Dopamine and serotonin transporter genotypes moderate sensitivity to maternal expressed emotion: the case of conduct and emotional problems in attention deficit/hyperactivity disorder

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Background: Mothers' positive emotions expressed about their children with attention deficit/hyperactivity disorder (ADHD) are associated with a reduced likelihood of comorbid conduct problems (CP). We examined whether this association with CP, and one with emotional problems (EMO), is moderated by variants within three genes, previously reported to be associated with ADHD and to moderate the impact of environmental risks on conduct and/or emotional problems; the dopamine transporter gene (SLC6A3/DAT1), the dopamine D4 receptor gene (DRD4) and the serotonin transporter gene (SLC6A4/5HTT). **Methods:** Seven hundred and twenty-eight males between the ages of 5 and 17 with a DSM-IV research diagnosis of combined type ADHD were included in these analyses. Parents and teachers rated children's conduct and emotional problems. Positive maternal expressed emotion (PMEE) was coded by

independent observers on comments made during a clinical assessment with the mother based on current or recent medication-free periods. **Results:** Sensitivity to the effects of PMEE on CP was moderated by variants of the DAT1 and 5HTT genes. Only children who did not carry the DAT1 10R/10R or the 5HTT l/l genotypes showed altered levels of CP when exposed to PMEE. The effect was most marked where the child with ADHD had both these genotypes. For EMO, sensitivity to PMEE was found only with those who carried the DAT1 9R/9R. There was no effect of DRD4 on CP or EMO. **Conclusion:** The gene–environment interactions observed suggested that genetic make-up can alter the degree of sensitivity an ADHD patients has to their family environment. Further research should focus on distinguishing general sensitivity genotypes from those conferring risk or protective qualities. **Keywords:** ADHD, gene × environment interaction, conduct problems, behaviour problems, emotional problems, emotional symptoms, expressed emotion, mothers, teachers, genetics, serotonin transports, dopamine transport.

Attention deficit/hyperactivity disorder (ADHD) frequently presents comorbid with conduct problems (CP; Biederman, 2005; Lahey, McBurnett, & Loeber, 2000) and emotional disorders (EMO; i.e., anxiety – Tannock, 2000; depression – Jensen et al., 2001). Children with comorbidity have poorer outcomes than children with ADHD alone (Hinshaw, 1992; Lynam, 1996). Multiple genetic and environmental risk factors are likely to drive the development of comorbidity in children with ADHD (Schachar & Tannock, 1995). For instance, childhood ADHD is associated with negative parent–child relationships (Pfiffner et al., 2005) and parental attitudes and actions expressed towards/about their child have been hypothesised to play an important role in the development of comorbidity (Johnston & Mash, 2001). The particular factors of significance may be different for CP and EMO (Vostanis et al., 1994). For CP, levels of positive/negative emotions expressed by a parent about/towards their ADHD child appear to be important (Baker, Heller, & Henker, 2000; Daley, Sonuga-Barke, & Thompson, 2003; Psychogiou et al., 2007). Taylor et al. (1996) found that low levels of 'warmth' and high levels of 'criticism', expressed by mothers about their children with pervasive symptoms of ADHD at age 7 years, predicted the later development of comorbid CP at the age of 17 years; whereas there was no developmental link between CP in 7-year-olds and the later development of ADHD by age 17 years. Conversely, high levels of parental warmth and low levels of criticism appeared to be protective for ADHD children with regard to CP. Such data is consistent with Patterson's (1982) model of the role of a coercive cycle of interaction between parent and child playing a key role in the development of CP in ADHD. In contrast, parental expressed hostility does not, in general, appear to predict the emergence of EMO (Stubbe et al. 1993; Vostanis et al., 1994). Over-protectiveness, insularity and discouragement (anxiety – Pfiffner & McBurnett, 2006; Kepley & Ostrander, 2007) and lack of monitoring and positive feedback (depression – Ostrander & Herman, 2006) seem distinctive elements of the parenting of ADHD children with internalising problems.

A number of genetic variants are reported to moderate the effects of environmental risk of comorbidity in ADHD (Thapar et al., 2007). The current paper examined three variable number tandem repeat polymorphisms (VNTRs) within genes involved in the regulation of the dopamine and serotonin neurotransmitter systems, as potential moderators of the effects of maternal expressed emotion on the development of CP and EMO in ADHD: the dopamine transporter gene (SLC6A3/DAT1); the dopamine D4 receptor gene (DRD4); and the serotonin transporter gene (SLC6A4/5HTT-LPR). For DAT1, most studies suggest that the risk of ADHD is increased in children homozygous for the 10R (Faraone et al., 2005). However, the evidence is far from consistent. Li et al. (2006) updated this work and failed to find evidence of an association with ADHD and the 10R allele in family-based studies, although there was significant evidence of heterogeneity between studies. The most recent meta-analysis of this gene found a small but significant association with ADHD for family-based, but not case–control studies (Yang et al., 2007). The heterogeneity in findings could arise at least in part from the additive or interactive effects of multiple functional variants within DAT1 (Asherson et al., 2007; Brookes et al., 2006a, 2006b). There is also evidence for interactions between this genotype and both prenatal environmental risk factors and psychosocial adversity (Becker et al., 2008; Kahn et al., 2003; Laucht et al., 2007; Neuman et al., 2007). Furthermore, some studies have implicated the 9R allele in aspects of ADHD such as cognitive impulsivity (Kim, Kim, & Cho, 2006). DAT1 has also been implicated in the aetiology of CP more generally, with inconsistencies regarding the risk genotype. Some have implicated the 9R allele rather than the 10R allele as being most significant (Lee et al., 2007; Young et al., 2002), while others have suggested that it is the heterozygous case (i.e., 9R/10R) which is at most risk. Given this pattern of results from previous studies, in the current analysis we compare the three most common DAT1 genotypes; 9R/9R, 9R/10R and 10R/10R.

