Dopamine-Related Genotypes and the Dose–Response Effect of Methylphenidate on Eating in Attention-Deficit/Hyperactivity Disorder Youths


Abstract

Objective: There are individual differences in the effects of methylphenidate (MPH), a dopamine (DA) transport inhibitor, on appetite in children with attention-deficit/hyperactivity disorder (ADHD). One potential moderating factor is variation in brain DA activity, which is influenced by dopamine-related genes: the DA transporter (DAT) (SLC6A3), the DA D2 receptor (DRD2), and the DA D4 receptor (DRD4) genes. The purpose of this study was to explore the relationship between dopamine-related gene polymorphisms and food consumption in ADHD children receiving varying doses of MPH.

Methods: In a randomized, within-subject, double-blind design, 58 ADHD children (ages 6–12 years) received placebo, 0.15, 0.3, or 0.6 mg/kg of MPH three times daily over 9 weeks. Observations of percent lunch consumed as a function of dopamine-related genotypes and MPH dose were analyzed using mixed effects regression models.

Results: A significant dose–response reduction in eating was observed across all genotypes (p < 0.001). There was an interaction of DAT SLC6A3 and DRD2 genotypes and dose, because 9/9 DAT children showed a stronger effect of dose when compared with the 9/10 and 10/10 children (p < 0.001) and DRD2 A2/A2 children showed a stronger effect of dose when compared with A1/A1 and A1/A2 children combined (p = 0.007). There was no significant interaction of dose by DRD4 genotype.

Conclusions: Lunch consumption decreased as a function of MPH dose. DA-related genotypes associated with greater brain DA signaling moderated the influence of drug on consumption. These results provide information relevant to predicting which children are likely to experience the greatest appetite suppression when taking MPH.

Introduction

Over the past decade there has been a rise in the prescription of medications for attention-deficit/hyperactivity disorder (ADHD) so that approximately 6% of boys and 2% of girls in the United States receive stimulants for the treatment of ADHD, the most common of which is methylphenidate (MPH) (Zuvekas et al. 2006). One of the most commonly reported side effects of MPH is anorexia with weight loss (Schertz et al. 1996; Schachter et al. 2001; Greenhill et al. 2002; Pliszka 2007). In some pediatric studies, weight loss dissipated after the first 3–6 months of MPH treatment (Spencer et al. 1998; Biederman et al. 2003; Wilens et al. 2003), but in others, decelerations in weight velocity persisted for as long as a clinically effective dose was given (Schertz et al. 1996; National Institute of Mental Health 2004; Charach et al. 2006; Swanson et al. 2006). Little is known about which children are most at risk for persistent anorexia and weight loss during treatment with stimulants like MPH because there are no prospective randomized studies that have identified phenotypic (e.g., age, gender) or medication-related (e.g., type of stimulant, dosing schedule, treatment duration) moderators for increased susceptibility to stimulant-induced anorexia and weight loss (Poulton 2005). Regardless of its duration, anorexia (and associated weight loss) is one of the leading reasons for dose reduction or discontinuation of stimulant therapy (Efron et al. 1997; Wigal et al. 2006; Zachor et al. 2006), with up to 12% of subjects stopping medication due to appetite loss (Zachor et al. 2006). In the Multimodal Treatment Study of Children with ADHD (MTA), which used a ½ dose in the afternoon, anorexia was still the most common reason for a dose reduction, leading 10% of subjects to lower their dose...
Studies of extended-release stimulants have also found that weight loss, anorexia, and related gastrointestinal problems are commonly reported side effects after 1–2 years of treatment and the primary reason cited for drug discontinuation (Wilens et al. 2005; McGough et al. 2005; Biederman et al. 2007). Side effects are the most common reason reported by children as to why they do not want to continue a stimulant (Efron et al. 1997). Because child refusal is one of the most common reasons for stopping medication (Swanson 2003), identifying predictors of drug tolerability may improve adherence and ultimately long-term outcomes in youth with ADHD.

