

Does parental expressed emotion moderate genetic effects in ADHD? An exploration using a genome wide association scan.

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Abstract

Background: Studies of gene x environment (GXE) interaction in ADHD have previously focused on known risk genes for ADHD and environmentally mediated biological risk. Here we use GXE analysis in the context of a genome-wide association scan to identify novel genes whose effects on ADHD symptoms and comorbid conduct disorder are moderated by high maternal expressed emotion (EE).

Method: 600,000 SNPs were genotyped in 958 ADHD proband-parent trios. After applying data cleaning procedures we examined 429,981 autosomal SNPs in 909 family trios. ADHD symptom severity and comorbid conduct disorder was measured using the Parental Account of Childhood Symptoms interview. Maternal criticism and warmth (i.e., EE) were coded by independent observers on comments made during the interview.

Results: No GXE interactions reached genome-wide significance. Nominal effects were found both with and without genetic main effects. For those with genetic main effects 36 uncorrected interaction p-values were less than 10^{-5} implicating both novel genes as well as some previously supported candidates. These were found equally often for all of the interactions being investigated. The observed interactions in SLC1A1 and NRG3 SNPs represent reasonable candidate genes for further investigation given their previous association with several psychiatric illnesses.

Discussion: We find evidence for the role of EE in moderating the effects of genes on ADHD severity and comorbid conduct disorder, implicating both novel and established candidates. These findings need replicating in larger independent samples.

Key Words: ADHD; Gene-by-environment interaction; FBAT; Genome-wide association; Hostility.

INTRODUCTION:

Behaviour genetic studies using twin and adoption designs provide compelling evidence for a genetic basis to Attention Deficit/Hyperactivity Disorder [Mick and Faraone 2008]. Molecular genetic studies focusing on candidate genes have identified a number of DNA variants showing statistically significant associations with the ADHD diagnosis [Faraone et al., 2005]. The most consistent effects are for variants at or near genes coding for dopamine function [Li et al., 2006]. However, the size of these effects for individual variants is typically very small and even in sum they account for only a small fraction of causal variance. Our recent Genome-Wide Association Scan (GWAS) of ADHD, including 600,000 individual SNPs identified a number of promising new genetic markers of nominal significance, however, no individual associations reached genome-wide significance [Neale et al., submitted]. This genome-wide negative result included SNPs in regions previously supported in candidate gene studies. This result is consistent

with the view that ADHD is a highly complex and heterogeneous genetic condition, with multiple genes of very small effect implicated to different degrees across affected individuals.

There are a number of promising approaches to partitioning genetic heterogeneity in ADHD [Thapar et al., 2007]. One approach, involves identifying phenotypic characteristics that define subgroups of patients affected by specific sets of genes so that a more direct mapping of specific genes to disorder can be made [Sonuga-Barke et al., 2008; Caspi et al., 2008; Crosbie et al., 2008]. Identifying alternative ADHD-related phenotypes, defined both a priori (e.g., hyperactive-impulsive vs. inattentive symptoms) and on empirical grounds using multivariate techniques, proved a useful way of identifying novel genetic candidates in an alternative analysis of the IMAGE GWAS data mentioned above [Lasky-Su et al., submitted; Neale et al., submitted; see

also Oades, 2002]. Genetic heterogeneity can also be partitioned in terms of variations in environment to which individuals are exposed. Genes interact with environments so that increased liability for a disorder associated with a gene may be seen more for individuals exposed to particular environmental risks [Rutter et al., 2006]. In fact, synergistic interaction of this sort, may mean a significant effect of a particular gene is present only for exposed individuals, with no apparent effect for those unexposed [Caspi et al., 2003; Khan et al., 2003]. In this case, the size of effect of an individual gene would be determined in part by the degree of exposure to the environmental risk operating within the affected population as a whole. This means even quite large genetic effects operating only within sub-populations affected by a specific environmental factor may not reach statistical significance when effects are aggregated across the ADHD population as a whole.

There has recently been considerable interest in gene x environment interactions (G x E) in ADHD following attempts to better understand the pathogenic mechanisms underpinning the disorder [Thapar et al., 2007]. The focus has been on known candidate genes and environmentally-mediated biological risk factors. For instance, pre-natal exposure to nicotine [e.g., Becker et al., 2008; Khan et al., 2003; Neuman et al., 2007] and alcohol [e.g., Brookes et al., 2006a] have both been shown to interact with variations in dopamine genes to increase the levels of ADHD symptoms. In the current paper we exploit the recent development of high throughput techniques that allow the genotyping of hundreds of thousands of single-nucleotide polymorphisms (SNPs) across the genome combined with new analytical techniques [Vansteelandt, 2008]. This allowed us to identify associations for further study including (i) novel GXE with known and novel genes that also have a nominally significant main effect (with potentially important consequences for models of ADHD pathophysiology) and (ii) G x Es that occur even though there are no genetic main effects: A

situation suggestive of genetic effects in environmentally defined sub-populations of ADHD children. Like other papers included in this special issue the reported analysis was based on 958 ADHD-parent trios from the International Multicenter ADHD Genetics Project (IMAGE) genotyped on a 600K SNP GWAS as part of the Genetics Analysis Information Network (GAIN)[http://www.fnih.org/GAIN2/home_new.shtml].

