The Role of Histamine Degradation Gene Polymorphisms in Moderating the Effects of Food Additives on Children’s ADHD Symptoms

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**Objective:** Food additives can exacerbate ADHD symptoms and cause non-immunoglobulin E-dependent histamine release from circulating basophils. However, children vary in the extent to which their ADHD symptoms are exacerbated by the ingestion of food additives. The authors hypothesized that genetic polymorphisms affecting histamine degradation would explain the diversity of responses to additives.

**Method:** In a double-blind, placebo-controlled crossover trial, challenges involving two food color additive and sodium benzoate (preservative) mixtures in a fruit drink were administered to a general community sample of 3-year-old children (N=153) and 8/9-year-old children (N=144). An aggregate ADHD symptom measure (based on teacher and parent blind ratings of behavior, blind direct observation of behavior in the classroom, and—for 8/9-year-old children only—a computerized measure of attention) was the main outcome variable.

**Results:** The adverse effect of food additives on ADHD symptoms was moderated by histamine degradation gene polymorphisms HNMT T939C and HNMT Thr105Ile in 3- and 8/9-year-old children and by a DAT1 polymorphism (short versus long) in 8/9-year-old children only. There was no evidence that polymorphisms in catecholamine genes COMT Val108M, ADRA2A C1291G, and DRD4-rs7403703 moderated the effect on ADHD symptoms.

**Conclusions:** Histamine may mediate the effects of food additives on ADHD symptoms, and variations in genes influencing the action of histamine may explain the inconsistency between previous studies. Genes influencing a range of neurotransmitter systems and their interplay with environmental factors, such as diet, need to be examined to understand genetic influences on ADHD symptoms.

(Am J Psychiatry Stevenson et al.; AiA:1–8)
It is uncertain which mechanisms might mediate these effects or which factors may make children more or less susceptible. Histamine is an interesting candidate neurotransmitter system for a number of reasons. The activity of central histamine H3 receptors have been shown to affect inhibition learning, to increase hyperactivity levels in mouse models, and to promote dopamine release in the frontal cortex (14). There is evidence that histamine might mediate the effects of artificial food colors on ADHD symptoms. Azo dyes have been shown to provoke urticaria in some individuals, independent of whether or not they are aspirin sensitive, providing clinical evidence that artificial colors may result in histamine release (15–17). It has been proposed that the effect of food additives is likely to be a nonspecific pharmacological effect that would be similar in children irrespective of their atopic status or other characteristics (12, 18). For these reasons histamine release and its effects on the CNS may play a crucial role in mediating the effects of artificial food colors on ADHD symptoms.

The present study examines whether the effect of food additives on ADHD symptoms is moderated by genetic differences between children. Genetic polymorphisms were selected from the dopamine (dopamine transporter [DAT1] and dopamine D4 receptor [DRD4]) and catechol O-methyltransferase (COMT) genes and adrenergic neurotransmitter systems (adrenergic receptor alpha 2A [ADRA2A]), since these have previously been implicated in ADHD (2, 19, 20). Since there is a suggestion that histamine may be involved in the effects of food additives, genetic polymorphisms from this system were also included (histamine N-methyltransferase [HNMT]) to address this hypothesis.

The first single nucleotide polymorphism (SNP) of HNMT, Thr105Ile (rs1801105), is the only nonsynonymous polymorphism identified in the gene in the Caucasian population. It is associated with reduced thermal stability and decreased activity of HNMT (21, 22). HNMT T939C (rs1050891) is in the 3′ untranslated region of the gene and correlates with HNMT activity, although given the strong linkage disequilibrium between this SNP with the exon III SNP in ADRA2A (rs740373), and one SNP in DRD4 (rs740373), and one SNP in ADRA2A -1291>G (ADRA2A C1291G [rs1800544]). It was also possible to genotypes one variable number tandem repeats: the short (9 or less repeats) and long (10 repeats or more) forms of DAT1. Extensive validation was undertaken to assess the feasibility of genotypeing the DRD4 repeat. However this revealed that there was bias in favor of amplification of shorter alleles. Therefore the SNP rs740373 was genotyped instead, since a significant association of the C allele with ADHD (p=0.008) has been reported (24) and there is evidence for linkage disequilibrium between this SNP with the exon III polymorphism (p=0.013) (25).