For DRD4, evidence of an association between ADHD and the 7R allele located within intron 3 of the gene reached genome-wide significance in a comprehensive meta-analysis of available data ($p > 5 \times 10^{-8}$; Li et al., 2006). Several potential gene by environment interactions involving the DRD4 polymorphism have been reported. Maternal insensitivity was associated with preschool externalising disorders only in children carrying the 7R allele

(Bakermans-Kranenburg & van IJzendoorn, 2006) and parental warmth was protective for externalising disorders only in the absence of the 7-repeat allele, and only for African-American children (Propper et al., 2007). In keeping with this literature our main DRD4 analysis compares individuals with and without the 7R allele, although preliminary analyses were carried out on other common alleles.

Support for the association between ADHD and the long (l) allele of an insertion/deletion polymorphism within the promoter region of the serotonin transporter gene (5-HTT-LPR) comes from several studies (Kent et al., 2002; Li et al., 2007). Interestingly for the current analysis, the short (s) allele of this polymorphism (the putative protective allele for ADHD) has been reported to interact with social adversity and other environmental factors to increase the risk for behavioural problems, including CP, in a number of different studies. The s allele is associated with increased risk for depression following exposure to stressful life events (Caspi et al., 2003; Kendler et al., 2005) and social adversity (Eley et al., 2004) and in children of low socioeconomic status (Cicchetti, Rogosch, & Sturge-Apple, 2007). It has also been associated with increased rates of drug abuse in the context of dysfunctional parenting (Gerra et al., 2007). This allele is associated with aggressive CP in middle childhood (Haberstick, Smolen, & Hewitt, 2006; although see Sakai et al., 2007) and interacts with an adverse childhood environment to increase the risk for violent conduct in young adults (Reif et al., 2007). In contrast, one study found that the presence of the l allele interacts with socioeconomic status to increase childhood externalising problems (Nobile et al., 2007). Given the inconsistency of findings, our analyses will compare the s/s, s/l and l/l genotypes.

Polymorphisms in the Monoamine Oxidase (MAOA) gene have been suggested to moderate the effects of childhood maltreatment on the development of CP (Kim-Cohen et al., 2006) while the Catechol-O-methyl transferase (COMT) gene appears to mark a subtype of ADHD patients more likely to have CP (Caspi et al., 2008). While these are two excellent additional candidates for the sort of analysis conducted here, the relevant polymorphisms had not been genotyped for the whole IMAGE sample at the time these analyses had been carried out.

In the current study we examined the moderating role of the variants in the three selected genes on the association between maternal EE (with specific reference to maternal warmth and criticism), and CP and EMO using a cross-sectional design. Based on the available literature that identifies a role for EE in the developmental link from ADHD to CP, we predicted that parental warmth and low levels of criticism would be associated with lower levels of CP, but not EMO. We did not measure factors such as intrusiveness or over-protectiveness that might be implicated in EMO in ADHD according to the literature. Our aim was to test the hypothesis that genotypic variations in our three candidate genes (DRD4, DAT1 and 5HTT-LPR) moderate the protective effect of positive maternal EE (PMEE) on the risk for CP but not EMO. Given the exploratory nature of these analysis and the mixed findings from previous studies, we made no directional predictions with regard to specific genotypes that might promote or suppress the effects of PMEE.

The analyses reported were part of an ongoing large-scale study of the molecular genetics of ADHD: The Multi-centre ADHD Genetics (IMAGE) study. Analyses of VNTRs in the IMAGE sample supported the association between ADHD and the 10-repeat allele and a specific haplotype of DAT1 (Asherson et al., 2007), but no association with VNTRs in the 5HTT gene (Xu et al., 2008). SNP-based analyses found evidence of association between ADHD and SNPs in both DAT1 and DRD4 (Brookes et al., 2006a), as well as an empirically derived quantitative trait measure of ADHD symptoms (Lasky-Su et al., 2007). For DAT1 two independent regions of association were identified in the 3′ and 5′ ends of the gene. Further analyses looking at interactions with clinical subtypes or environmental risk measures found that neither DAT1 or DRD4 was associated with IQ (Sonuga-Barke et al., 2008a), DAT1 did not show an interaction with exposure to prenatal smoking (Altink et al., 2008) and DRD4 was not moderated by season of birth (Brookes et al., 2008). Analysis of ADHD with and without comorbid CP demonstrated that the DAT1 associations with ADHD were restricted to the pure ADHD group, which did not have comorbidity with CP (Zhou et al., 2008). The EE variables used in the current analysis has been employed in a secondary analysis of genome-wide association scan data in which partitioning by EE allowed us to identify a number of new candidate genes for ADHD and CP (although none reached genome-wide significance; Sonuga-Barke et al., 2008b). Finally, an unpublished analysis supports a differential role for maternal and paternal EE in relation to CP and AMO (Psychogiou et al., submitted).