MPH raises synaptic dopamine (DA) (Volkow et al. 2001) by inhibiting the dopamine transporter (DAT) protein (Volkow et al. 1998), which regulates synaptic DA levels by transporting DA back into the presynaptic neuron (Bannon et al. 1995). Individual differences in the DAT may interact with drug effects to influence drug action differentially for different polymorphisms of the dopamine transporter. The DAT SLC6A3 gene has three common genotypes: 10/10, 9/10, and 9/9 (Gerlentner et al. 1994). There is evidence that the DAT 9 allele is associated with less DAT protein and thus with more synaptic dopamine signaling compared with genotypes containing a 10 allele (VanNess et al. 2005), with the expectation that lower levels of DA activity would lead to greater energy intake and that higher levels of dopaminergic activity would lead to lower energy intake (Epstein and Leddy 2006). Genotypes associated with reduced brain DA signaling have been implicated in ADHD (Cook et al. 1995) as well as in addictive behaviors (Comings et al. 1996) and appetite regulation (Wang et al. 2001; Epstein et al. 2002). Brain DA signaling is one of the determinants of the motivation to eat (Berridge 1996; Epstein and Leddy 2006), and there are dose-dependent effects of stimulants on appetite suppression and weight gain (Poulton 2005; Charach et al. 2006; Pliszka et al. 2006; Wigal et al. 2006). It is possible that ADHD children who have a DAT genotype associated with greater synaptic DA signaling (i.e., containing two 9 alleles) would develop greater appetite suppression during treatment with MPH than children with one or no 9 alleles. In adults with binge-eating disorder, Davis et al. (2007) reported that subjects with at least one copy of the 9-repeat DAT allele showed a significant suppression of appetite in response to MPH when compared with controls with this allele or with subjects having the 10/10 genotype.

Pharmacological data show that reducing brain DA activity using antagonists leads to increased eating and weight gain (Baptista 1999), and that increasing brain DA activity using DA agonists or reuptake inhibitors leads to a reduction in eating and weight loss (Schartz et al. 1996; Leddy et al. 2004). Dopamine D2 and D4, two of the five types of DA receptors, are coded by the DRD2 and DRD4 genes, respectively. The DRD2 gene has three polymorphisms: A1/A1, A1/A2, and A2/A2. The presence of the DRD2 Taq1 A1 allele is associated with a lower density of DRD2 receptors (Thompson et al. 1997; Jonsson et al. 1999) and therefore to reduced brain DA signaling. The DRD2 A1 allele has been shown to be associated with changes in energy intake and body weight. For example, we have shown that the DRD2 A1 allele influences food intake both in smokers (Epstein et al. 2004) and in obese and nonobese nonsmokers (Epstein et al. 2007), whereas others have shown that obese individuals have a higher prevalence of the DRD2 Taq1 A1 allele (Noble et al. 1994).

The D4 receptor has been related to cue-elicited craving for food (Sobik et al. 2005), and pharmacological evidence implicates the DRD4 gene in eating regulation. For example, clozapine, which binds with high affinity to DRD4, can lead to increased food consumption and weight gain (Van Tol et al. 1991). A functional VNTR (variable number of tandem repeats) polymorphism has been identified in the third exon in the DRD4 gene. The genetic variant is a 16-amino-acid repeat polymorphism, which is repeated 2–11 times, with four repeats (the “short” allele) being by far the most common allele (Van Tol et al. 1992). The in vitro studies suggest that the exon III DRD4 7-repeat allele (7R, the “long” allele) has decreased affinity for dopamine and transmits weaker intracellular signals in comparison with other exon III alleles (Asghari et al. 1995).

The purpose of this study was to explore the relationship between DA-related genotypes and food consumption in children who received varying doses of MPH or placebo in a double-blind, random fashion during a summer treatment program for ADHD. We hypothesized that ADHD children would demonstrate a dose–response reduction in food intake on MPH and that children with DA genotypes associated with greater brain dopamine signaling (i.e., DAT SLC6A3 9/9; DRD2 A2/A2; and the “short” DRD4 allele) would reduce intake with increasing MPH dose more than those with genotypes associated with reduced brain dopamine signaling (i.e., DAT SLC6A3 9/10 or 10/10; DRD2 A1/A1 and A1/A2; and the “long” DRD4 allele).

