Gene-Environment interactions in the development of combined type ADHD: Evidence for a synapse-based model

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KEYWORDS
ADHD • DAT • DRD4 • CHRNA4 • brain development

ABSTRACT

To determine the mechanism of interaction of prenatal smoking exposure and child genotype in the development of attention deficit/hyperactivity disorder (ADHD), polymorphisms in the CHRNA4 gene were tested for interactions with prenatal smoking exposure on risk for ADHD subtypes using multiple logistic regression. An exon 5 polymorphism demonstrated a significant interaction with history of maternal smoking during pregnancy for increasing risk for severe combined type ADHD (OR = 3.0, 95% CI 1.1-8.4 for population-defined severe combined type, OR = 3.9 95% CI 1.2-13.1 for DSM-IV defined combined subtype ADHD). This interaction increased the effects of previously reported interactions for the DRD4 and DAT1 genes with prenatal smoking exposure. Given the known functions and the known areas of expression of these three genes at the dopaminergic synapse in the pre-frontal cortex, the results are compatible with a synapse-based model of the development of this form of ADHD. The subtype specificity of these findings supports the concept that ADHD is composed of a group of distinct disorders. © 2007 Wiley-Liss, Inc.

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ARTICLE TEXT
INTRODUCTION

The attention deficit/hyperactivity disorder (ADHD) syndromes are a common but impairing group of disorders starting in early childhood which are associated with problems in attention span, distractibility, hyperactivity and impulsivity. A variety of candidate genes have been proposed for ADHD [reviewed in Faraone et al., [2005]] As part of a genetic epidemiology study of ADHD we collected prenatal exposure data for a birth-records identified sample of twins [Neuman et al., [2007]]. Focusing on the effects of prenatal exposure to alcohol and cigarette smoke [Linnet et al., [2003]], multiple logistic regression models were developed to determine if genetic effects and prenatal smoking or alcohol use interacted to increase risk for ADHD subtypes after taking into account potential confounders. Assessing the relationship between prenatal smoking or alcohol exposure and two well-established candidate gene polymorphisms (3’ DAT1 440 and 480 bp alleles (SLC6A3) and the DRD4 exon 3 7-repeat allele) [Faraone et al., [2005]] we found significant evidence that interaction effects between genotypes at both loci and prenatal smoking exposure increased the risk for a severe combined type ADHD, defined using population based criteria. The overall risk of developing severe combined type ADHD for a child carrying the DRD4-7 repeat allele and the DAT1-440 bp allele was increased ninefold in the presence of pre-natal maternal smoking [Neuman et al., [2007]]. In this report we extend these findings to the CHRNA4 gene and propose a synapse mediation model to explain the observations.

Using birth records-based sampling of twins in the state of Missouri and general population-based twin registries in Australia and latent class analysis [a form of non-parametric cluster analysis; McCutcheon, [1987]] we identified naturally occurring clusters of the 18 DSM-IV ADHD symptoms which are more phenotypically homogeneous than DSM-IV ADHD subtypes [Todd et al., [2001]; Rasmussen et al., [2002], [2004]; Neuman et al., [2005]; Volk et al., [2006]]. Three of the population defined ADHD subtypes appear to be clinically relevant based upon impairment in school, home and other functioning [Todd et al., [2003a]; Volk et al., [2006]]. These population-based subtypes appear to be family specific as judged by monozygotic and dizygotic concordance rates, suggesting that the subtypes may be distinct [Todd et al., [2001]; Rasmussen et al., [2004]; Volk et al., [2006]]. Subtype genetic independence has been supported by the finding of specific candidate gene associations with the severe inattentive [Todd et al., [2003b]] and the severe combined population-based subtypes [Todd et al., [2005]] as well as the presence of subtype specific gene × gene and gene × environment interactions with prenatal exposure to smoking described above [Neuman et al., [2007]]. The population-based ADHD types only partially overlap with the DSM-IV conceptualizations with the largest overlap being with the DSM-IV combined type ADHD and the severe combined problems latent class [Todd et al., [2001]; Rasmussen et al., [2002]]. Essentially all DSM-IV combined type cases occur in the severe combined population-based ADHD subtype. About 20% of DSM-IV predominantly inattentive ADHD cases fall in this class and about 20% of the class is composed of individuals that do not meet criteria for a formal DSM-IV ADHD diagnosis. For the severe attentive problems population-based ADHD subtype, about 60% of DSM-IV predominantly inattentive cases occur in this subtype along with many individuals who do not qualify for a DSM-IV ADHD diagnosis [Todd et al., [2001]; Rasmussen et al., [2002]]. In terms of symptom counts, the severe combined population-based ADHD subtype has more than seven inattentive and seven hyperactive/impulsive symptoms on average while the severe attention problems population-defined ADHD class has more than six inattentive symptoms but two or fewer hyperactive/impulsive symptoms. The few symptoms class includes individuals with endorsement of one or no ADHD symptoms.