The family environment is the focus for the current analyses. ADHD children are exposed to raised levels of negative parent-child relationships, family dysfunction, parenting stress and psychopathology [Johnston and Mash, 2001], effects which seem stronger where ADHD is comorbid with externalizing problems [Pfiffner et al., 2005]. Evidence already exists for G x E effects on externalizing problems involving the family environment. Pressman and colleagues [Pressman et al. 2006] demonstrated high levels of conflict in the families of children with ADHD and showed that these effects appeared to mediate familial patterns of impairment in siblings. More direct evidence of G x E effects comes from studies showing that maternal insensitivity is associated with preschool externalizing disorders only in children carrying the 7-repeat allele of DRD4 [Bakermans-Kranenburg and van Ijzendoorn, 2006], and that parental warmth was protective for externalizing disorders only in the absence of the 7-repeat allele, but only for African-American children [Propper et al., 2007].

Expressed emotion (EE) is an important marker of parental negative attitudes and behavior directed towards their children [Vaughn and Leff, 1976]. It incorporates aspects of criticism, hostility or emotional over-involvement and is unusually high in ADHD [Baker et al., 2000; Daley et al., 2003; Psychogiou et al., 2007]. Current

evidence suggests that EE does not play a main causal role in ADHD but rather that ADHD elicits high levels of EE from some parents [Taylor et al. 1996; Schachar et al., 1987]. However, it remains possible that high EE may affect ADHD levels in a genetic sub-group of ADHD patients perhaps exacerbating already existing problems, increasing the severity of the condition or its persistence into adolescence and adulthood. Furthermore there is good evidence that EE associated with ADHD is implicated in the emergence of comorbid conduct disorder over time [Taylor et al., 1996]. In an analysis of the IMAGE sample there was a consistent pattern of association between parental criticism and lack of warmth and oppositional defiant and conduct disorder [Psychogiou et al., submitted] an association that was moderated by variations in serotonin and dopamine transporter genes [Sonuga-Barke et al., submitted]. In the current paper we explored the moderating effects of EE on genetic effects for ADHD severity and comorbid conduct problems. Our aim was to identify novel G X E effects. Through this we hoped to illustrate the power of this approach for partitioning genetic heterogeneity and identifying novel genes whose effects may be confined to EE-defined subgroups.

METHODS:

Subjects

Families were collected by the International Multicenter ADHD Genetics (IMAGE) project. Families were identified through ADHD probands aged 5 to 17 attending outpatient clinics at the data collection sites in Europe and Israel. A total of 958 affected proband-parent trios were initially selected for GWAS. Family members were Caucasians of European origin from Belgium, Germany, Ireland, the Netherlands, Spain, Switzerland, and the United Kingdom, as well as Israel. Of these, 936 probands were initially ascertained as having DSM-IV combined type ADHD. Twenty-two probands who did not meet combined subtype ADHD diagnosis were included because they either met the criteria for the inattentive or hyperactive subtypes, or they missed the DSM-

IV combined type diagnosis by a single item. Since our analysis is primarily based on ADHD diagnosed individuals, we can only generalize our findings to this group of individuals.

Phenotypic Outcome

Two quantitative traits intended to measure ADHD and conduct disorder (CD) severity were generated as the outcome phenotypes for use in the association analyses using the Parental Account of Childhood Symptom (PACS). The PACS is a reliable, semi-structured interview that measures children's behavior [Taylor et al. 1986a; Taylor et al. 1986b]. The PACS was administered by investigators at each center to the parents of the affected child. There was centralized training for all who administered the PACS and the coding of responses to questions were standardized. Parents were asked to describe their child's symptoms during medication-free periods, both currently and over the past two years. The PACS assesses the DSM-IV symptoms for both ADHD and CD in children and adolescents. The inattentive and hyperactive-impulsive symptoms measured by the PACS for ADHD include: 1) inability to pay attention to details; 2) difficulty with sustained attention in tasks or play activities; 3) listening problems; 4) difficulty following instructions; 5) problems organizing tasks and activities; 6) avoidance or dislike of tasks that require mental effort; 7) tendency to lose things like toys, notebooks, or homework; 8) distractibility; and 9) forgetfulness in daily activities, 10) fidgeting or squirming; 11) difficulty remaining seated; 12) restlessness; 13) difficulty playing quietly; 14) always seeming to be "on the go"; 15) excessive talking; 16) blurting out answers before hearing the full question; 17) difficulty waiting for a turn or in line; and 18) problems with interrupting or intruding. The CD symptoms include: 1) severity of destructiveness; 2) frequency