Methods

Participants

Fifty eight ADHD children who participated in the Summer Treatment Program (STP), a therapeutic summer camp for ADHD, between the years of 2002 and 2004 participated in this study. STP participants were referred by physicians, schools, or self-referred through advertisements. STP inclusion criteria included ages 6–12 years, Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) (American Psychiatric Association 1994) diagnosis of ADHD, and an intelligence quotient (IQ) ≥80. Diagnoses were made using a combination of ratings from parents and the child’s primary school teacher on the Disruptive Behavior Disorders Rating Scale (DBD) (Pelham et al. 1992) and parent report on the Diagnostic Interview Schedule for Children (DISC) (Shaffer et al. 2000). The DBD rates all 45 DSM symptoms of ADHD, conduct disorder (CD), and oppositional defiant disorder (ODD) on a 0–3 scale. Symptoms counted as positive if they were endorsed on either or both measures.

In addition, ADHD-related impairment in at least two realms using the Parent and Teacher versions of the Impairment Rating Scale (IRS) was required for entry into the STP. The IRS is an eight-item visual-analog scale with established psychometrics that evaluates the child’s problem level and need for treatment in developmentally important areas, such as peer relationships, adult–child relationships, academic performance, classroom behavior, and self esteem (Fabiano et al. 2006).

The presence of ADHD as the primary disorder responsible for the majority of the reported impairment was confirmed during a semistructured interview designed specifically for
the STP intake process and administered by a graduate-level clinician to the parent and child. This interview is designed to identify the child’s primary behavioral impairments, which are then used to develop individual behavior goals for that subject during the STP (Pelham et al. 2005b). Exclusion criteria included: (1) History of seizures or other serious neurological problems; (2) severe psychopathology such as pervasive developmental disorder (PDD), schizophrenia, or other psychotic disorders. Subjects could have ODD or CD, because they commonly occur with ADHD (Waxmansky 2003). Any child requiring psychotropic medication for treatment of a comorbid disorder was excluded.

Of the 58 STP subjects enrolled in this protocol, 95% had combined-type ADHD, 3% inattentive and 2% hyperactive/impulsive, while 52% had ODD and 10.5% had CD. This was similar to the entire STP cohort (n = 154, 91% combined type, 5% inattentive, 3% hyperactive/impulsive, 55% ODD, and 27% CD). During the school year prior to the STP, 19% of the 58 subjects were not medicated for ADHD, 37% were taking medications only during school, and 44% were taking medication on weekends and/or after school (in the evening), similar to the entire STP cohort (80% were previously medicated; 47% medicated after school).

Procedures

All 2002–2004 STP participants were enrolled in a placebo-controlled trial of behavior modification (BMOD) and MPH (Pelham et al., in preparation). During the STP, both treatments varied on a within-subjects basis to evaluate children’s response to each treatment given the presence and intensity of the other treatment. The STP ran for 9 weeks, Monday–Friday, from 9 am to 5 pm. Activities included sports games and practice, academic classroom periods, art class, and swimming. BMOD varied every three weeks and included three conditions: no behavior modification (NBM), low-intensity behavior modification (LBM), and high-intensity behavior modification (HB). LBM resembles the behavior intervention used in the summer component of the MTA (The MTA Cooperative Group 1999). LBM resembles traditional interventions implemented in community mental health settings. The primary difference between high versus low was the use of daily versus weekly contingency rewards and the use of individualized behavior plans in HB. NBM used non-contingent weekly rewards and did not implement time-out procedures (see Pelham et al. 2005a for further detail). Drug dose varied daily on a randomized basis and included four conditions: placebo, 0.15 mg/kg per dose of MPH three times daily (t.i.d.), 0.3 mg/kg per dose MPH t.i.d., and 0.6 mg/kg per dose MPH t.i.d. Each condition occurred at least once per week for a total of 12 days for placebo, 0.15, and 0.3 conditions, and 9 days for the 0.6 mg/kg condition.