Methods

Participants

The sample was drawn from the participants in the IMAGE study recruited through 12 specialist ADHD clinics in eight countries: Belgium, Germany, Netherlands, Ireland, Israel, Spain, Switzerland, and the United Kingdom, as part of the NIMH-funded International Multi-centre ADHD Genetics (IMAGE) project (Brookes et al., 2006a).

The current analysis was limited to male ADHD probands whose research assessment and diagnosis was made on the basis of a current or recent period off-medication (N = 728; mean age 11.0 years (SD = 2.8). Two hundred and fifty-one male probands who were being continuously medicated at the time of study were

excluded from this analysis as their assessment was based on retrospective accounts by parents which may have compromised the veracity of the EE assessment. Female probands (N = 127) were excluded as there were insufficient in number to provide the necessary statistical power to identify anticipated interactions between genotype gender and maternal EE. Probands were of European/Caucasian ancestry and between the ages of 5 and 17 years at the time of entry into the study. Entry criteria for ADHD cases were: a clinical diagnosis of DSM-IV combined-subtype ADHD; having one or more full siblings (although these were not included in these analyses) available for ascertainment of clinical information and DNA collection; access to one or both biological parents for DNA collection. Exclusion criteria applying to both ADHD cases included autism, epilepsy, IQ <70, brain disorders, and any genetic or medical disorder associated with externalising behaviours that might mimic ADHD. The Parental Account of Children's Symptoms interview (PACS; see below) was conducted with all probands. The DSM-IV combined type ADHD diagnosis was confirmed by the PACS in 94.5% of cases, while 1.8% had the predominantly inattentive subtype and 3.7% had the predominantly hyperactive/impulsive subtype; 15.5% of cases were diagnosed with a probable comorbid mood disorder and 23.6% with comorbid conduct disorder. Interviewer ratings of maternal EE warmth and criticism were available for 673 of the 728 male probands. In order to examine the effects of age, boys were divided into two groups; 11 years and below (56.2%) and 12 years of age and above (43.8%). Genotypic data was available for a large proportion of the original 728 probands (DAT1 N = 668; DRD4 N = 684; 5HTT N = 681). Parental genotypes were also available (DAT1 N = 663; DRD4 N = 673; 5HTT N = 675). Given that the availability of data varied for different genes, the numbers included varied from analysis to analysis.

The IMAGE project procedures were approved by the Ethics Committees at all data collection sites and by the Institutional Review Board at the coordinating site (SUNY Upstate). All enrolled parents provided informed consent for the participation of their families in the project.

Measures

Research diagnosis was established using the PACS interview (Taylor et al., 1991; Chen & Taylor, 2006). This is a semi-structured interview used to collect parents-based detailed information on children's behaviour. It is divided into four sections: Mood Disorders, ADHD/hyperkinetic disorder, disruptive behaviour problems and additional problems. In the ADHD/Hyperkinetic Disorder section, the interviewer asks parents to describe their child's behaviour in different settings; the interviewer then rates the severity and frequency of the behaviour according to previously defined criteria. The settings were selected to represent common unstructured (watching TV, reading or playing alone), semi-structured (meals, outings or shopping) and structured (home tasks, homework or getting ready) daily life situations. In this study, parents were asked to focus on examples of their child's behaviour during current or recent medication-free periods. The

interviewers made their own coding on the basis of a formal training and written definitions of the behaviours, on a 4-point scale (0 to 3) of severity and frequency in the previous week and previous year. A standardised diagnostic algorithm based on the DSM-IV criteria was applied to the information from PACS and from the teacher-rated ADHD subscale from the Conners' Teacher Rating Scale (Conners, 2003). The algorithm included behavioural symptoms, age of onset, situational pervasiveness and clinical impairment. Previous studies have shown high inter-rater reliability for this instrument (product–moment correlations between .76 and .96 (Chen & Taylor, 2006). In order to ensure cross-site consistency within the IMAGE project in measurement and coding of PACS, all interviewers from each site attended a 5-day PACS training course in the UK. The chief investigator at each site attended an annual inter-rater reliability exercise and was responsible for reliability in their native site. A mean Kappa coefficient across all sites of .88 (range .71––1.00) and an average agreement percentage of 96.6% (range 78.6–1.00) were obtained, indicating a substantial level of inter-rater agreement (Brookes et al., 2006a).

Conduct and emotional symptoms. For the current analysis, which required data from both teacher and parent, these were derived using the parents and teacher version of the Strengths and Difficulties Questionnaire (SDQ; Goodman, 1997). This is a brief behavioural screening questionnaire that can be completed by parents or teachers of children aged 4 to 16. Both the CP and EMO scales contain 5 items, each with a 3-point response scale, ranging from 0 (Not True) to 2 (Certainly True). Cut-offs are standardised to identify the top 10% of children within the UK (Goodman, 1997). The scale is well validated and has good test–retest reliability (α = .85), with similar psychometric properties in different countries (Achenbach et al., 2008).

Parental expressed criticism and warmth. Assessment of mothers' expressed criticism and warmth was made using codings derived from the Camberwell Family Interview on the basis of parental responses over the extended period of the entire clinical assessment (i.e., > 1 hour). Warmth was assessed by the tone of voice, spontaneity, sympathy, and/or empathy toward the child. *A great deal of expressed warmth (0)* was coded when there was definite warmth, enthusiasm, interest in, and enjoyment of the child. *Quite a lot of demonstration of warmth (1)* was coded when there was definite understanding, sympathy, and concern but only limited warmth of tone. *Moderate demonstration of warmth (2)* was coded when there was a detached and rather clinical approach, with little or no warmth of tone, but moderate understanding, sympathy, and concern. *Little warmth (3)* was coded when there was only a slight amount of understanding, sympathy, or concern or enthusiasm about or interest in the child or when parents did not display any of the qualities of warmth described above. Inter-rater reliability has been satisfactory, ranging from .78 to .91 (Schachar et al., 1987).