Method

The study was registered as a clinical trial with Current Controlled Trials (Registration number ISRCTN74481308) and was approved by the Local Research Ethics Committee (Ref. No. B/ Q1702/61). After providing full information about the study and its dietary implications, written informed consent was obtained from the parents. Each participating early years setting received £250 and each school £500 as a contribution toward school funds for the benefit of all children.

Participants

Details of recruitment and participation in the study are provided in figures S1 and S2 in the data supplement that accompanies the online version of this article. The sample of 3-year-old children was drawn from a population of preschool children aged between 3 years and 4 years 2 months registered in early years settings (nurseries, day nurseries, preschools, playgroups) within the Southampton City Council area. The older sample was drawn from children between 8 and 9 years of age attending schools within the city of Southampton.

Parents who returned an expression of interest form were contacted by phone and a convenient time for a home visit was arranged. The study dietitian also obtained a report based on a 24-hour recall by the parent of the child’s pre-study diet, which allowed an assessment of baseline levels of the number of foods containing additives consumed by the child in the previous 24-hour period.

Of the 3-year-old children enlisted in the study (N=153), 16 (10.5%) failed to complete. In only one case was this related to problems with the child’s behavior. Of the 8/9-year-old children enlisted in the study (N=144), 14 (9.7%) failed to complete. In no case was this related to problems with the child’s behavior. For both samples, age and gender of the child and marital status of the parents had no effect on study completion, and children were no more likely to drop out during active compared to placebo challenge weeks.

Study Design and Challenge Protocols

Children were entered into this randomized, double-blind, placebo-controlled food challenge with a within-subject cross-
over. There were two active mixes of additives. Mix A contained sunset yellow, carmoisine, tartrazine, and ponceau 4R; mix B contained sunset yellow, carmoisine, quinoline yellow, and allura red AC. In addition, both additive mixes incorporated sodium benzoate, which had been included in the challenge in our earlier study (12) and in previous studies (10, 26). After a week on their normal diet, the target food colors and sodium benzoate were excluded from their diet over a 6-week period when challenge and withdrawal occurred as follows: week 0: baseline/normal diet; week 1: withdrawal period but receiving placebo; weeks 2, 4, and 6: challenge with randomization to two active and one placebo period; weeks 3 and 5: washout continuing on placebo. Full details of the challenges have been published previously (13) and are also given in supplementary data. We set a minimum of 85% of drinks consumed to constitute a per protocol population and the analyses in the present paper were restricted to these 132 3-year-old and 119 8-year-old children.

**Global Hyperactivity Aggregate**

For the 3-year-old children, three measures of behavior were used to calculate the global hyperactivity aggregate. The first was the Abbreviated ADHD Rating Scale–IV (Teacher version) (27, 28). A total score was obtained for 10 of the 18 items (inattentive=5, hyperactive=5) in this questionnaire, which was completed by the early year setting practitioner who described the frequency of the specific behaviors displayed over the past week for each week of the study. The second was a parent behavior measure: Abbreviated Weiss-Werry-Peters hyperactivity scale (29) . This scale has been used in a number of studies to assess ADHD symptoms (30, 31). Interparent agreement is good (r=0.82) (32). Parents rated their child's behavior over the previous week for 7 items previously used (12) and a total score was obtained. The third measure was the Classroom Observation Code (33). This instrument assesses the occurrence of 12 mutually exclusive behaviors during structured didactic teaching and during periods of independent work under teacher supervision. The interrater reliability of the method, tested prior to and in mid-study, exceeded 0.87. Each child was observed for a total duration of 24 minutes each week (three observation sessions each of 8 minutes duration) and a total weekly mean score was derived from the total score over each session. The code was slightly modified for use in the present study, since preschool children in the U.K. have relatively little structured or didactic teaching sessions and tend to engage in "activities" rather than "tasks."