of destructiveness; 3) severity of aggressiveness; 4) frequency of aggressiveness; 5) bully; 6) start fights; 7) used weapon; 8) cruel to animals; 9) cruel to people; 10) stay out at night; 11) tried to set fire to something; 12) run away from home; 13) broken into a building or car; 14) truant from school; 15) threatened anyone with a gun; 16) mugging, extortion, or robbery; and 17) forced someone into sexual activity. Each symptom is given a value of 0 or 1 to demarcate the absence or presence of the symptom respectively. The total symptom counts for both ADHD and CD were generated by summing over all symptoms, making the maximum symptom count 18 and 17 respectively. The symptom counts for ADHD and CD were used as the phenotypic traits in the GXE analyses.

Environment measures

Parental Expressed Emotion: Assessment of mothers' and fathers' expressed criticism and warmth was made using codings derived from the Camberwell Family Interview on the basis of parental responses during the clinical assessment. In this analysis, we only used the measures of maternal expressed criticism and warmth, as these variables were more complete. 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.

Criticism was assessed by statements which criticized 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 dislikes, resents, disapproves of, or is angered or annoyed by the child's behavior 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 baseline. 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 indication that the parent did not like or approve of the child's behavior. *Some criticism (2)* was coded when there were statements of dissatisfaction indicating that the parent was bothered, irritated or upset by the child's behavior 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 or dislikes child's behavior. Inter-rater reliability on these codings has been satisfactory; ranging from .79 to .86 [Schachar et al., 1987]. In order to ensure cross-site consistency within the IMAGE project in measurement and coding of PACS scales including those relating to expressed emotion all interviewers from each site attended a 5-day training course in the UK. The chief investigator at each site attended annual inter-rater reliability exercise and was responsible for reliability in their native site. A mean Kappa coefficient across all sites of 0.88 (range 0.71-1.00) and an average agreement percentage of 96.6% (range 78.6-1.00) were obtained indicating a substantial level of inter-rater agreement (Chen et al., 2008).

Genotyping

Details of the genotyping and data cleaning process were reported elsewhere [Neale et al., submitted]. Briefly, genotyping was performed by Perlegen Sciences using the Perlegen platform. The Perlegen Array uses which has 600,000 tagging SNPs designed to be in high linkage disequilibrium with untyped SNPs for the three HapMap populations. Genotype data cleaning and quality control procedures were done by The National Center for Biotechnology Information (NCBI) using the GAIN QA/QC Software Package (version 0.7.4) developed by Gonçalo Abecasis and Shyam Gopalakrishnan at the University of Michigan. A copy of the software is available by e-mailing gopalakr@umich.edu or goncalo@umich.edu. Data were removed on the basis of the following quality control metrics: 1) sample genotype call rate < 95%; 2) gender discrepancy; 3) per-family Mendelian errors >2%; 4) sample heterozygosity < 32%; 5) genotype call quality score cutoff of > 10%; 6) a combination of SNP call rate and minor allele frequency (MAF) (a) $0.01 \leq \text{MAF} < 0.05$ and call rate $\geq 99\%$; b) $0.05 \leq \text{MAF} < 0.10$ and call rate $\geq 97\%$; and c) $0.10 \geq \text{MAF}$ and call rate $\geq 95\%$; 7) deviation from Hardy-Weinberg equilibrium with a p-value < 0.000001; and 8) duplicate sample discordance. The removal of additional SNPs based on other GAIN samples that was reported in the initial manuscript were not applied to the data presented here [Neale et al., in press].

Statistical Analyses

We apply the FBAT1-Interaction methodology proposed by Vansteelandt et al. [Vansteelandt et al., 2008] for nuclear families where gene-by-environment interactions can be modeled using SNPs, quantitative phenotypes, and complex exposure variables. Vansteelandt et al.'s [Vansteelandt et al., 2008] method uses causal inference to derive estimating equations that generate an estimate of the main genetic effect, β_1 , and the gene-by-environment interaction, β_2 after accounting for the main genetic effect. The general principle behind FBAT-Interaction is that after removing the

overall main genetic effect, the phenotype should not depend on the genotypes conditional on the environmental exposure under the null hypothesis. Such a test is therefore valid regardless of the estimate for the main genetic effect. This class of estimates and tests is available in the program PBAT [Lange et al., 2004] [<http://biosun1.harvard.edu/~clange/pbat.htm>].