Criticism was assessed by statements which criticised or found fault with the child based on tone of voice

and critical phases. *A lot of expressed criticism (4)* was coded when the parent mentioned critical comments indicating that the respondent disliked, resented, disapproved of, or was angered or annoyed by the child's behaviour or characteristics. High criticism was also based on harsh tone of voice, even if the statement did not meet the content criteria. For a statement to be considered critical, the inflection, pitch and/or rate of speech had to be dramatically different from the preceding and usual level of speech in the interview. The tone had to strongly indicate resentment and/or anger about the topic being discussed. *Quite a lot of expressed criticism (3)* was coded when there were indications that the parent did not like or approve of the child's behaviour. *Some criticism (2)* was coded when there were statements of dissatisfaction indicating that the parent was bothered, irritated or upset by the child's behaviour or characteristics. *Very little expressed criticism (1)* and *no expressed criticism (0)* were coded when there was no evidence during the interview that the parent disapproves of or dislikes the child's behaviour. Inter-rater reliability has been satisfactory, ranging from .79 to .86 (Schachar et al., 1987).

DNA extraction and genotyping. DNA was extracted directly from blood samples or cell lines at Rutgers Cell line and DNA repository in the US. In a few cases we used a mouth swab sampling technique and extracted the DNA at the SGDP laboratories in London. For genotyping of the VNTR markers we used a standard PCR method according to previous optimised protocols for the markers used in this study. Based on previous studies of ADHD and gene × environment interactions, our analyses focused on the following genotypes: (1) for DRD4 we compared the group with either one or two copies of the 7-repeat allele with those with no copies of the 7R allele. (2) For DAT1 and 5HTT-LPR: given the inconsistency of previous results with regard to risk for ADHD and gene × environment interaction, we compared the three common genotypes in each case. For DAT1 we compared the 9R/9R, 9R/10R and 10R/10R and for the 5HTT-LPR analyses were compared s/s, s/l and l/l.

Analytical strategy

Maternal warmth and criticism were negatively correlated ($r = -.54$). In keeping with the notion of EE as a composite variable encompassing both elements, these two scores were combined into a single factor (positive maternal EE: PMEE) using equal weightings. For the current analysis this score was dichotomised to form a binary variable (i.e., high PMEE vs. low PMEE) using a median split in the PMEE score in order to facilitate the G×E analyses. A number of preliminary analyses were conducted to explore the associations between child and parent genotypes and PMEE. To test for genotype × maternal PMEE interaction repeated measures ANOVAs models were run with genotype (DAT1–9R/9R, 9R/10R, 10R/10R; DRD4 – +7 R/−7R; 5HTT-LPR – ll, ls, ss), and PMEE (high, low) and age (under 12 years vs. 12 years and over) as the between-subject variables, and rater (parent or teacher) as the within-subject variable. One set of analyses had SDQ CP, and a second, SDQ EMO as the dependent variable. We also conducted supple-

mentary analyses on the disaggregated measures of warmth and hostility. These analyses (which are available from the authors) produced similar patterns of effects as for the combined PMEE measure but failed to reached significance overall.

Results

Among the ADHD cases, frequencies for common genotypes were similar to those found in previous studies and the data were all found to be in Hardy Weinberg Equilibrium: (*DRD4*: +7 – $N = 232$; −7 – $N = 452$; *DAT1*: 9R/9R – $N = 72$; 9R/10R – $N = 233$; 10/10 – $N = 363$. 5HTT-LPR: s/s – $N = 124$; s/l – $N = 338$; l/l – $N = 219$). The genotypes for the VNTRs were not significantly associated with each other ($\chi^2 < 5.57$; ps > .2). There was no significant association between PMEE and child genotypes (DAT1 $\chi^2(2) = .83$, $p = .660$; DRD4 $\chi^2(1) = 1.92$, $p = .166$; 5HTT $\chi^2(2) = 2.84$, $p = ..241$) or maternal genotypes (DAT1 $\chi^2(2) = .563$, $p = .771$; DRD4 $\chi^2(1) = .32$, $p = .572$; 5HTT $\chi^2(2) = .27$, $p = .873$). There was an effect of national centre on PMEE ($F(10,673) = 3.68$; $p < .001$). This effect appeared to be due entirely to Spain having significantly higher levels of PMEE than the other groups (Scheffé's tests $p < .02$). However, national grouping did not interact with PMEE with respect to CP and so subject scores were pooled across national groups for the analyses. Furthermore, excluding the children from the Spanish cohort had no effect on the findings.