For the 8/9-year-old children, four measures of behavior were used to calculate the global hyperactivity aggregate. Two were the same as for the 3-year-old children: the Abbreviated ADHD Rating Scale–IV (Teacher version) and the Classroom Observation Code. Parental ratings of behavior were obtained using a parent version of the Abbreviated ADHD Rating Scale–IV (unpublished 1994 instrument, C.J. DuPaul et al.), which has the same format as the teacher version. The final component was Connors’ Continuous Performance Test II (34). This is a test using visual stimuli of 14 minutes duration and is widely used to evaluate attention and the response inhibition component of executive control. Four measures (standard error of reaction time, percentage of commission errors, d’, and β) were used to derive a weekly Continuous Performance Test II aggregate score. This subset of indicators has been shown to be highly correlated with the ADHD Rating Scale employed in this study (35).

**Statistical Analysis**

The weekly scores from the teacher, parent, Classroom Observation Code, and, for 8/9-year-old children only, the Continuous Performance Test II measures for each child were standardized to time 0 at baseline (T0) for the same measure:

\[ \text{Weekly standardized (z) aggregate score} = \frac{\text{score} - \text{mean at T0}}{\text{SD at T0}} \]

The primary outcome measure, the global hyperactivity aggregate, is an unweighted aggregate of the weekly teacher, parent, Classroom Observation Code, and Continuous Performance Test II z scores. This was calculated only when at least two (for 3-year-old children) or three (for 8/9-year-old children) of these component behavior scores were present for any week, one of which had to be the Classroom Observation Code score. A high global hyperactivity aggregate indicates more ADHD symptoms. Preliminary analyses using a Kolmogorov-Smirnov one-sample test showed that these aggregate scores and the difference between the scores under placebo and active mix challenges were normally distributed.

Linear mixed-model methods (36, 37), with a compound symmetry covariance matrix for 3-year-old children and unstructured covariance matrix for 8/9-year-old children (the best fit models for each age group), were used to analyze data. The study was powered to detect differences between the active and placebo periods so that in each case the effects of mix A and mix B can be compared to the effect of placebo. With a sample of 120 children there was 80% power at α=0.05 to identify an effect size of 0.32, i.e., the magnitude of the difference in mean score

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**TABLE 1. Association of Genotype and Global Hyperactivity Aggregate at Baseline for 3- and 8/9-Year-Old Children Before Consumption of Challenge Drinks Containing Food Additive Mixtures and Sodium Benzoate**

<table>
<thead>
<tr>
<th>Group and Genotype</th>
<th>SNP Present</th>
<th>SNP Absent</th>
<th>Main Effect of Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mean</td>
<td>SD</td>
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<tr>
<td>3-year-old children</td>
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<tr>
<td>HNMT Thr105Ile (T allele)</td>
<td>30</td>
<td>0.09</td>
<td>0.66</td>
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<tr>
<td>HNMT T939C (C allele)</td>
<td>39</td>
<td>0.11</td>
<td>0.61</td>
</tr>
<tr>
<td>COMT Val108Met (A allele)</td>
<td>76</td>
<td>-0.13</td>
<td>0.64</td>
</tr>
<tr>
<td>ADRA2A C1291G (G allele)</td>
<td>52</td>
<td>-0.14</td>
<td>0.72</td>
</tr>
<tr>
<td>DAT1 (short allele)</td>
<td>63</td>
<td>-0.11</td>
<td>0.71</td>
</tr>
<tr>
<td>DRD4 rs740373 (C allele)</td>
<td>26</td>
<td>0.31</td>
<td>0.62</td>
</tr>
<tr>
<td>8/9-year-old children</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HNMT Thr105Ile (T allele)</td>
<td>23</td>
<td>-0.24</td>
<td>0.77</td>
</tr>
<tr>
<td>HNMT T939C (C allele)</td>
<td>44</td>
<td>0.09</td>
<td>1.17</td>
</tr>
<tr>
<td>COMT Val108Met (A allele)</td>
<td>79</td>
<td>0.03</td>
<td>0.94</td>
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<tr>
<td>ADRA2A C1291G (G allele)</td>
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<td>-0.04</td>
<td>1.11</td>
</tr>
<tr>
<td>DAT1 (short allele)</td>
<td>75</td>
<td>0.03</td>
<td>1.10</td>
</tr>
<tr>
<td>DRD4 rs740373 (C allele)</td>
<td>39</td>
<td>0.00</td>
<td>0.76</td>
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</tbody>
</table>
FOOD ADDITIVES AND ADHD SYMPTOMS