Each child’s primary STP counselor observed the subject during lunch (which the children brought from home) and recorded the percentage of lunch eaten daily using a scale of 0 (none), 1 (about 25%), 2 (about 50%), 3 (about 75%), and 4 (100%). Daily ratings were averaged across the 11–12 days on each dose to create a summary rating for placebo and each MPH dose. Other studies have successfully used similar observation methods to determine food consumption in children (Sallis et al. 2003). Lunch began between 11:45 am and 12:00 pm and medication was given between 7:30 and 7:45 a.m., 11:35 and 11:45 am, and 3:35 and 3:45 pm. At the end of each day, counselors completed the Pittsburgh Side Effects Rating Scale (Pelham et al. 2005c), which rates commonly occurring stimulant-induced adverse events as 0 (none), 1 (mild), 2 (moderate), or 3 (severe). All subjects had their height (in cm) and weight (in kg) measured without shoes during their first week of the STP.

In the summer of 2006, (2–4 years after subjects completed the STP), research assistants visited subject’s homes to obtain the additional data needed for this study. The study was approved by the University at Buffalo Institutional Review Board, and subjects and parents provided informed consent. Current ADHD symptoms were ascertained using parent ratings on the DBD (Pelham et al. 1992). All subjects met full criteria for a current diagnosis of ADHD upon reassessment. Parents reported the type, dose, and frequency of psychotropic usage during the past school year for treatment of ADHD or any other psychiatric condition. DNA was then collected by buccal brushing. Once collected, the two brushes were labeled, stored, and sent to the laboratory for release of DNA using standard established methods.

The DNA samples were labeled with an accession number corresponding to the informed consent form and a “RESEARCH ONLY” label. The DNA was isolated using a commercially available DNA purification kit (Gentra, Minneapolis, MN). The buccal cell collection and isolation yielded 20 μL of DNA at a concentration of 25–50 ng/μL. After DNA purification, each sample was assigned a new accession number and stored at 4°C for later analysis. Analysis of the DAT1 (SLC6A3) gene was determined by the variable number of tandem repeats (VNTR) according to Vandenbergh et al. (1992). The DRD2 TaqI polymorphism was detected using methods of Grandy et al. (1993). The primers were modified to sense: 5’-CCC TTC CTG AGT GTC ATC A-3’; antisense 5’-CGG CTG GCC AAG TTG TCT-3’. The PCR amplification produced a 304-bp amplicon. The product was digested using TaqI endonuclease (Fermentas Inc, Glen Bernie, MD). The A2 allele is cut by the enzyme and yields 177- and 12-bp fragments. The A1 allele remains intact. The DRD4 48-bp VNTR of exon 3 was analyzed as previously reported by Ebstein et al. (1996).

Four control DNA samples were added to each batch of samples. The control samples were amplified along with the subjects’ DNA and included three sequenced, known controls. For the DAT1 VNTR polymorphism, the three controls were 9, 9, 10, and 10/10 VNTR; for DRD2, A1/A1, A1/A2, and A2/A2; and finally for DRD4, 4/4, 4/7, and 7/7 VNTR. The fourth control sample of each PCR run contained no DNA and served as a negative control (“water blank”).

Analytic plan

One-way analyses of variance (ANOVA’s) were used to look for differences in age, body mass index (BMI), and percent of food consumed on placebo between genotypes. Gender differences by genotypes were assessed using a chi-squared frequency analysis. The primary analysis was a mixed-effects regression model to evaluate the percent of food consumed at lunch as a function of SLC6A3 (9/9, 9/10, and 10/10), DRD2 (A1/A1 + A1/A2 vs. A2/A2) and DRD4 (short and long) genotypes and MPH medication dose (placebo, 0.15, 0.30, and 0.60 mg/kg, corresponding to low, medium, and high doses), and the interaction of genotype by
medication dose. DRD2 A1/A1 and A1/A2 are usually combined in genetic studies because of the small number of A1/A1 genotypes in the population (Epstein et al. 2007). Mixed-effects regression models use all the available data to account for serial correlations between repeated observations as well as differences in variability over repeated observations and, unlike ANOVA, do not assume equal variance at each repeated measure (Hedeker and Gibbons 1997). Additional mixed-effects regression models were used to test differences in dose × genotype × gender (m/f) dummy codes and genotype × dose interactions. The genotypes were dummy-coded for the regression model. All mixed models used a random intercept and had fixed-effect predictors of MPH dose, genotype, and interactions of genotype by dose, with age, gender, and BMI as covariates. Bonferroni corrections were used to correct p values for the three additional models by dividing z = 0.05 by the number of comparisons (z = 0.017). As with the overall model, intercepts were treated as random effects and all other effects and interactions were treated as fixed effects.