Using this methodology we assessed possible SNP-by-environment interactions using the GWAS data. There were two environmental variables, a measure of mother's warmth and mother's criticism, and two quantitative phenotypes, ADHD symptom count and CD symptom count, resulting in four different SNP-by-environment interaction analyses: 1) Mother's warmth with ADHD symptom count; 2) Mother's warm with CD symptom count; 3) Mother's criticism with ADHD symptom count; and 4) Mother's criticism with CD symptom count. These analyses were run looking at additive, dominant, and genetic modes or inheritance. Given that there were four phenotype/environmental exposure combinations and three genetic analyses, in total 12 GWAS were analyzed. The most significant associations are presented.

We also examined the interaction p-values for SNPs in a set of pre-specified ADHD candidate genes that was generated by the IMAGE study investigators [Brookes et al., 2006a]: NR4A2, PER2, SLC6A1, DRD3, SLC9A9, HES1, ADRA2C, ADRB2, ADRA1B, DRD1, HTR1E, DDC, STX1A, ADRA1A, NFIL3, ADRA2A, ADRB1, SLC18A2, TPH1, BDNF, FADS1, FADS2, ADRBK1, ARRB1, DRD2, HTR3B, TPH2, SYT1, HTR2A, SLC6A2, ARRB2, PER1, PNMT, CHRNA4, COMT, ADRBK2, and CSNK1E.

When correcting for the overall number of statistical tests, there are two environmental variables, two phenotypes,

and all of the GWAS SNPs. Given this information, a p-value less than 1.14×10^{-8} would achieve genome-wide significance. When considering the candidate genes alone, a significant finding would need to be less than 9.06×10^{-6} , which corrects for all of the comparisons at all candidate genes and all phenotype/environmental exposures. In this report we display our findings but look to future replications to verify or refute the nominally significant results.

RESULTS:

After the quality control procedures, 438,784 markers were available for analytic use. The G x E interaction analysis in PBAT cannot analyze sex-linked markers. Consequently, we restricted our statistical analysis to 429,981 autosomal markers. A total of 2803 individuals, 1865 founders and 938 non-founders were included after the cleaning process. Of these individuals, 29 offspring did not have clinical data and/or parental genotypes resulting in 909 individuals used in the analysis. A summary of the sample is listed in table 1

G x E interaction found in the context of genetic main effects: No SNP achieved genome-wide significance, i.e., $p < 10^{-8}$. The 37 interaction p-values $< 10^{-5}$ are summarized in tables 2 and 3. Among these association tests, the interaction of SNPs in PIWIL4 and KIF6 and mother's warmth were associated with ADHD symptom count. For CD symptom count, strong interactions between mother's warmth and SNPs in RIT1, ADH1C, SLC6A1, A2BP1, and MFHAS1 were observed. One of these findings is in one of the ADHD candidate genes that had been selected *a priori* (SLC6A1, the gamma-aminobutyric acid transporter). Finally, interactions between SNPs in PPM1K and ZBTB16 and mother's criticism were observed when the CD symptom count was used as the phenotype. For all of these interaction associations, the association p-value for the main genetic effect is less than 0.01.

Table 1:

Descriptive Statistics of the Individuals used in the GWAS analyses (Standard Deviation, SD)

Number of parents	1865
Number of children	938
Number of ADHD diagnosed children	933
Gender distribution of the offspring	
Male (percent)	816 (86.99)
Female (percent)	122 (13.01)
Age among offspring (SD)	10.88 (2.81)
Average total number ADHD symptoms (SD)	16.1 (1.92)
Average total number of CD symptoms (SD)	4.55 (2.64)
Mean overall measure of maternal warmth (min, max, SD)	1.43 (0, 3, 0.87)
Mean overall measure of maternal criticism (min, max, SD)	1.74 (0, 4, 0.91)

The lowest p-value associations for each candidate gene are listed in table 4. At a nominal significance level of 0.01, we found associations at the following ADHD candidate genes: ADRA1A, ADRA1B, ADRA2C, ARRB1, DBH, DDC, DRD3, FADS2, HTR2A, HTR3B, SLC6A1, SLC6A2, SLC6A3, SLC6A4, SLC9A9, SYT1, TPH1, and TPH2. One association in SLC6A1, rs9990174, was significant after adjusting for the comparisons among the 2 phenotypes, 4 environmental variables, and all of the candidate gene SNPs (p-value = 5.91×10^{-6}).

GXE interaction found in the absence of genetic main effects: There were also nominally significant G x Es found without main genetic effect ($p > 0.05$). A summary of these findings with an interaction p-value < 0.001 are listed in table 5. Nineteen of these related to CD while only 6 related to ADHD severity. A number of these were in genes previously associated with psychiatric disorders thought unrelated to ADHD. For instance, rs10974610 located in 9p24 in an intronic region of neuronal/epithelial high affinity glutamate transporter (SLC1A1) and rs17746658, located in an intronic region of Neuregulin-3 (NRG3) which were both implicated in G x E for CD have been implicated in the pathophysiology of several psychiatric disorders.