<table>
<thead>
<tr>
<th>Genotype</th>
<th>Mix A Versus Placebo</th>
<th>3-Year-Old Children</th>
<th>8/9-Year-Old Children</th>
<th>Mix B Versus Placebo</th>
<th>3-Year-Old Children</th>
<th>8/9-Year-Old Children</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>95% CI</td>
<td>p</td>
<td>Estimate</td>
<td>95% CI</td>
<td>p</td>
</tr>
<tr>
<td>HNMT Thr105Ile</td>
<td>–0.53</td>
<td>–1.04 to –0.02</td>
<td>0.04</td>
<td>–0.10</td>
<td>–0.35 to 0.14</td>
<td>0.40</td>
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<tr>
<td>HNMT T939C</td>
<td>–0.46</td>
<td>–0.94 to 0.02</td>
<td>0.06</td>
<td>–0.24</td>
<td>–0.44 to 0.02</td>
<td>0.02</td>
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<tr>
<td>COMT Val108Met</td>
<td>–0.23</td>
<td>–0.75 to 0.29</td>
<td>0.38</td>
<td>–0.02</td>
<td>–0.20 to 0.24</td>
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<td>0.01</td>
<td>–0.44 to 0.47</td>
<td>0.74</td>
<td>–0.05</td>
<td>–0.26 to 0.15</td>
<td>0.61</td>
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<tr>
<td>DAT1</td>
<td>0.08</td>
<td>–0.39 to 0.54</td>
<td>0.74</td>
<td>0.11</td>
<td>–0.11 to 0.34</td>
<td>0.32</td>
</tr>
<tr>
<td>DRD4 rs740373</td>
<td>–0.27</td>
<td>–0.82 to 0.27</td>
<td>0.33</td>
<td>–0.08</td>
<td>–0.29 to 0.13</td>
<td>0.44</td>
</tr>
</tbody>
</table>

a The main effects of challenge type, genotype, week during study, gender, global hyperactivity aggregate in baseline week, number of additives in pre-trial diet, maternal educational level, and social class were included in the model but are not tabulated.

b Mix A contained sunset yellow, carmoisin, tartrazine, and ponceau 4R; mix B contained sunset yellow, carmoisin, quinoline yellow, and allura red AC. Both food additive mixtures incorporated sodium benzoate.

Changes (in SD units), a slightly smaller effect size to that found previously (12).

Genotyping

In the course of the study, buccal swabs were collected from the children for genotype analysis. These samples were refrigerated and packed in ice to be sent to the laboratory for DNA extraction and genotyping. The genotyping of the HNMT Thr105Ile (rs1801105), HNMT T939C (rs1050891), COMT Val108Met (rs4680), ADRA2A C1291G (rs1800544), and DRD4 (rs740373) SNPs was performed by Kbiosciences (Herts, U.K.) using a KASPar assay system (http://www.kbioscience.co.uk).