Side effects of nausea and appetite loss may be associated with higher doses of MPH and may be predictive of who is at risk for growth suppression in ADHD children treated with MPH (Kramer et al. 2000). Severity ratings of stomachache and loss-of-appetite side effects and percentages of participants experiencing these symptoms were assessed with a mixed-effects regression model with a random intercept and fixed effects of MPH dose, genotype dummy codes, and genotype by dose interactions. The relationship between ratings of stomachache and loss of appetite and percentage of lunch consumed were explored using mixed-effects regression to predict lunch intake with side-effect ratings, dose, and genotype and the interactions between these variables as predictors. Analyses were conducted using SYSTAT (Systat Software, 2004).

Results

Subject characteristics are presented in Table 1. There were no significant differences between genotypes for gender, BMI, or percent of food consumed on placebo. Age, however, significantly differed among SLC6A3 genotypes (p < 0.02), with contrasts showing that 9/9 children were older than 9/10 and 10/10 children (p < 0.01). Age was used as a covariate in the mixed regression models to control for the differences by genotype. SLC6A3 genotype frequencies for the 58 children with DAT genotypes available were as follows: 12% (7/58) were 9/9, 43% (25/58) were 9/10, and 45% (26/58) were 10/10, consistent with the distribution of these genotypes in other studies of DAT in children with ADHD (Stein et al. 2005). We were able to obtain DRD2 genotypes in 53 children and DRD4 genotypes in 55 children. The frequencies for DRD2 were as follows: 57% (30/53) were A1/A1 + A1/A2 and 43% (23/53) were A2/A2. For DRD4, 75% (41/55) had the “short” allele and 25% (14/55) had the “long” allele. The frequency of these genotypes is consistent with that found in the general population (VanTol et al. 1992; Epstein et al. 2007) and in studies of ADHD children (Mill et al. 2003). The SLC6A3, DRD2, and DRD4 alleles were all in Hardy–Weinberg equilibrium.

Although a numerically greater percentage of 9/9 subjects versus subjects with 9/10 or 10/10 genotypes had co-morbid ODD/CD (88% vs. 55%, χ² = 3.1), were previously medicated (100% vs. 78%, χ² = 1.9), and were of ethnic/racial minority status (29% vs.14%, χ² = 1.1), none of the group differences was significant (except for a trend toward a higher rate of externalizing co-morbidities in the 9/9 subjects, p = 0.079). Similarly, there were no statistically significant differences in the prevalence of ODD/CD (54.5% vs. 54.5% χ² = 0), previous medication exposure (76.5% vs. 82.6%, χ² = 0.23), or ethnic/racial minority status (82.4% vs. 82.6% Caucasian, χ² = 0.20) for the DRD2 combined A1/A1 + A1/A2 genotypes versus the A2/A2 genotype, respectively.

In the analysis of the interaction of DAT by dose, there was a main effect of dose (estimate = −38.52, p < 0.001) as well as an interaction of dose × INN genotype (estimate = −13.52, p < 0.001). Regression models to test the interaction between dose and genotype showed significant differences in the rate of change as a function of dose for 9/9 children versus 9/10 (estimate = −39.89, p < 0.001) or 10/10 (estimate = 38.91, p < 0.001) children, with no differences between 9/10 and 10/10 (estimate = −0.95, p = 0.78) (Fig. 1, top). No main effects of SLC6A3 genotypes on eating were observed.