Table 2:A Summary of the Interaction association p-values less than 10^{-5} using total ADHD symptoms as the phenotype

Environmental Variable	SNP	Allele (Freq)	Genetic Model	# of Info Families	Main Genetic Effect (p)	Inter-action Effect Estimate	Inter-action p-value	SNP Position	Gene/Function
Mother's Criticism	rs2825388	A (0.40)	Recessive	348	3.27E-05	1.06	7.82E-06	chr21:19458520	
	rs2827093	A (0.72)	Additive	555	3.37E-05	0.72	4.78E-06	chr21:22142959	
Mother's Warmth	rs2212361	C (0.26)	Dominant	511	6.96E-06	-0.97	9.08E-07	chr11:93951971	PIWIL4: Intron
	rs1161463	C (0.79)	Additive	476	1.20E-06	-1.72	1.45E-06	chr13:28329338	
	rs4714261	T (0.17)	Dominant	416	9.41E-06	0.95	2.03E-06	chr6:39647185	KIF6: Intron
	rs1161457	T (0.83)	Additive	424	8.34E-06	-1.41	2.14E-06	chr13:28332854	
	rs1792040	A (0.17)	Dominant	411	1.74E-05	0.99	2.33E-06	chr13:28274943	
	rs1161453	T (0.79)	Additive	482	2.26E-06	-1.47	2.33E-06	chr13:28315484	
	rs7126782	G (0.28)	Dominant	522	2.12E-05	-0.95	2.42E-06	chr11:93963488	PIWIL4: Intron
	rs1161457	C (0.17)	Dominant	405	2.41E-05	0.95	2.75E-06	chr13:28332854	
	rs1792040	A (0.83)	Additive	430	1.75E-05	-1.25	3.05E-06	chr13:28274943	
	rs1161463	T (0.21)	Dominant	449	3.18E-05	0.84	3.34E-06	chr13:28329338	
	rs4714261	T (0.83)	Additive	429	1.36E-05	-1.30	4.10E-06	chr6:39647185	KIF6: Intron
	rs11752175	T (0.16)	Dominant	410	1.17E-05	0.99	4.65E-06	chr6:39625468	KIF6: Intron
	rs1161453	C (0.21)	Dominant	454	5.05E-05	0.82	5.53E-06	chr13:28315484	
	rs2212361	T (0.74)	Additive	571	2.53E-05	0.69	5.78E-06	chr11:93951971	PIWIL4: Intron
	rs2360997	C (0.86)	Additive	370	6.32E-05	-1.30	7.79E-06	chr14:75882244	
rs10049246	G (0.59)	Additive	662	3.25E-05	0.60	8.09E-06	chr3:187169435		
rs4875598	G (0.34)	Dominant	543	1.31E-05	-0.94	8.91E-06	chr8:5449161		

Table 3:A Summary of the Interaction association p-values less than 10^{-5} using total CD symptoms as the phenotype

	SNP	Allele (Freq)	Genetic Model	No. Inform. Families	Main Genetic Effect (p)	Inter-action Effect Estimate	Inter-action p-value	SNP Position	Gene/Function*
Mother's Criticism	rs893971	C (0.40)	Dominant	482	7.54E-06	1.23	3.57E-06	chr4:89422694	PPM1K: Intron
	rs719593	T (0.86)	Additive	373	6.90E-05	-2.05	4.83E-06	chr2:41858501	
	rs4321143	G (0.28)	Dominant	524	0.000135	1.13	6.57E-06	chr15:93957372	
	rs893971	T (0.60)	Additive	634	6.46E-06	-1.15	6.88E-06	chr4:89422694	PPM1K: Intron
	rs7162535	C (0.28)	Dominant	522	0.00014	1.12	7.04E-06	chr15:93959602	
	rs16880441	G (0.08)	Dominant	230	7.37E-05	1.63	8.56E-06	chr6:89032696	
rs17116334	T (0.16)	Dominant	392	2.28E-05	1.30	8.99E-06	chr11:113466972	ZBTB16: Intron	
Mother's Warmth	rs13188771	A(0.17)	Recessive	83	0.008	-4.24	1.70E-06	chr5:100976194	
	rs6500744	C (0.53)	Additive	648	3.65E-05	0.91	2.54E-06	chr16:6053662	A2BP1: Intron
	rs1789891	A (0.14)	Dominant	346	3.44E-05	1.47	3.17E-06	chr4:100469442	ADH1C: Downstream
	rs1229863	T (0.14)	Dominant	350	4.57E-05	1.45	3.77E-06	chr4:100471409	ADH1C: Downstream
	rs332034	A (0.85)	Additive	382	6.55E-05	1.05	5.77E-06	chr8:8754417	MFHAS1: Intron
	rs9990174	T (0.33)	Recessive	257	3.56E-05	-2.52	5.91E-06	chr3:11015439	SLC6A1: Intron
	rs2395528	T (0.23)	Dominant	486	4.94E-05	-1.46	6.17E-06	chr10:79770867	
	rs2282301	A (0.23)	Recessive	139	4.90E-06	2.88	7.01E-06	chr1:154135249	RIT1: Downstream
	rs17664267	T (0.19)	Dominant	426	2.24E-05	1.39	7.10E-06	chr18:39062796	
rs8073783	C (0.49)	Dominant	417	1.56E-05	-1.76	7.81E-06	chr17:48592940		
rs604381	A (0.32)	Dominant	500	4.24E-05	1.26	8.29E-06	chr2:37830521		