Conduct problems

Table 1 shows the level of CP and EMO as a function of PMEE group and genotype. For all three genes there was a main effect of PMEE on CP ($F^{DRD4}(1,689) = 10.40$, $p = .001$; $F^{DAT1}(1,608) = 11.36$, $p = .001$; $F^{5HTT}(1,618) = 10.97$, $p = .001$): Children in the high PMEE group had less CP. There was no effect of age (Fs < .14, ps > .24) and age was not involved in any interactions with PMEE or genotype (Fs < 2.20, $p > .130$). For the analyses of DRD4 there was no main effect of genotype on CP levels ($F(1,625) = .08$, $p = .779$) and no genotype × PMEE interaction ($F(1,625) = .13$, $p = .718$). Also, no three-way interaction between these factors and rater was observed ($F(1,605) = .76$, $p = .383$). Supplementary analyses found no effects for other common DRD4 genotypes (e.g., 2R) or of the presence of two as opposed to just one 7R allele. For DAT1 there was no main effect of genotype on CP levels ($F = 2,608) = .28$, $p = .758$). However, there was a significant interaction between genotype and PMEE ($F(2,608) = 3.81$, $p = .023$) which was independent of rater ($F(2,588) = .65$, $p = .523$). This interaction is illustrated in Figure 1a. There was a significant simple main effect of PMEE for probands with 9R/10R genotype ($F(1,213) = 17.79$; $p < .001$) and a trend for those with the 9R/9R genotype ($F(1,62) = 3.39$; $p = .07$), despite the small sample size for this group. There was no effect of PMEE for
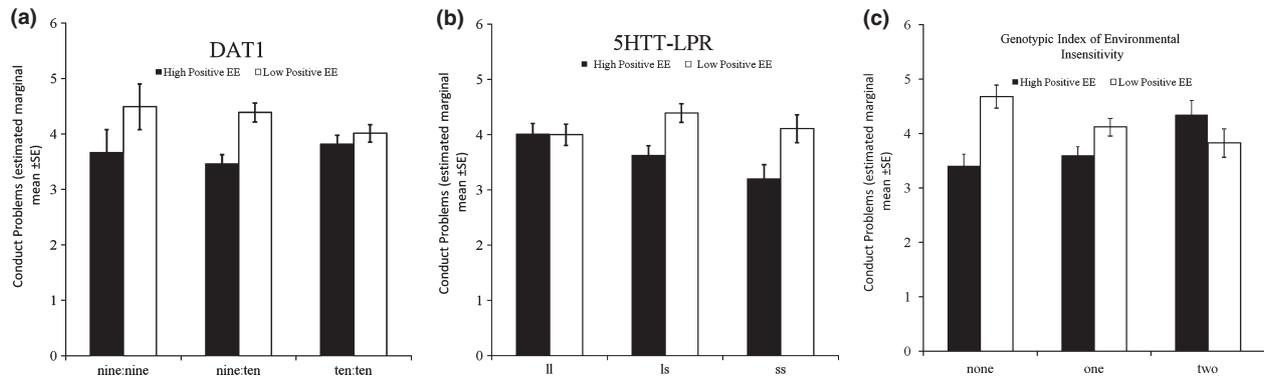
Figure 1 (a) The interaction between DAT1 genotypes and positive maternal expressed emotion for conduct problems. (b) The interaction between 5HTT genotype and positive maternal expressed emotion for conduct problems. (c) The interaction between the Genotypic Index of Environmental Insensitivity positive maternal expressed emotion for conduct problems. This measure ranges for 0 (neither l/l and 10R/10R), 1 (either 1/1 or 10R/10R) and 2 (both l/l and 10R/10R)

those in 10R/10R group ($F(1,333) = .70$; $p = .404$). From the figure it appeared that the 10R/10R group were protected from the negative effects of low PMEE. However, there was no significant effect of genotype in either the high or the low PMEE groups ($F^{high}(2,334) = 1.09$; $p = .337$; $F^{low}(2,280) = 1.73$; $p = .179$).

A somewhat similar pattern of results was observed for the 5HTT gene (Figure 1b). There was no main effect of genotype on CP ($F(2,618) = 1.55$, $p = .213$) but the interaction between genotype and PMEE on risk for CP was significant ($F(2,618) = 3.13$, $p = .045$). This again was independent of rater ($F(2,598) = .46$, $p = .629$). High PMEE conferred a protective advantage for s/s and s/l genotypes ($F^{s/l}(1,306) = 12.04$, $p = .001$; $F^{s/s}(1,112) = 6.23$, $p = .014$) but not 1/1 genotypes ($F(1,200) = .01$, $p = .964$). From Figure 1b it seemed that those with 1/1 failed to benefit from the protective effects of PMEE. Consistent with this view, there was an effect of genotype in the high PMEE environment ($F(1,356) = 3.04$, $p = .049$) but not the low one ($F(1,325) = 1.57$, $p = .210$).

Given the similarity of the findings for the 5HTT and the DAT1 groups, we explored the cumulative effects of the two genes in determining sensitivity to PMEE. To do this we first identified the 1/1 genotype of the 5HTT and the 10R/10R genotype of the DAT1 as those associated with insensitivity to PMEE in terms of the development of CP. We then created a genotypic index of PMEE insensitivity (GIPI) by adding these two scores: probands with neither genotype associated with insensitivity scored '0' ($N = 212$), those with one but not the other scored '1' ($N = 343$) and those with both scored '2' ($N = 118$). We then employed this cumulative score as the independent variable in the ANOVA model described above (Figure 1c). There was a main effect of PMEE ($F(1,612) = 5.83$, $p = .016$) and a highly significant GIPI × PMEE interaction ($F(2,674) = 7.44$, $p = .001$). This effect was independent of rater ($F(2,592) = 2.02$, $p = .133$) and age

($F(2,612) = .87$, $p = .481$). There were simple main effects of PMEE for groups with scores '0' ($F(1,191) = 22.06$; $p < .001$) and '1' ($F(1,316) = 4.97$; $p = .027$) but not a score of '2' ($F(1,105) = 1.95$; $p = .166$). Figure 1c suggests that compared to those with either none or one insensitivity genotypes, those with both were less likely to be affected by the negative effects of low PMEE and less likely to benefit from the effects of high PMEE. This was confirmed by the finding that there was an effect of the GIPI in both the high PMEE group ($F(2,333) = 4.23$; $p = .015$) and the low PMEE group ($F(2,279) = 3.80$; $p = .024$).