The analysis for DRD2 also showed a main effect of dose (estimate = −52.40, p < 0.001) as well as an interaction of dose × DRD2 genotype such that children with the A2/A2 genotype had a greater dose–response reduction in eating when compared with children with an A1 allele (A1/A1 + A1/A2) (estimate = 15.55, p = 0.007) (Fig. 1, bottom). The analysis of DRD4 again showed a main effect of dose

### Table 1. Descriptive Characteristics by DAT SLC6A3, DRD2, and DRD4 Genotypes (Mean ± SD)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Alleles</th>
<th>Gender (m/f)</th>
<th>Age (years)</th>
<th>BMI</th>
<th>% Food consumed (placebo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DATa</td>
<td>9/9</td>
<td>6/1</td>
<td>10.9 ± 1.5b</td>
<td>17.6 ± 2.5</td>
<td>82.4 ± 14.46</td>
</tr>
<tr>
<td></td>
<td>9/10</td>
<td>20/5</td>
<td>9.1 ± 1.9</td>
<td>18.2 ± 4.1</td>
<td>70.0 ± 20.88</td>
</tr>
<tr>
<td></td>
<td>10/10</td>
<td>24/2</td>
<td>8.5 ± 2.2</td>
<td>18.0 ± 4.5</td>
<td>69.6 ± 15.78</td>
</tr>
<tr>
<td>DRD2c</td>
<td>A1/A1 + A1/A2</td>
<td>15/5</td>
<td>8.9 ± 2.0</td>
<td>19.2 ± 4.6</td>
<td>75.6 ± 15.7</td>
</tr>
<tr>
<td></td>
<td>A2/A2</td>
<td>28/5</td>
<td>9.4 ± 2.0</td>
<td>19.6 ± 4.9</td>
<td>73.5 ± 16.4</td>
</tr>
<tr>
<td>DRD4d</td>
<td>Short</td>
<td>34/7</td>
<td>9.1 ± 2.2</td>
<td>18.1 ± 4.3</td>
<td>74.1 ± 18.4</td>
</tr>
<tr>
<td></td>
<td>Long</td>
<td>13/1</td>
<td>8.7 ± 1.7</td>
<td>18.2 ± 3.9</td>
<td>68.9 ± 14.3</td>
</tr>
</tbody>
</table>

a n = 58.
b Significant difference versus 9/10 and 10/10 subjects.
c n = 53.
d n = 55.

Abbreviations: DAT = Dopamine transporter; SD = standard deviation; m = male; f = female; BMI = body mass index.
were significant interactions of DRD2 genotype by dose (estimate = 4.100, p = 0.008) and of DRD4 genotype × dose (estimate = 0.3850, p = 0.007). Regression models also showed that appetite suppression ratings (estimate = 0.385, p = 0.01) were associated with percent of lunch consumed. Reports of stomachache were also related to food consumption (estimate = –6.88, p < 0.02), but stomachache did not interact with dose and genotypes to predict food consumption (p = 0.50) and thus could not mediate the effect of dose by genotype on consumption.

Discussion

Using a within-subject, randomized, placebo-controlled design, we have shown that specific DAT SLC6A3 and DRD2 genotypes interact with MPH dose to affect food intake in children with ADHD. There was a main dose–response effect of MPH on food intake as all children reduced eating with increasing doses of MPH. Across all genotypes, when compared with placebo, the 0.3 mg/kg dose reduced lunch consumption by 15% while the 0.6 mg/kg dose reduced consumption by 27%. Children with the SLC6A3 9/9 DAT polymorphism, however, ate significantly less of their lunch meal in response to MPH than children with the more common 9/10 and 10/10 polymorphisms, who did not differ in their food intake in response to MPH. Controlling for age and weight, children with the 9/9 genotype did not differ in their food consumption on placebo when compared with the 9/10 and 10/10 children, yet they showed a greater dose–response reduction in eating on MPH (by 28% and 49% of consumption on placebo, for the 0.3 mg/kg and 0.6 mg/kg doses, respectively). Children with the DRD2 A2/A2 polymorphism ate significantly less of their lunch meal than children with an A1 DRD2 allele, consistent with the DAT results in that children with DA-related genotypes associated with greater brain DA signaling showed a dose–response reduction in energy intake on increasing doses of MPH versus children with DAT and D2 genotypes associated with lower DA signaling. We did not observe an effect of DRD4 polymorphisms on eating in this study, which is not completely unexpected as the evidence for an effect on energy intake with this genotype is not nearly as strong as with DAT SLC6A3 (Epstein et al. 2004; Epstein and Leddy 2006; Epstein et al. 2007a) or DRD2 (Epstein and Leddy 2006; Epstein et al. 2004; Epstein et al. 2007a; Epstein et al. 2007b).