* A blank indicates that the SNP is not within a gene

Table 4. The most significant interaction associations at alpha = 0.01 or better for the *a priori* specified ADHD candidate genes

Candidate Gene	Number of SNPs per Gene	Number of associated test by phenotype							
		ADHD symptom Count				CD symptom Count			
		Criticism	SNP	Warmth	SNP	Criticism	SNP	Warmth	SNP
ADRA1A	47	0.00277	rs10503800						
ADRA1B	18					0.0023	rs17455628		
ADRA2C	2	0.0066	rs7692883						
ARRB1	2	0.00186	rs687652						
DBH	21	0.0044	rs2797851	0.0021	rs2797851	0.0098	rs1611115		
DDC	24	0.000998 0.0034 0.0013 0.0061 0.0062 0.0035	rs11974297 rs11489734 rs7804365 rs7807335 rs921451 rs978784	0.000587 0.0047 0.0099 0.0078 0.0064 0.0049 0.0033	rs7804365 rs10499694 rs11238133 rs11974297 rs1470750 rs17634958 rs4602840				
DRD3	15							0.00495 0.0099	rs3773678 rs324032
FADS2	9	0.00778	rs174626			0.0056	rs174570		
HTR2A	40			0.00091 0.001 0.0036	rs1923886 rs6561333 rs7330636	0.0042 0.0077	rs1885884 rs1002513.		
HTR3B	10					0.0019	rs4936286		
SLC6A1	23					0.001 0.0067	rs1710892 rs1919075	5.91E-06	
SLC6A2	27							0.009	rs16955708
SLC6A3	13	0.0056	rs3863145			0.0065	rs458334.	0.00144	rs2550936
SLC6A4	6			0.008	rs2020939				
SLC9A9	181	0.0099	rs11916000	0.00063 0.0021 0.0091 0.0064 0.0025 0.004 0.0015	rs11721078 rs11916000 rs16854336 rs1868183 rs1875463 rs4839595 rs746351	0.0024 0.0026 0.006 0.004 0.0047	rs2293328 rs11916000 rs1242076 rs1554671 rs9289668	0.0054 0.0065 0.0083 0.0057	rs11916000 rs9289667 rs9332458 rs9848371
SNAP25	29			0.0092	rs6133855				
SYT1	16			0.0066	rs7300645				
TPH1	7					0.00396	rs623580		
TPH2	19							0.000499 0.0038 0.0011	rs11179050 rs1487275 rs4488237

DISCUSSION:

Environmental exposures may moderate the degree of association between genetic markers and ADHD, its severity and its comorbidity [Thapar et al., 2007]. Therefore studying the interaction between environmental exposures and multiple genetic markers from across the genome can facilitate the search for novel genes and help elucidate underlying pathogenic mechanisms. The current analysis using a quantitative phenotype for ADHD severity and CD comorbidity as well as environmental measures of maternal warmth and criticism in a GXE interaction GWAS analysis, represents the first attempt at such an analysis. No GXE interactions met genome-wide significance after adjusting for all comparisons; however, there are several findings of potential interest among the association tests with the lowest p-values. In general a number of aspects of the results were noteworthy.

First, the results highlight the possible putative role that the family environment may have as a moderator of genetic effects in ADHD. Previous research has largely focused on biologically mediated effects such as those associated with prenatal exposure to nicotine [Khan et al., 2003] and alcohol [Brookes et al., 2006b]. Specifying the biological mechanism that might be responsible for gene x social environment interactions is beyond the scope of the current paper. Future research should focus on fundamental mechanisms underpinning the environmental factors that regulate gene expression. For instance, the hypothesis that adverse social environments (here marked by maternal hostility) may 'switch-off', or socially benign environments 'switch-on' genetic effects [Peedicayil, 2007] is a hypothesis worth exploring further. 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 may occur through epigenetic effects such as DNA methylation [Parent et al., 2005; Diorio and Meaney, 2007]. However, for these effects to account for the current finding would require the marker of environmental adversity, in this case an interview-based measure of EE, to be indexing a broadly based, stable and powerful social influence of long-standing, that can induce fundamental changes in gene expression. If this were the case then chronic exposure to these sorts of histories of aversive family interactions could conceivably have the potency to alter gene expression.