Emotional problems

When EMO was analysed as the dependent variable no effects of either PMEE ($Fs < 1.35$; $p > .24$), genotype ($Fs < .26$; $p > .600$) or genotype × PMEE interaction ($Fs < .94$, $p > .300$) were found in the analyses for DRD4 or 5HTT-LPR genotypes. For DAT1 there were a significant effect of PMEE ($F(1,608) = 5.56$; $p = .019$), a significant trend toward a genotype effect ($F(2,608) = 2.98$; $p = .051$) and a significant PMEE × genotype interaction ($F(1,608) = 3.28$; $p = .040$). Figure 2 plots this interaction. There was a significant main effect of PMEE only for the 9R/9R genotype ($F(1,62) = 6.72$; $p = .040$). It appeared that those with the 9R/10R and the 10R/10R genotype were protected from the negative effects of low PMEE. This was supported by the analysis showing an effect of genotype on emotional problems for the low PMEE group ($F(2,277) = 3.89$; $p = .022$) but not for the high PMEE group ($F(2,331) = 1.48$; $p = .229$).

Discussion

Our results are consistent with a complex model of risk and resilience in the development of comorbidity in ADHD. First, they confirm an association between parenting factors, in this case PMEE, and the pres-
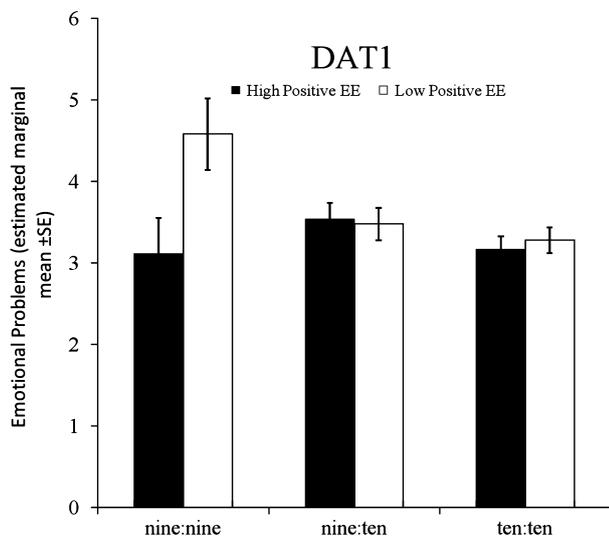
Figure 2 The interaction between DAT1 genotypes and positive maternal expressed emotion for emotional problems

ence of CP in ADHD children. Previous data suggest that these effects are driven, at least in part, by early-onset ADHD and associated hard-to-manage behaviour and that parental EE is in part a response to this and in part an exacerbating factor which leads to the escalation of CP (Taylor et al., 1996). Second, the association between PMEE and CP was significantly moderated by genetic factors. While those with the 9/9R and 9/10R of the DAT1 genotype and the s/s and the s/l 5HTT-LPR genotype showed sensitivity to the effects of PMEE (the low PMEE group had more CP), those with the DAT 10R/10R or the 5HTT-LPR l/l genotypes did not. While the size of these interaction effects were small individually, the effects were much greater when the genotypes associated with insensitivity to PMEE were added together to create a cumulative index. Importantly, these effects were independent of whether a parent or a teacher was rating the child's behaviour, did not vary significantly across national settings and appeared similar in childhood and adolescence (i.e., there was no effect of age category). As predicted, there was no main effect of PMEE on EMO in the current sample. However, there was a gene × environment interaction for DAT1, with an effect being found for those children with the 9R/9R genotype, as was seen for CP, but no effect at all for those with 9R/10R (differing from that seen for CP) and the 10R/10R genotypes. In supplementary analysis we also looked at the impact of maternal warmth and criticism separately and found that while the pattern of effects was similar for these two components of EE, the disaggregated measures were far less powerful at predicting the presence of comorbidity than was the combined measure – a finding which appears to support the value of the broader construct of EE as a combination of warmth and criticism.

There are in principle a number of possible ways to interpret the reduced sensitivity to PMEE seen for the l/l and the 10R/10R genotypes (and the 9R/10R for EMO). First, there could be a protective effect of the genotype in terms of reducing the negative effects of parental hostility and lack of warmth. Second, there could be a risk element associated with the genotype expressed as a reduction of the positive effect of high PMEE. Third, it could be that the genotype produces a more general insensitivity to environmental factors, whether they have positive or negative effects in those without the genotype. The current results are rather mixed in this regard. For DAT1 the data favour this latter sort of explanation, with those with 10R/10R having lower CP under the low PMEE condition than those patients with 9R/9R and 10R/9R genotypes and higher levels of CP than these under high PMEE. For 5HTT-LPR it seemed that those with l/l genotype failed to benefit from the high PMEE as those patients with the other genotype did. The overall pattern of results for the cumulative index also supports a general insensitivity hypothesis, with those with both 'insensitivity' genotypes showing less CP under the low PMEE and more CP under the high PMEE than those with either one or no insensitivity genotypes. The data for EMO and DAT1 provides evidence for the protective value of the 9R/10R and the 10R/10R genotypes in the current study.