To better understand the interaction of early nicotine exposure with dopaminergic pathways, we investigated four single nucleotide polymorphisms, SNPs, in the region of exon/intron 5 of the neural nicotinic acetylcholine receptor alpha 4 subunit gene (CHRNA4) previously identified using mutation screening approaches in the same twin population [Todd et al., [2003b]]. One of these SNPs, rs1044396, is a synonymous substitution polymorphism in exon 5 which has been reported to be associated with smoking addiction in two independent studies [Feng et al., [2004]; Li et al., [2005]]. The CHRNA4 protein product forms part of the high affinity neural nicotinic acetylcholine receptor complex which is located, among other sites, on presynaptic dopaminergic neurons. Though other acetylcholine receptors have been implicated in smoking, the α4β2 high affinity receptor complex increases dopamine release when stimulated by levels of nicotine as low as that found in a single cigarette [Cao et al., [2005]].

MATERIALS AND METHODS

All procedures were approved by the local human studies committee and written consent obtained from all subjects and legal guardians. The study sample was drawn from twins identified from birth records of the state of Missouri who...
had participated in a genetic epidemiology study of the prevalence and heritability of ADHD [described in detail in Neuman et al., [2005]; Volk et al., [2005]]. The total sample included 812 complete male and female twin pairs and six individual twins ages 7-19 years at the time of interview. As detailed in previous reports, a screening interview assessed the presence of three past or present inattentive symptoms for each twin [Neuman et al., [2005]; Volk et al., [2006]]. Families were invited into the study if the parent of either twin of a twin pair endorsed three or more inattentive symptoms in the twin (n = 563 families). Complete data on both twins was collected on 528 families and complete data on one twin was collected on 6 families (94.1% completion rate on a twin basis). In addition, 104 families in which at least one child had a Child Behavior Checklist [Achenbach, [1991]] anxious/withdrawn subscale score ≥95 percentile, and 183 families randomly selected across birth years were enrolled in the study to keep interviewers blind to diagnosis and to serve as disease contrast and control subjects, respectively [described in detail in Neuman et al., [2005]]. Parents were interviewed about their twins using the Missouri Assessment of Genetics Interview for Children (MAGIC) to obtain DSM-IV diagnoses and individual symptom information [Todd et al., [2003b]]. The interview did not include asking parents about their own psychopathology including possible ADHD symptoms.

Population-based ADHD subtype assignment using the 18 DSM-IV ADHD symptoms was made using the program LCAP-CA (available at http://hardy.wustl.edu) and class assignment parameter values derived from the combined analysis of the previously described twin studies [Todd et al., [2001]; Rasmussen et al., [2002]]. Parent report of the presence or absence of their offspring’s current ADHD symptoms was used for class assignments. The current analyses include individuals (n = 1,441) assigned to the few, severe inattentive or severe combined latent classes and individuals who qualified for DSM-IV no ADHD, predominately inattentive ADHD or combined type ADHD.

As previously reported [Neuman et al., [2007]] over 24% of mothers (n = 191) self-reported smoking cigarettes during their pregnancy. Of these over 75% smoked during all three trimesters and another 10% smoked two of the three trimesters. A binary variable for prenatal smoking was created indicating those who smoked at all during their pregnancy and those who did not. In univariate analyses this simple exposure variable was found to be as or more predictive of ADHD than number of cigarettes used or questions about in which trimester exposure occurred. As detailed in Neuman et al. ([2007]), the average number of DSM-IV ADHD symptoms was significantly higher in the offspring who were exposed to prenatal smoking than those who were not, 4.2 versus 3.4 (P = 0.006). In univariate analyses prenatal smoking was associated with the child having any DSM-IV ADHD diagnosis or population-defined severe combined type ADHD [defined in Rasmussen et al., [2002]]. There was no association between alcohol use and ADHD risk or number of ADHD symptoms in this sample [Neuman et al., [2007]].