Second, we provide preliminary evidence that G x E interactions *may* occur both in the presence or the absence of a genetic main effect. This is important as it clearly suggests that in addition to potentially facilitating the understanding of the pathophysiology of ADHD and related conditions, studying G x E interactions *may* have the potential to identify novel genes in its own right, most likely by helping to partition the genetic heterogeneity in the sample. While further research is required to elucidate the specifics of these effects, one hypothesis worth exploring is that there are environmentally defined sub-groups with a distinctive pattern of genetic risk.

Third, possible G x E effects were observed for both ADHD severity and conduct disorder comorbidity, although none of these findings survived the multiple testing correction. Previous studies of ADHD and EE have focused on conduct disorder as the primary outcome measure and have suggested that EE may do little to alter the fundamental expression of ADHD. The current results suggest, in contrast, that maternal expressions of warmth and criticism may

act together with genetic factors to alter the severity of ADHD. Given the cross sectional nature of the data in the current study it is impossible to disentangle the direction of causation, to say whether criticism and low warmth combine with specific genes to increase the severity of ADHD, or whether children with high levels of ADHD who carry specific alleles elicit high levels of EE from parents. In this regard it is interesting that the relative proportion of nominal effects for ADHD severity and CD was different for GXE effects that were and were not accompanied by genetic main effects. There were proportionately fewer GXE effects for ADHD severity in the case of no genetic main effects. One possible interpretation of this is that for ADHD severity variations in EE typically act to 'fine tune' genetic main effects while for CD the EE levels mark a distinctive genetic subtype.

Fourth, the two components of EE measured may play different and distinctive roles in relation to the two outcomes. First, although both warmth and criticism were implicated for each phenotype, these two elements may be interacting with different genes. Second, levels of warmth appear a more powerful marker of the moderators of ADHD severity, with only a small number of interactions with criticism reaching nominal significance for this outcome. For conduct disorder, both warmth and criticism were equally likely to be implicated. These results highlight the value of looking at warmth and criticism separately when exploring GXE interactions.

The data relating to individual genes are also of some note. Of the 37 uncorrected interaction p-values less than 10^{-5} , the most compelling finding is the interaction of rs9990174 and CD symptoms with a p-value of 5.19×10^{-6} .

Rs9990174 is in an intronic region of solute carrier family 6, member 1 (SLC6A1, encoding the gamma-aminobutyric acid (GABA) transporter) located on 3p25-p24. This is a candidate gene that was selected a priori by our investigators because, early in development, it plays a role in both brain maturation and later functions as a neurotransmitter [Kandel et al., 1991]. There are also two additional SNPs (rs1710892, rs1919075) in SLC6A1 that interact with mother's criticism (p-values < 0.01) that are associated with CD symptoms. Of the recent GWAS analyses using the IMAGE data, SLC6A1 emerges in the gene by environment analyses more strongly than using any other phenotype or analytic method.

There were several notable nominally significant associations among the list of ADHD candidate genes. Given the number of SNPs at each gene, DDC had the largest percentage of nominally significant associations (p-value < 0.01) among the candidate genes with 11 of the 24 SNPs in DDC having an association p-value less than 0.01. All of these associations were observed with the ADHD symptom counts and both environmental measures. SLC9A9 had association p-values < 0.01 for all phenotypes and environmental measures. This is not surprising; however, as 181 SNPs were genotyped in SLC9A9 to characterize the gene and therefore one would expect association p-values less than 0.01 by chance. Several of the neurotransmitter transporters (SLC6A1, SLC6A2, SLC6A3, SLC6A4, SLC6A6, and SLC9A9) have gene by environment interaction p-values less than 0.01, suggesting that these transporters may have important interaction effects.

Turning to the G x E effects that occurred without genetic main effects as mentioned above these are predominantly for CD. Rs10974610 is located in 9p24 in an intronic region of neuronal/epithelial

high affinity glutamate transporter (SLC1A1) and was found to have a nominally significant GXE interaction with CD symptom count and overall maternal warmth. SLC1A1 has been implicated in the pathophysiology of several psychiatric disorders including schizophrenia, obsessive compulsive disorder, and bipolar disorder and is therefore a plausible candidate gene for ADHD and CD. An associations study found this gene and schizophrenia to be associated [Nudmamud-Thanoi et al., 2007] and microarray data suggest that there is decreased transcriptional expression among individuals diagnosed with schizophrenia [McCullumsmith and Meador-Woodruff, 2002]. The chromosomal region containing this gene has also been identified in a linkage analysis of early-onset obsessive-compulsive disorder [Veenstra-Vander Weele et al., 2001]. Finally, a translocation in SLC1A1 was found to segregate with bipolar disorder [Baysal et al., 1998]. SLC1A1 presents a good biologic candidate for CD because it is a neuronal and epithelial glutamate transporter. Although it has been studied in relation to other psychiatric disorders, currently there is no research of this gene in relation to CD. Rs17746658, located in an intronic region of neuroregulin-3 (NRG3), was associated in a gene by environment interaction with CD and overall maternal criticism. Although neuregulin-1 has most commonly been associated with psychiatric disorders, NRG3 has also been implicated in several psychiatric disorders, most notably schizophrenia, and has also been found to have gene-gene interaction with other neuroregulin genes [Benzel et al., 2007]. Of the GWAS studies with the IMAGE data to date, no other phenotypes have identified SNPs in either SLC1A1 or NRG3 as possible candidate genes to follow-up in replication samples [Neale et al. in press; Lasky-Su et al., in press]. Although