The variable number tandem repeat analysis of DAT1 was performed in our laboratory using a total volume of 10 µl including 20 ng DNA template, 2 µl 5X Herculase II buffer containing 2 mM MgCl₂, 0.5 µmol/l of each primer, 0.1 µmol of Herculase II DNA polymerase (Stratagene), 8% DMSO, 1 M Betaine, and 250 µmol/l of each deoxynucleotide triphosphates. The polymerase chain reaction conditions were 3 minutes at 98°C, followed by 35 cycles each of 20 seconds at 98°C, 20 seconds at 65°C, 30 seconds at 72°C, and finally 3 minutes at 72°C in 96-well plates. DAT1 primer sequences were DAT1f 5’- GCC ACT CAG GCA GCC TGT G-3 and DAT1r 5’-6FAM- AGG ACC CTC ATG GCC TTC T-3’. Amplified fragments were sized using capillary electrophoresis on a CEQ8800 (Beckman Coulter, UK). Genotype data were assessed for concordance with the Hardy–Weinberg equilibrium law using a χ² test with one degree of freedom. All genotypes were in Hardy–Weinberg equilibrium.

Results

There was evidence that the HNMT T939C and the DRD4 rs740373 polymorphisms were related to the overall level of the global hyperactivity aggregate at baseline in the 3-year-old children (Table 1). There were no significant effects of any of the polymorphisms on the overall global hyperactivity aggregate level at baseline in the 8/9-year-old children.

The results of the general linear mixed model analysis are summarized in Table 2 for both the 3- and 8/9-year-old children. We have previously shown that there were significant effects of challenge type on global hyperactivity aggregate (13). Here, interest centers on the interaction between challenge type and genotype. These interaction effect estimates in Table 2 are based on the difference in global hyperactivity aggregate score while challenged by an additive mix and by a placebo and specifically whether such an effect is different for the two genotypes for each gene (i.e., it estimates the magnitude of the difference of two differences and the associated 95% CI). If the latter include zero then the interaction is not significant at the 5% level. The interpretation of the interactions can be determined from the mean estimated marginal global hyperactivity aggregate values presented in Figure 1 (3-year-old children) and Figure 2 (8/9-year-old children). To test the effect of the genotype-by-challenge interactions, a number of other factors that may be related to overall the global hyperactivity aggregate needed to be taken into account in the mixed models analysis and were slightly different for the two age groups. In total, these included baseline global hyperactivity aggregate scores, age, gender, maternal education level, and prestudy diet. However, for the 3-year-old children these were gender, baseline global hyperactivity aggregate, prestudy diet, in addition to challenge and genotype. In this age group there were no significant interactions between these factors and the effects of challenge. For the 8-year-old children the factors in the analysis were week during study and baseline global hyperactivity aggregate in addition to challenge and genotype. In this age group there were no significant interactions between these factors and the effects of challenge.

Considering 3-year-old children first, the estimated marginal means plotted in Figure 1 show that the effects of
Mix A (relative to placebo) on ADHD symptoms were limited to those children not carrying T (Ile) allele of HNMT Thr105Ile (p=0.04) and to children not carrying the carrying the C allele of HNMT T939C, although this interaction just failed to reach significance (p=0.06). The effects of mix B (relative to placebo) were the same regardless of the HNMT polymorphisms in this age group. The effects of mix A and mix B were not moderated in 3-year-old children.
FIGURE 2. Effect of Genotype in Moderating Impact of Food Additive Challenge on Global Hyperactivity Aggregate in 8/9-Year-Old Children

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Mix A</th>
<th>Mix B</th>
<th>Placebo</th>
<th>Mix A</th>
<th>Mix B</th>
<th>Placebo</th>
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</table>

*a Mix A contained sunset yellow, carmoisine, tartrazine, and ponceau 4R; mix B contained sunset yellow, carmoisine, quinoline yellow, and allura red AC. Both food additive mixtures incorporated sodium benzoate. Mean est. marg.: mean estimated marginal mean when baseline global hyperactivity aggregate, week during study, challenge, and genotype were included in the mixed model analysis.