The four CHRNA4 exon/intron 5 SNPs were found to be in high linkage disequilibrium (D’ ≥ 0.85) with two being completely associated (r² = 1.0). Therefore, our analysis focused on SNP rs1044396 C/T synonymous substitution polymorphism in exon 5 which had been associated with nicotine addiction [Feng et al., [2004]; Li et al., [2005]] and haplotypes composed of three of the four SNPs. Haplotypes were estimated and analyzed using the program WHAP [Purcell et al., [2007]]. Analyses of possible interactions of the CHRNA4 polymorphism with pre-natal smoking exposure used four mutually exclusive levels: (1) twins without the risk allele and without prenatal exposure was the reference group; (2) twins with the risk allele but without prenatal exposure; (3) twins without the risk allele but with prenatal exposure; and (4) twins with both risk factors. All models were adjusted for gender, negative expressed emotion in the family and DSM-IV diagnoses of oppositional defiant disorder (ODD) and conduct disorder (CD). These variables had previously been found to increase ADHD risk in this sample but were independent of smoking or genotypic risk. Models were fit with and without an interaction term. Logistic regression was carried out using STATA, version 7.0, with confidence intervals corrected for clustered data. One MZ twin was randomly removed from the dataset if the twins had the same ADHD subtype. The fit of all models was assessed with the Homer-Lemeshow goodness-of-fit Chi-square test. Analyses were then expanded to include our previously typed DRD4 and DAT loci [Neuman et al., [2007]], with SNP rs1044396.

RESULTS

Individual and family characteristics of the genotyped sample are shown in Table I by ADHD subtype.

<table>
<thead>
<tr>
<th>Table I. Characteristics of Participants by ADHD Subtype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population-baseda</td>
</tr>
</tbody>
</table>

...
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All</th>
<th>Few symptom</th>
<th>Severe combined</th>
<th>Severe inattentive</th>
<th>P-value</th>
<th>NO ADHD Combined</th>
<th>Inattentive</th>
<th>P-value^c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Twin</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of twins</td>
<td>1,540</td>
<td>838</td>
<td>121</td>
<td>94</td>
<td>-</td>
<td>1,320</td>
<td>85</td>
<td>113</td>
</tr>
<tr>
<td>Zygosity (% monozygotic)</td>
<td>24.8</td>
<td>22.4</td>
<td>17.6</td>
<td>17.6</td>
<td>0.3940</td>
<td>24.3</td>
<td>16.0</td>
<td>20.2</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>62.0</td>
<td>51.6</td>
<td>78.5</td>
<td>81.9</td>
<td>&lt;0.0001</td>
<td>58.3</td>
<td>87.1</td>
<td>84.1</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>13.0</td>
<td>13.3</td>
<td>11.9</td>
<td>13.1</td>
<td>&lt;0.0001</td>
<td>13.1</td>
<td>11.7</td>
<td>12.8</td>
</tr>
<tr>
<td>European American (%)</td>
<td>84.3</td>
<td>85.2</td>
<td>83.3</td>
<td>86.8</td>
<td>0.7921</td>
<td>84</td>
<td>89.3</td>
<td>85.6</td>
</tr>
<tr>
<td>Conduct or ODD (% yes)</td>
<td>15.1</td>
<td>4.0</td>
<td>58.1</td>
<td>33.0</td>
<td>&lt;0.0001</td>
<td>9.8</td>
<td>66.7</td>
<td>39.6</td>
</tr>
<tr>
<td>Very low birth weight (%) (&lt;1,500 g)</td>
<td>4.7</td>
<td>4.8</td>
<td>5.8</td>
<td>4.3</td>
<td>0.8650</td>
<td>4.8</td>
<td>7.1</td>
<td>3.5</td>
</tr>
<tr>
<td>Prescribed ADHD medication (% yes)</td>
<td>19.4</td>
<td>4.1</td>
<td>75.2</td>
<td>43.6</td>
<td>&lt;0.0001</td>
<td>12.4</td>
<td>78.8</td>
<td>54.0</td>
</tr>
<tr>
<td>Prescribed methphenidate (% yes)</td>
<td>15.1</td>
<td>3.2</td>
<td>59.2</td>
<td>31.9</td>
<td>&lt;0.0001</td>
<td>9.7</td>
<td>60.0</td>
<td>40.7</td>
</tr>
<tr>
<td>Family</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal prenatal smoking (% yes)</td>
<td>24.4</td>
<td>22.7</td>
<td>32.4</td>
<td>28.6</td>
<td>0.0632</td>
<td>23.5</td>
<td>32.0</td>
<td>26.9</td>
</tr>
<tr>
<td>Maternal prenatal alcohol Use (% yes)</td>
<td>5.0</td>
<td>5.2</td>
<td>4.6</td>
<td>13.2</td>
<td>0.0173</td>
<td>5.1</td>
<td>5.3</td>
<td>6.7</td>
</tr>
<tr>
<td>Mother's age at birth (mean years)</td>
<td>26.9</td>
<td>27.1</td>
<td>26.4</td>
<td>27.3</td>
<td>0.3638</td>
<td>26.9</td>
<td>26.9</td>
<td>26.7</td>
</tr>
<tr>
<td>Negative expressed emotions at home (% yes)</td>
<td>36.0</td>
<td>29.5</td>
<td>56.2</td>
<td>48.9</td>
<td>&lt;0.0001</td>
<td>32.6</td>
<td>61.2</td>
<td>50.4</td>
</tr>
<tr>
<td>Median family income ($)^d</td>
<td>33,958</td>
<td>34,358</td>
<td>33,238</td>
<td>37,194</td>
<td>0.1411</td>
<td>33,877</td>
<td>33,977</td>
<td>35,406</td>
</tr>
</tbody>
</table>