This pattern of findings for the two significant genotypes and for their aggregation is rather different from the pattern seen in previous studies. Those studies typically reported a synergistic interplay between genetic and environmental factors in the development of disorder (Rutter, Moffitt, & Caspi, 2006), where children carrying a particular genotype are at increased risk for disorder when exposed to a particular environmental risk (Caspi et al., 2003). That is, the environmental effect is manifest for those with one but not another type of genotype. This was not the case in the current paper where the presence of different genotypes led to opposite effects depending on the operating environmental conditions, vis-à-vis risk – in high-risk settings (e.g., low PMEE) one genotype may have a protective effect while under low risk or positive settings it produced a negative or an antagonistic effect (Ottman, 1996). The most parsimonious explanation may be that certain genotypes simply reduce (and others increase) the sensitivity to environmental effects in a general way. Distinguishing such insensitivity genotypes from genotypes with more specific risk and protective properties seems an important goal for future research (Belsky, Bakermans-Kranenburg, & van IJzendoorn, 2007).

Generally there are a number of plausible biological and psychological mechanisms that might account for these sorts of gene by environment effects. For example, genetic factors may 'block' the exposure of children to, or determine their degree of sensitivity to, the beneficial effects of positive parenting or the harmful effects of parental dysfunction. Genetic factors may reduce the receptivity of children to the experience of maternal warmth and

Table 1 The SDQ conduct and emotional scores for DAT1, DRD4 and 5HTT-LPR genotypes as a function of level of positive maternal expressed emotion (PMEE)

	Conduct Problems				Emotional Problems			
	High PMEE		Low PMEE		High PMEE		Low PMEE	
	P	T	P	T	P	T	P	T
DAT1								
9R/9R	4.29 ± 2.3	3.09 ± 2.1	5.52 ± 2.0	3.07 ± 2.1	3.26 ± 2.3	3.06 ± 2.7	4.42 ± 3.0	3.57 ± 2.6
$N = 72$								
9R/10R	4.07 ± 2.2	2.83 ± 2.3	5.56 ± 2.5	3.32 ± 2.3	3.81 ± 2.5	3.32 ± 2.4	4.24 ± 2.6	2.74 ± 2.5
$N = 233$								
10R/10R	4.35 ± 2.3	3.23 ± 2.5	4.87 ± 2.2	3.09 ± 2.3	3.58 ± 2.5	2.71 ± 2.2	3.88 ± 2.4	2.63 ± 2.2
$N = 363$								
DRD4								
−7R	4.26 ± 2.2	3.00 ± 2.3	5.16 ± 2.3	3.24 ± 2.3	3.6 ± 2.5	2.87 ± 2.3	4.13 ± 2.5	2.91 ± 2.3
$N = 233$								
+7R	4.22 ± 2.3	3.16 ± 2.5	5.22 ± 2.1	2.95 ± 2.3	3.67 ± 2.4	3.07 ± 2.3	4.06 ± 2.6	2.5 ± 2.3
$N = 452$								
5HTT								
s/s	3.72 ± 2.2	2.76 ± 2.4	5.12 ± 2.1	3.07 ± 2.4	3.48 ± 2.6	3.12 ± 2.0	4.05 ± 2.7	2.73 ± 2.3
$N = 124$								
s/l	4.18 ± 2.2	3.06 ± 2.2	5.37 ± 2.3	3.3 ± 2.3	3.59 ± 2.4	3.07 ± 2.3	3.92 ± 2.5	2.68 ± 2.5
$N = 338$								
l/l	4.66 ± 2.3	3.29 ± 2.6	4.91 ± 2.3	3.00 ± 2.3	3.68 ± 2.5	2.62 ± 2.5	4.34 ± 2.6	2.94 ± 2.2
$N = 219$								

Note: P = parent SDQ; T = teacher SDQ.

criticism or the impact that this has on their difficult and challenging behaviour. Alternatively, genetic factors may alter the extent to which attitudes and emotions, expressed about the child in an interview setting, actually result in parenting behaviour (be it positive or negative) being expressed towards the child. Observation of mother–child interaction would be necessary to test these two hypotheses.

A second class of explanations focuses more on the possibility that high- or low-risk environments (such as those characterised by low and high PMEE respectively) alter the expression or effect of genes. First, risk environments may have powerful effects that may 'swamp' smaller and less robust genetic effects. This may be a problem especially for polygenic disorders (such as ADHD and CP) where effects are determined by many genes of small effect acting together. On the other hand, a more biologically interesting possibility derives from the hypothesis that adverse social environments may 'switch off', or socially benign environments 'switch on' genetic effects through epigenetic mechanisms such as DNA methylation (Mill & Petronis, 2008). While almost nothing is known empirically about the power of the family environment to impinge on gene expression within the human infant, recent animal models suggest that such effects are plausible (Parent et al., 2005; Diorio & Meaney, 2007).