Several prior studies failed to find an association between DAT genotypes and the therapeutic response to stimulants when combining homozygotes for the 9 allele with heterozygotes, likely due to the low frequency of the 9/9 genotype (Winsberg and Comings 1999; Roman et al. 2001), but more recent studies (Kirley 2004; Lott et al. 2005; Stein et al. 2005; Joober et al. 2007) have shown that 9/9 ADHD subjects differ in their therapeutic response from subjects who have genotypes containing a 10 allele, suggesting that the 9/9 genotype may represent a unique group of ADHD patients. In the recent Preschool ADHD Treatment Study (PATS) (McCough et al. 2006), the 9/9 genotype was associated with poorer response on parent ratings of ADHD symptoms, although this effect disappeared when parent and teacher ratings were combined into a single index. PATS did, however, find evidence of other pharmacogenetic influences on drug tolerability, including motor tics and irritability. In a study of

FIG. 1. Percent of lunch consumed by dopamine transporter (DAT) and DRD2 genotypes as a function of methylphenidate (MPH) dose. DAT 9/9 children ate less of their lunch food than both 9/10 (p < 0.001) and 10/10 (p < 0.001) children, and DRD2 A2/A2 children ate less of their lunch food than A1/A1 + A1/A2 (p = 0.007) children, as MPH dose increased.

(estimate = –41.17, p < 0.001) but no significant interaction of dose × DRD4 genotype (p > 0.05).

There was a main effect of MPH dose on the incidence of both stomachache (estimate = 0.193, p = 0.009) and loss of appetite (estimate = 1.36, p < 0.0001), but there was no main effect of any of the genotypes on stomachache or loss of appetite. There was no interaction of appetite ratings by DAT genotype by dose (estimate = –0.0003, p = 0.121), but there
adults with ADHD (Mick et al. 2006), there was no clear relationship between DAT polymorphisms and efficacy or self-reported appetite loss, but specific measures of food consumption were not collected.

The biological mechanism whereby the DAT SLC6A3 9/9 and DRD2 A2/A2 genotypes affect appetite on MPH is not known. Given the mechanism of action of stimulants, however, it is logical to consider brain appetite control systems that are partly under the control of DA (Berridge 1996). DA has been linked to the etiology of ADHD (Volkow et al. 2007) and structural and functional imaging studies in children (Cheon et al. 2003) and adults (Krause et al. 2000) with ADHD have found that DAT levels correlate with ADHD symptom severity. Adoption and twin studies suggest that genetics accounts for >70% of the heritability of ADHD symptoms (Biederman and Faraone 2002). The transmission mode is unknown but the etiology is likely to be polygenic (Biederman and Faraone 2002). DA genes are prime candidates for genetic susceptibility and treatment response in ADHD because a majority of the specific genes implicated in ADHD encode components of the DA signaling system: The DAT (Cook et al. 1995) as well as the D4 and D5 DA receptors 9 (Swanson et al. 2000).

Our data suggest that MPH-induced appetite suppression may relate to differences in synaptic DA signaling among the DAT SLC6A3 and DRD2 genotypes. The accumulating evidence suggests that DAT 9/9 genotypes are associated with lower DAT protein levels and/or expression and therefore higher levels of brain synaptic DA when compared with genotypes having a 10-repeat allele (VanNess et al. 2005) and that the DRD2 A2/A2 polymorphism is associated with greater brain DA signaling when compared with polymorphisms containing an A1 allele (Noble et al. 1991). Thus, ADHD children with DAT 9/9 and/or DRD2 A2/A2 genotypes may have a relative abundance of synaptic DA, which would rise with increasing dosages of MPH. Amplifying the DA synaptic signal to high levels would reduce eating by curbing appetite, as is often seen in the case of chronic drug abuse (Coehran et al. 1998). Consistent with this, the chronic administration of MPH reduces the reinforcement value of drugs of abuse and of natural rewards such as sucrose (Bolanos et al. 2003). Conversely, the DAT VNTR or DRD2 alleles may be in linkage disequilibrium with other functional genetic variants that are responsible for the observed effect on eating in response to MPH.