^a Only includes twins in three out of eight population defined classes.

^b DSM-IV predominately hyperactive/impulsive diagnosis twins are not included.

^c All P-values are for comparisons within DSM-IV or population-based ADHD designations.

^d Median Income by home USA postal code at time of birth from 2000 Census Records.

After adjusting for covariates, there was no main effect of the CHRNA4 polymorphism on ADHD risk in our data. However, a significant interaction between prenatal smoking and child genotype at the rs1044396 C allele on risk for combined type ADHD was observed (OR = 3.0, 95% CI 1.1-8.4 for population defined severe combined type ADHD; OR = 3.9, 95% CI 1.2-13.1 for DSM-IV defined combined type ADHD). No significant interactions were found for other ADHD subtypes by either nosology. This is unlikely to be due to subtype sample size differences. For example, for DSM-IV the largest group is predominately inattentive type ADHD (n = 113) and the smallest group is combined type...
ADHD (n = 85, Table 1). For haplotype analyses, there were five estimated haplotypes, two with a combined frequency of 6%. In an omnibus test without the rare haplotypes, there were no main effects on ADHD risk or evidence for interactions with prenatal smoking exposure. The rs1044396 C allele was divided across two haplotypes with frequencies of 0.36 and 0.16. Haplotypes were not considered further.

Though samples sizes for particular combinations of genotypes at several loci and prenatal risk factors were limited, there was evidence for an increasing effect of interactions for (a) CHRNA4 and DRD4 polymorphisms with prenatal smoking (both individual interaction ORs of 3.0) with a two-gene X smoking interaction OR = 5.5 (95% CI 1.4-22.5, population-defined severe combined type ADHD, non-significant for DSM-IV combined type) and (b) CHRNA4 and DAT1 440 bp allele with prenatal smoking (individual interaction ORs of 3.0 and 2.6, respectively) with a two-gene X smoking interaction OR of 6.0 (95% CI 1.2-28.9 for DSM-IV combined type ADHD, not significant for severe combined type ADHD). For the interaction of prenatal smoking exposure with all the three gene polymorphisms, the odds ratio for having severe combined type ADHD was 14.9 (95% CI 1.6-136.1) but was not significant for DSM-IV combined type ADHD. This last subtype difference may be due to the smaller simple size of the DSM-IV versus population defined combined ADHD group (n = 85 vs. n = 121, Table 1). The subtype specificity of these interactions supports the concept of multiple distinct ADHD syndromes [Rasmussen et al., [2002]; Todd et al., [2003b]; Reiersen et al., [in press]].