Previous research has implicated the 5HTT gene in both externalising (i.e., aggression; Haberstick, Smolen, and Hewitt, 2006) and internalising problems (i.e., depression; Eley et al., 2004) and pointed to it as one of the best examples to date of a genetic moderator of environmental adversity (Caspi et al., 2003; Kendler et al., 2005). In contrast to most

previous research, in the current study the l/l genotype rather than the s/s genotype was associated with greater risk for CP in the low-risk environmental setting (Kendler et al., 2005), although as discussed above the l allele has been associated with risk for ADHD (see also Nobile et al., 2007). Furthermore, surprisingly, given the extensive literature linking 5HTT-LPR to depression (Eley et al., 2004), the effects found for CP with this gene in the current study did not extend to EMO. It should also be borne in mind that the SDQ EMO phenotype in the current study neither specifically probed depression nor allowed a clinical diagnosis (Goodman et al., 2000). The presence of the 5HTT-LPR genotype was especially potent when it was accompanied by the DAT1 10/10 genotype, suggestive of synergies between serotonin and dopamine systems (Oades, 2002) consistent with the recent paper by Schmidt and colleagues (Schmidt, Fox, & Hamer, 2007).

Consistent with the current findings, previous studies have implicated DAT1 in the aetiology of CP. However, there have been inconsistencies regarding the identity of the risk genotype, with a number of studies implicating the 9R allele rather than the 10R allele as most significant (Lee et al., 2007; Young et al., 2002). However, Guo et al. (2007) recently found evidence for a significantly increased risk for CP associated with the presence of at least one 10R allele. The current study supports the significance of the 10R/10R as operating in a different way from the other common genotypes – although it would not be accurate to describe it as a risk genotype for CP as it did not significantly increase risk of disorder in the low-risk environment. In all these studies it is unclear how specific these effects are to CP rather

than ADHD. What is distinctive in the current study is that levels of ADHD are constant in the sample, which suggests a specific role in relation to CP in addition to any role in ADHD. The finding showing that the 9R/9R allele can increase risk of EMO in the high-risk setting provides the first evidence linking this gene to emotional problems. Further research with more refined and clinically informative EMO phenotypes is required. Although studies have previously demonstrated a possible role for DRD4 in moderating the effects of the child's early social environment, this was not the case here. However, PMEE, the construct employed in the current study, is not directly related to those employed in previous studies (i.e., maternal insensitivity; Bakermans-Kranenburg & van IJzendoorn, 2006).

Our study had a number of limitations. First, the study was limited to male participants with combined type ADHD and mother's PMEE. A comparison of the effects of maternal and paternal EE in the IMAGE study suggested that there may be different effects by gender for parental EE and these may also be affected by the gender of the proband (Psychogiou et al., submitted). Second, the participants in the IMAGE study are all patients and a significant number were receiving, or had in the past received, medication for their condition. There are potentially important implications of this. First, treatment may alter the relationship between PMEE and conduct and emotional problems in the sample and also the extent to which these are moderated by genetic factors. Second, assessments of EE and the presence of conduct and emotional problems may be biased if they are based on observations made during periods when participants were receiving medication. Because systematic data on medication history was not available, an analysis of the association between medication history and the effects of PMEE on conduct and emotional problems was not possible. We did attempt to limit the potential biasing effects of assessment based on periods of active medication by limiting the current analysis only to those whose EE and psychopathology evaluation was made on the basis of current or recent medication-free periods. Ideally the current analyses would be repeated in a medication-naive group – unfortunately this was not feasible in the current study given the need for the very large numbers of probands required to test for gene by environment effects. Third, despite the fact that these effects were independent of whether data about CP and EMO was derived from teachers or parents, there remained a possibility of shared-method variance. In future analyses it would be good if the assessment of EE towards the patient was based on both the mother's responses during an interview and on direct observation of actual behaviour during mother–child interaction. Fourth, the effects of only three genes were assessed. While this is a strength, as the selection of genes was based on hypothesis rather than a data trawl, quite clearly

there may be many other genes implicated in the relation between CP and ADHD. MAOA and COMT are obvious examples but these had not been genotyped for the whole IMAGE sample at the time of the analyses in this paper. Finally, this was a cross-sectional study and therefore the causal relationship between EE and CP and EMO would need to be established in a longitudinal follow-up of the current sample.

In summary, the current results demonstrated a role for gene × family environment interaction in determining the presence of CP (and to a lesser extent EMO) in ADHD children in a very large nationally diverse cohort of ADHD patients (both children and adolescents). 5HTT and DAT1 genotypes appeared to moderate the impact of PMEE by reducing both the negative effects of low PMEE and the positive effects of high PMEE – perhaps by promoting a generalised insensitivity to this particular environmental factor. CP and EMO are a major source of clinical impairment in ADHD and an important target for both scientific study and clinical intervention. We have known for some time that variations in the quality of family environments may be implicated in the aetiology of these comorbidities (Taylor et al., 1996). The current results refine this understanding by illustrating the possibility that the genetic make-up of an individual may alter the degree to which a person is sensitive to their environment.

Acknowledgements

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Key points

- Individuals with ADHD frequently also display conduct and emotional difficulties.
- Family and parenting factors alter the risk that individuals with ADHD will develop such comorbidity. Genetic factors may make some individuals more susceptible to such influences.
- In the current study in general ADHD individuals whose mothers spoke with positive emotion about them had fewer conduct and emotional problems. Sensitivity to these protective effects varied as a function of the patient's dopamine and serotonin transporter genotypes.
- Parental expressed emotion may be a marker of a broader parenting style which can represent a target for treatment in ADHD. The therapeutic effects of altering expressed emotion may be determined by genetic factors.

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