium (Wellcome Trust Case Control, 2007), with high/ low AB+/CU+ status. Quantile–quantile plots of *p*-values derived from a Student's *t*-test show no evidence of association of the SNPs falling within genomic regions found to be differentiated by geographical region in the UK and AB+/CU+ (Online Appendix 2). The RAS scores from each SNP were analysed for association with high/low status using Student's *t*-test (two-tailed) to examine the mean differences in RAS scores for high vs. low pools. The negative log base 10 *p*-value associated with the test statistic was used to rank the SNPs in order of evidence for association.

Power analyses suggest that a pooling study of this kind retains around two-thirds of the power of the equivalent sample individually genotyped (Barratt et al., 2002), which implies that a study of this type has 80% power to detect an additive SNP accounting for 1.7% of the variance where the causal allele is typed and alpha is 5×10^{-7}.

The 3000 top-ranked SNPs from the first stage were taken forward to the second stage. Again, the negative log base 10 *p*-value associated with Student's *t*-test (one-tailed) for each of the 3000 SNPs was used to rank the SNPs in order of evidence for association with high/ low status.

Results

Stage 1

Figure 1 is a standard quantile–quantile (QQ) plot showing the *p*-values obtained from Student's *t*-test plotted against theoretical quantiles. In the case of no association, the points will lie along the line $y = x$. It can be seen that *p*-values are deflated across the range and lie below the line, indicating that Student's *t*-test was a conservative test of association in this sample. This conservativeness of the Student's *t*-test was confirmed by finding that of the 642,432 autosomal SNPs with a MAF >.05 typed in Stage 1 of the study, 25,049 (4%) achieved $p < .05$. No SNPs approached genome-wide significance.

However, the primary aim of this study was to generate a list of candidate genes for verification and further investigation in other community and clinical samples, so we adopted the rationale of Purcell et al. (2009) and selected a liberal threshold for follow-up in Stage 2. Following this approach, which increases the likelihood of capturing multiple variants of small effect, the top 3000 most strongly associated SNPs were tested for association in the same direction in Stage 2.

Stage 2

The top 3000 SNPs carried forward from Stage 1 show evidence for enrichment of associations when

the experiment is repeated in the second sample (Stage 2). Of the 3000 most strongly associated SNPs, 1920 show the same direction of effect in both stages (i.e., association with high/low status), of which 273 (14.2%) show $p < .05$ (one-tailed *t*-test). This is shown in Figure 2, which is a QQ plot using one-tailed *p*-values for the 3000 SNPs. As in Stage 1, no associations reached genome-wide significance (lowest *p*-value (one-tailed) = 4.77×10^{-5}; genome-wide significance threshold = 5×10^{-7}), but a large number show positive deviations from expectation under the null hypothesis.

Approximate *p*-values of the top 30 ranked SNPs in Stage 2 (out of the 3000 most strongly associated in Stage 1) are plotted against the background of *p*-values achieved in each stage with respect to genomic position (Figure 3). These SNPs are annotated in Table 1.

Discussion

We undertook the first GWA study of AB+/CU+ in the general population, using dimensional measures of AB and CU. Our main finding is that although we had power to detect genes of large effect size, we did not find such genes for AB+/CU+. None of our associations reached genome-wide statistical significance, which would require an association that accounted for more than 1% of the variance. Despite the lack of evidence for statistically significant associations in Stage 1, we found that the top 3000 SNPs from Stage 1 showed a greater than expected number of positive results in the same direction when we replicated these SNPs in Stage 2. From Stage 2, we identified 273 SNPs that showed nominally significant associations in the expected direction and focused on the 'top' 30 of these associations (as defined by rank order *p*-values). These include SNPs near several neurodevelopmental genes, such as ROBO2, which is interesting given the emerging data from our group and others suggesting that children with AB+/CU+ differ from typically developing children in their brain structure and function (e.g., De Brito et al., 2009; Jones et al., 2009; Marsh et al., 2009) and may have aberrant cortical maturation (De Brito et al., 2009). Recent findings from our group also suggest that some of the structural brain differences are driven by the same genetic influences that increase risk for AB+/CU+ (Rijsdijk et al., in press). However, it is premature to speculate how the tentative associations we find might translate to the structural and functional brain differences associated with AB+/CU+. Nevertheless, these SNPs may be leads that are worth following up if our findings are replicated and extended in other samples.