DISCUSSION

Given that dopamine acts as a neuronal morphogen during frontal cortical development [Todd, [1992]] and that ADHD associated dopaminergic deficits in prefrontal cortex have been postulated [Sonuga-Barke, [2003]; Sagvolden et al., [2005]], these observations suggest a synaptic model for pre-natal nicotine exposure interacting with genotypes at three loci in modulating risk for severe combined ADHD (Fig. 1: the localization of the DRD4 receptor to postsynaptic pyramidal and non-pyramidal neurons in frontal cortex has been confirmed in primate brain by immunohistochemistry) [Mrzljak et al., [1996]; Reiersen et al., [in press]]. We propose that nicotine from maternal smoking stimulates fetal pre-synaptic high affinity D2/2 neuronal nicotinic receptor complexes resulting in increased dopamine release from developing dopaminergic neurons which results in post-synaptic DRD4 receptor mediated changes in neurite outgrowth and branching. These developmental changes in neuronal maturation are proposed to result in lasting changes in neuronal organization and function. The magnitude of nicotine induced dopamine release in this model is dependent on nicotine exposure levels and CHRNA4 isoforms due to genetic variations. The subsequent magnitude of the morphogenic effects of increased dopamine release on DRD4 receptors is dependant on presynaptic dopamine transporter number and function (terminating the effects of release by re-uptake) and by post-synaptic DRD4 receptor number and function, both proteins being affected by genetic variations (Fig. 1).

Figure 1. Diagram of a dopaminergic synapse, illustrating the functional interaction of DRD4 (a D2 class dopamine receptor), DAT (dopamine transporter) and α4β2 nAChR (high affinity neural nicotinic acetylcholine receptor complex) in dopaminergic neurotransmission. Polymorphisms of DAT and DRD4 may interact to influence the degree and quality of dopaminergic neurotransmission at the synapse. Nicotine can also influence dopaminergic neurotransmission by increasing the amount of dopamine released into the synapse. The effect of nicotine is mediated through α4β2 nAChR, located at the dopaminergic neuronal cell body and neuron terminal. Polymorphisms of the CHRNA4 gene coding for the α4 subunit may influence dopamine release in response to circulating levels of nicotine. Figure redrawn from Reiersen et al. [in press].

[Normal View 34K | Magnified View 50K]

Though prenatal exposure to cigarette smoke has many potential deleterious effects and the breadth of the confidence intervals for the observed three- and four-way interactions emphasizes the need to examine larger sample sizes to confirm these results, the proposed model ties together several observations on the development of the forward projecting dopaminergic pathway and its possible role in the etiology of combined type ADHD. First, though dopamine is not required for the development of midbrain dopaminergic neurons and their projections per se [Zhou and Palmiter, [1995]], stimulation of dopamine D2 family receptors (which include DRD2, DRD3 and DRD4 receptors) on transfected neurons [Swarzenski et al., [1994]] and fetal cortical neurons [Todd, [1992]] increases neurite outgrowth and branching. Second, others have presented evidence that the studied DAT1 and DRD4 polymorphisms are functional [Vanness et al., [2005]]. That these variations can developmentally modify fronto-striatal gray matter
volumes in combined type ADHD has been recently demonstrated [Durston et al., [2005]]. Finally, though the associated CHRNA4 polymorphism is an exon 5 synonymous substitution polymorphism and has not been associated with functional receptor complex changes, this same polymorphism has been linked to smoking addiction in two studies [Feng et al., [2004]; Li et al., [2005]], suggesting that it, or another sequence variation in tight linkage disequilibrium, does affect response to smoking. Finally, Kotimaa et al. [[2003]] have found a dose response relationship between frequency of prenatal smoking exposure and degree of subsequent hyperactivity in youth. Functional changes in DRD4 receptors and in dopamine transporter number due to genetic variations coupled with genetic variations in nACh receptor complex responsiveness to nicotine represent a neurodevelopmental mechanism to explain the interaction of CHRNA4, DRD4, and DAT1 polymorphisms with prenatal nicotine exposure to increase risk for this severe form of ADHD.

The proposed model, due to the limited data it is based on, cannot be considered a complete etiologic explanation of combined type ADHD. Undoubtedly, other genes [such as reviewed in Faraone et al., [2005]], environmental influences and interactions also contribute to this form of ADHD. Similarly, given the subtype specificity of our current findings, different factors and interactions are likely to contribute to other forms of ADHD. That the effects are due to nicotine rather than other known deleterious effects of smoking is supported by the association with a coding region CHRNA4 polymorphism and by the lack of difference in frequency of low weight births across the ADHD and control groups (Table I). That rs1044396 C containing haplotypes did not show evidence for gene × smoking interaction effects may be secondary to the smaller group sizes of these haplotypes. Conversely, this is also compatible with the rs1044396 C allele being causative, as haplotype analysis is well known to decrease evidence for association when a causative DNA sequence variation is part of the haplotype definition. Given the neuroanatomical proximity of the products of these genes in brain regions linked to attention [Todd and Botteron, [2001]], this hypothesis has some face validity and is amenable to direct testing in vitro and in vivo.

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