**FOOD ADDITIVES AND ADHD SYMPTOMS**

By the **COMT Val108Met**, **ADRA2A C1291G**, **DAT1** or **DRD4 rs740373** polymorphisms.

For the 8/9-year-old children, there were no significant genotype-by-challenge interactions for **COMT Val108Met**, **ADRA2A C1291G**, or **DRD4 rs740373**. However, as for the 3-year-old children, interactions were present for **HNMT Thr105Ile** and **HNMT T939C**. For mix A (relative to placebo) the effects were limited to those without the C allele of **HNMT**
T939C (p=0.02). The effects of mix B (relative to placebo) were found only for children without the T allele of HNMT Thr105lle (p=0.05), the C allele for HNMT T939C (p=0.03), and those with the short form allele of DAT1 (p=0.05)

Discussion

These findings indicate a link between histamine and ADHD symptoms, with polymorphisms in the HNMT gene moderating behavioral responses to food additives. They suggest that the current focus on catecholamines in studies of ADHD needs to be extended to other neurotransmitters. They open up one avenue of explanation as to why the genes so far shown to be associated with ADHD explain so little of the known variance. The histamine risk alleles in this study have two actions: to influence the overall level of the global hyperactivity aggregate in the study (significantly so in the case of HNMT T939C for the younger children), and to make the child more vulnerable to the effects on behavior of food additives in the diet.

There are limitations to these findings. Although there is internal replication, in that the same pattern of results were found in both 3- and in 8/9-year-old children, there is need to replicate the HNMT and food additive interactions in additional samples. The importance of such replication in gene/environment interactions has been emphasized (38). The effects we have shown are relatively short-term in that they reflect a 1-week exposure. We cannot tell if the effects of food additives on ADHD symptoms and the moderation by HNMT polymorphisms would hold for longer-term exposures. A strength of the study is that the environmental risk of food additive exposure was experimentally manipulated and the effects shown using within-person difference in a crossover design. Moreover, the gene/environment interaction was found for polymorphisms in a gene that had been postulated to be involved rather than on the basis of wider genome search. These two features of the design of the study were recently recommended as ways to promote the study of gene-environment interactions using hypothesis-based molecular genetic studies with candidate genes (39).

The role of genes in influencing behavior needs to be understood not just by their main effects of raising levels, for example, of ADHD symptoms but also by the interplay both with each other in gene-gene interactions, and also by interactions with environmental factors such as diet (40). HNMT polymorphisms impair histamine clearance (21) and a food additive challenge causes histamine release (17), and therefore the interaction found here is neurobiologically plausible. The presence of H3 receptors in the brain provides a potential mechanistic explanation for the effect (41). Moreover it has become apparent that both methylphenidate and atomoxetine, widely used treatments for ADHD, may have effects mediated via the histamine system (42, 43). Many environmental factors will increase histamine release including many food items as well as infections. This would explain the frequent claim that food allergy/intolerance is a cause of ADHD symptoms and the effects of infections in aggravating aberrant behavior (44). This clearly supports a potential target for therapeutic intervention in ADHD focused on the H3 receptor (45, 46).

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Dr. Sonuga-Barke reports speaker board participation for Shire Pharma and UCB Pharma; consultancy work for UCB Pharma and Shire; research support from Janssen Cilag, Shire, and Qbtech; advisory board participation for Shire, Flynn Pharma, UCB Pharma, and AstraZeneca; and conference support from Shire. Professor Warner reports speaker board participation for UCB Pharma, Danone, Novartis, and Merck; research grants from UCB Pharma, Danone, Novartis, and Merck; and advisory board participation for UCB Pharma, Danone, Novartis, and Merck. The remaining authors report no financial relationships with commercial interests.

Supported by a grant from the U.K. Food Standards Agency (Grant Ref. T07040).

The authors acknowledge the help and assistance received from the children, families, and teachers in the participating early years settings and schools in the Southampton area.


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