Our study was not without limitations. We used relatively short, teacher-rated screening measures to identify AB and CU. However, the AB measure we used is an extremely well-validated screen of conduct problems (Goodman, 1997). In addition, previ-

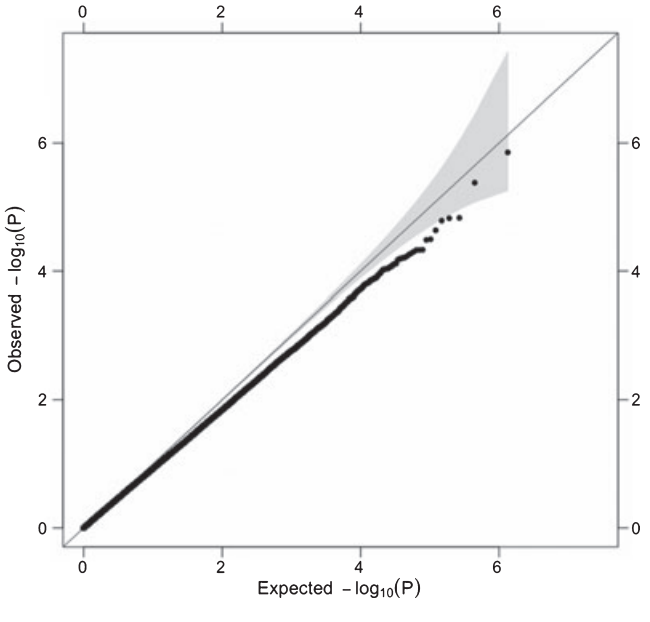

Figure 1 Quantile-quantile plot for sample 1. Negative log base 10 P values are plotted against theoretical quantiles from the null distribution. The line $y = x$ represents the null distribution, and the grey area represents the 95% confidence interval. The plot shows genome-wide SNPs passing quality control in Sample 1 with no evidence of association, and suggests that the t statistic is conservative in this sample

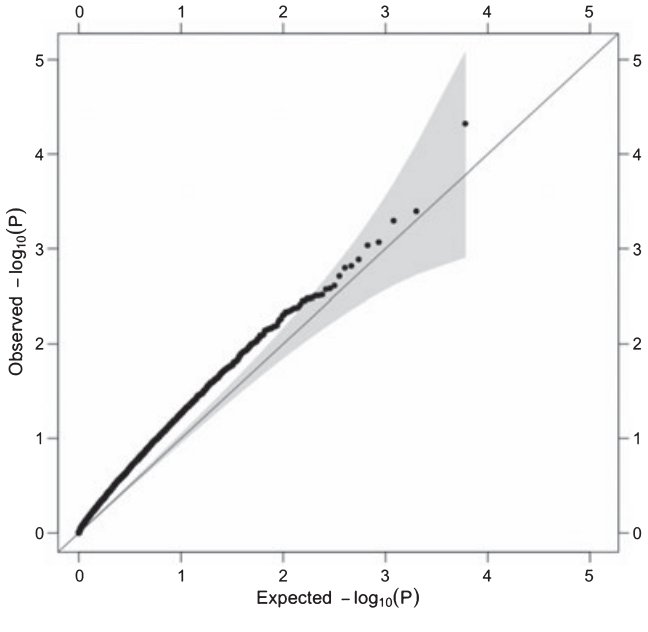

Figure 2 Quantile-quantile plot for sample 2. Negative log base 10 P values are plotted against theoretical quantiles from the null distribution. The line $y = x$ represents the null distribution, and the grey area represents the 95% confidence interval. The plot shows the top 3000 most strongly associated SNPs from Sample 1 tested in Sample 2 (one-tailed). The positive deflection from the line y = x, suggests the SNPs tested in the second sample are enriched for associations