these SNPs were identified by having low nominal p-values, their confirmation requires replication. Clearly these findings are just preliminary and future replications are the only way to verify or refute.

In summary the current GWAS analysis provides some initial evidence, at nominal levels of significance, for the role of parental EE as a moderator of novel genetic effects in relation to both ADHD severity and the presence of comorbid conduct disorder. Of particular note are the effects that occur without a genetic main effect typically related to CD that implicate previous candidates for psychiatric disorders and may be related to an ADHD subtype susceptible to social environmental influence for CD. These effects need to be replicated in larger independent samples and to be explored in more detail using a range of different measures on mother's attitudes and mother child interaction.

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Table 5: A Summary of the Interaction association p-values less than 0.001 and no main genetic effect ($p > 0.05$) using total ADHD and CD symptoms as the phenotype.

Environmental Variable and Phenotype	SNP	Allele (Freq)	Model	Info Fam	Main Genetic Effect (p)	Interaction Effect Estimate	Interaction p-value	SNP position	Gene(s)	Role
Mother's Warmth/CD Symptom Count	rs10974610	G (0.12)	Recessive	44	0.061	-5.90	0.00021812	chr9:4530588	SLC1A1	Intron
	rs1871437	T (0.10)	Recessive	23	0.105	-12.66	0.000622013	chr15:90536919		
	rs10980865	C (0.12)	Recessive	36	0.063	-8.2	0.000712936	chr9:113112797		
	rs17714301	A (0.13)	Recessive	44	0.071	-18.81	0.000809531	chr19:61260662	NALP5	Intron
	rs1335204	C (0.10)	Recessive	35	0.111	-12.57	0.000819165	chr10:25946497		
	rs12776431	C (0.10)	Recessive	35	0.111	-12.57	0.000819179	chr10:25937098	GPR158	Downstream
	rs2921007	T (0.13)	Recessive	58	0.132	-30.50	0.000890434	chr8:8269681		
	rs12108098	T (0.12)	Recessive	42	0.125	-19.27	0.000978051	chr3:61487959		
Mother's Criticism/CD Symptom Count	rs6889324	A (0.11)	Recessive	36	0.064	-6.27	0.000193258	chr5:139420647		
	rs16960350	C (0.13)	Recessive	48	0.053	-3.00	0.000600393	chr17:62321449	CACNG5	Downstream
	rs12603171	T (0.14)	Recessive	61	0.071	-5.61	0.00073312	chr17:78566042	B3GNTL1	Intron
	rs17127947	G (0.20)	Recessive	130	0.062	-6.07	0.000750021	chr11:123282512	OR8D4	Coding exon
	rs17127950	G (0.21)	Recessive	128	0.063	-6.04	0.000782704	chr11:123282622	OR8D4	Coding exon
	rs17746658	C (0.09)	Recessive	30	0.090	-15.00	0.000832163	chr10:84561249	NRG3	Intron
	rs4422732	G (0.14)	Recessive	75	0.067	-4.52	0.000891919	chr8:82966209		
	rs6877423	A (0.11)	Recessive	33	0.122	-5.25	0.000975995	chr5:139418290		

	rs847971	G (0.09)	Recessive	29	0.053	-7.42	0.000982706	chr7:12448530		
	rs290439	G (0.19)	Recessive	106	0.080	-2.86	0.00081694	chr20:52116797	BCAS1	Intron
Mother's Warmth/ADHD Symptom Count	rs17492765	T (0.16)	Recessive	73	0.056	-2.30	0.000188326	chr15:93842747		
Mother's Criticism/ADHD Symptom Count	rs1590106	G (0.12)	Recessive	52	0.079	-3.17	0.000352921	chr18:66841766		
	rs11609420	A (0.25)	Recessive	164	0.062	-1.07	0.000798172	chr12:93676075		
	rs2476509	G (0.08)	Recessive	35	0.089	-4.48	0.000877486	chr13:21733572		
	rs10865184	G (0.83)	Additive	423	0.073	0.54	0.00093328	chr2:42749320	MTA3	Intron