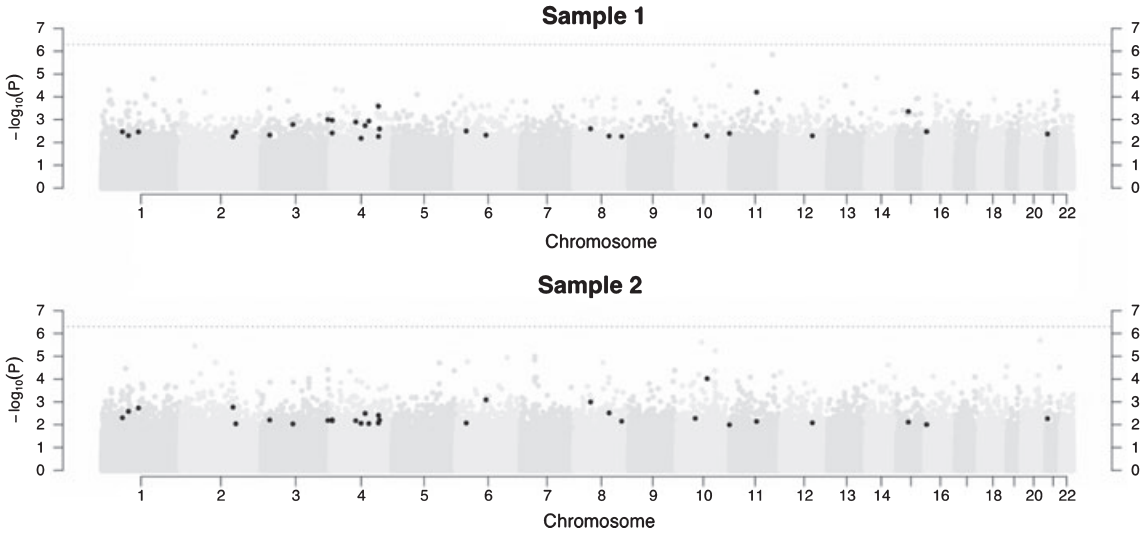

Figure 3 Genome-wide signal plots. Negative log base 10 P values (two-tailed) from a Student's t test of mean differences in relative alleles scores for AB+/CU+ high and low pools, plotted against genomic position for both samples. Highlighted SNPs are the top 30 selected in Sample 2 of the 3000 carried forward from Sample 1. Only SNPs with the same direction of effect in both samples are shown. The dotted line represents the genome-wide significance threshold of $P = 5 \times 10^{-7}$

Table 1 Thirty top-ranked SNPs in sample 2, of 3,000 taken forward from sample 1. The table is ordered by one-tailed *p*-value achieved in Stage 2. This is an approximate *p*-value taken from a standard Student's *t*-test of mean differences in pools of low and high AB+/CU+, used simply as a statistic to rank SNPs by evidence for association

						Sample 1		Sample 2		
SNP rsID	Chr	Position	Strand	Allele A/B	CEPH MAF	RAS diff	*p*-value	RAS diff	*p*-value	Candidate gene
rs12761718	10	79162976	–	C/T	.20	–.06	5.16E-03	–.07	4.77E-05	KCNMA1
rs9343557	6	77845553	–	C/T	.38	.07	4.76E-03	.06	4.02E-04	HTR1B
rs10103840	8	29475632	–	A/C	.12	.05	2.50E-03	.07	5.05E-04	
rs11682518	2	153932192	+	A/C	.38	.06	5.56E-03	.06	8.55E-04	**ARL6IP6**
rs12411132	1	112279235	+	A/G	.29	.05	3.43E-03	.07	9.25E-04	**KCND3**
rs7531603	1	83018311	–	C/T	.32	–.07	4.98E-03	–.09	1.30E-03	LPHN2, TTLL7
rs2514788	8	95212920	+	A/G	.46	.05	5.20E-03	.04	1.53E-03	**CDH17**
rs4383690	4	107586296	+	C/T	.28	.06	1.80E-03	.06	1.60E-03	DKK2, MGC16169, SCYE1
rs6846114	4	152824008	+	A/G	.48	–.05	5.47E-03	–.03	1.95E-03	**PET112L**
rs2376016	1	65806584	–	A/G	.26	.06	3.36E-03	.05	2.46E-03	**LEPR**
rs2311846	10	44865947	+	C/G	.22	.06	1.23E-03	.05	2.63E-03	C10orf10, RASSF4, ZNF22
rs11088618	21	17420297	–	A/T	.46	–.07	4.22E-03	–.04	2.69E-03	
rs41516949	4	6975910	–	A/C	.18	–.08	3.82E-03	–.05	3.06E-03	**TBC1D14**
rs7640807	3	15660141	–	A/G	.37	–.05	4.71E-03	–.03	3.12E-03	**BTD**
rs1490666	4	155622735	–	G/T	.15	.07	2.55E-03	.05	3.13E-03	**DCHS2**
rs293844	3	192614640	+	C/T	.38	.07	9.90E-04	.05	3.26E-03	CCDC50
rs4241597	4	78121149	+	A/G	.18	–.09	1.27E-03	–.08	3.33E-03	**SEPT11**
rs6446569	4	7177419	+	A/C	.49	–.06	1.06E-03	–.04	3.34E-03	PSAPL1, SORCS2
rs1441990	8	130075654	+	A/G	.28	.06	5.44E-03	.06	3.52E-03	
rs1893815	11	79536511	–	A/C	.44	–.10	6.20E-05	–.08	3.58E-03	
rs11637779	15	55998112	–	C/T	.13	.09	4.34E-04	.07	3.86E-03	ALDH1A2, GRINL1A
rs10859716	12	93435384	–	A/G	.42	.07	5.08E-03	.04	4.13E-03	
rs17835633	6	21252098	+	A/G	.14	–.06	3.14E-03	–.04	4.21E-03	**CDKAL1**
rs4479686	4	152618774	+	C/T	.43	.07	2.58E-04	.05	4.22E-03	**FAM160A1**
rs10050093	4	94942802	+	A/G	.48	.08	6.57E-03	.06	4.35E-03	GRID2
rs12647756	4	119550717	+	C/G	.20	–.11	1.16E-03	–.08	4.47E-03	CEP170L, PRSS12
rs1345959	2	163536361	+	C/T	.19	–.05	3.50E-03	–.06	4.55E-03	KCNH7
rs13064369	3	77621908	–	A/G	.49	.06	1.64E-03	.04	4.61E-03	**ROBO2**
rs8059231	16	5790794	+	A/C	.49	–.08	3.33E-03	–.05	4.91E-03	ALG1, FAM86A, LOC440337
rs6560704	10	133648503	–	C/T	.14	–.06	4.00E-03	–.04	5.00E-03	**BNIP3**, C10orf39, JAKMIP3 KCNMA1

Note: SNP rsID = db SNP rsID; Chr = chromosome; Position = physical position; Strand = Affymetrix strand naming convention; Allele A/B = Affymetrix assignment to A and B depends on which allele name comes first alphabetically. Underlined allele is the minor allele; CEPH MAF = minor allele frequency in HapMap Caucasian sample; RAS Diff = high – low relative allele score (RAS = A/(A+B)); *p*-value = *p*-value from Student's *t*-test, two-tailed in Sample 1, one-tailed in Sample 2; candidate genes in bold within 20KB of SNP = annotation was based on data provided by UCSC (March 2006 assembly).

ous quantitative genetic work has demonstrated that our CU measure designates an aetiologially different subgroup of children with highly heritable AB (Viding et al., 2005; Viding, Jones, Frick, Moffitt, & Plomin, 2008). It is critical to note that the high heritability of AB in this group is not attributable to simply having more severe AB (Viding et al., 2005), which underscores the sensitivity of our CU measure in capturing a distinct AB subgroup. There are general limitations to GWA research, such as a focus on common variants and neglect of rare variants and structural variants. Also, similar to other GWA research, our study was underpowered to detect very small effect size; a specific limitation of our study is the additional reduction of power caused by the loss of information from DNA pooling. We fully expect that a proportion of the top associations will be false-positive signals, although the two-stage pooling design is designed to go some way towards ameliorating this issue. For example, of the 3000 SNPs selected in Stage 1, two-thirds showed an effect in the same direction in Stage 2, suggesting that there are likely to be true findings among the candidate SNPs. However, replication of the findings in large well-powered independent samples is crucial before we can draw firm conclusions about the association of these SNPs with psychopathic tendencies. Once associations have been replicated, the next step is to refine the genetic signal and identify the functional variants that underlie susceptibility to the trait.

Conclusion

This paper reported the first GWA study of psychopathic tendencies. In line with most common disorders, no SNPs with large or even medium effect sizes were found for psychopathic tendencies. However, this study generated a list of SNPs that should be followed up in subsequent studies. Our data are in line with the notion that genetic vulnerability to psychopathic tendencies is conferred by multiple genes of small effect size that probabilistically increase the risk for poor behavioural outcome.

Supplementary material

The following supplementary material is available for this article:

Appendix 1. Scatter plot of teacher rated CP and CU scores at age 7 (Word document)

Appendix 2. Quantile–quantile plot of SNPs within ancestry-informative regions (Word document)

This material is available as part of the online article from:

http://www.blackwell-synergy.com/doi/abs/10.1111/j.1469-7610.2010.02236.x

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Key points

- Twin studies indicate that psychopathic tendencies are strongly heritable in children.
- This paper reports the first genome-wide association study of psychopathic tendencies and has generated a list of SNPs that should be followed up in subsequent studies. These include SNPs near neurodevelopmental genes, such as ROBO2.
- In line with most common disorders, no SNPs with large or even medium effect sizes were found for psychopathic tendencies.
- Although preliminary, these findings have translational relevance. Our data suggest that the genetic vulnerability to psychopathic tendencies is conferred by multiple genes of small effect size that probabilistically increase the risk for poor behavioural outcome. This finding underscores the importance of characterising environmental buffers that can be used to moderate the effects of risk genes.

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