Stomachache and loss of appetite may be important to monitor during MPH treatment as one study found them to be predictors of subsequent growth suppression (Kramer et al. 2000). We found that loss of appetite and stomachache varied with MPH dose, that appetite ratings were related to food intake, and that appetite ratings were modified by DRD2 and DRD4 genotypes, suggesting that these genotypes may influence appetite with increasing MPH dosage. There are some differences in the relationship of dose x genotype for the eating versus appetite ratings. The amount consumed was related to the interaction of dose x genotype for the DAT and DRD2 genotypes, but the appetite ratings were related to the interaction of dose by DRD2 and DRD4. This points to the often inconsistent relationship between appetite ratings and amount of food consumed (Gray et al. 2002; Gray et al. 2003) and shows the necessity of measuring intake rather than focusing only on subjective ratings of appetite. In this study, both the side effects and amount of food consumed were measured by the participant’s counselor, who may have used the objective measure of amount of food consumed to estimate loss of appetite. Further research on the influence of genotype on the relationship between patient-reported loss of appetite and food consumption is needed.

The strengths of this study are its randomized blind design using multiple doses of MPH dosed by body weight. Although there is concern over a potential association of stimulant-induced anorexia with growth suppression (Poulton 2005), we did not measure growth in this study, and the daytime appetite suppression observed here may not translate to clinically relevant growth suppression. Because stimulant-induced anorexia has been found to be one of the side effects most likely to persist over time (Charach et al. 2004; Wilens et al. 2005; Wigal et al. 2006) and is one of the most frequent reasons that children discontinue treatment with stimulants (Efron et al. 1997; McGough et al. 2005; Zachor et al. 2006), a reliable marker of appetite suppression may have clinical utility for predicting treatment tolerability and adherence even if found not to be predictive of changes in growth velocity.

The 9/9 polymorphism appears to be present in approximately 10–12% of children with ADHD (Stein et al. 2005). Thus, our findings are limited by the small n (7) of the 9/9 group in comparison with the other genotypes, although our sample size is comparable to those reported in other pharmacogenetic studies of therapeutic response in pediatric ADHD (McGough 2005; Stein et al. 2005; McGough et al. 2006). Although the differences were not significant, 9/9 subjects were more likely to have ODD/CD or have been previously medicated. These differences may have reached significance in a larger sample. However, the entire sample is similar to others drawn from the STP (Pelham et al. 1999a; Pelham et al. 1999b; Pelham et al. 2005a; Pelham et al. 2005c). The finding that all of the 9/9 subjects had previous stimulant exposure should have predicted less appetite suppression, not more, versus the other DAT genotypes that included some subjects who were stimulant naïve. Hence, it is unlikely that the difference in previous medication usage contributed to the differential rates of appetite suppression. In contrast, the DRD2 findings are not limited by the relatively rare frequency of one of the alleles and the unbalanced level of ODD/CD and prior medication usage across the genotypes.

Additional limitations include the use of observers to rate food intake rather than weighing food before and after eating to get a more quantitative measure of intake, and the fact that lunches were sent from home and were therefore different for each child. Parents may have provided different amounts of food based on the weight of the child or the child’s recent eating, which may have reduced the impact of MPH on intake. It is also possible that additional variables not accounted for in this protocol may have caused the differential appetite suppression seen in this sample. For example, blood levels of d-MPH after a set oral dose vary widely across subjects due to differences in the rates of absorption and elimination (Tiecher et al. 2006). This protocol employed short-acting stimulants, which have been replaced by extended-release stimulants as the standard first-line medication treatment for pediatric ADHD (Pliszka 2007).

Whereas there is no clear evidence that extended-release stimulants have differential rates of anorexia versus short-acting versions (Pelham et al. 2001; Weisler et al. 2007), these data cannot be definitively applied to modern stimulant for-
mulations. Similarly, dose varied from 0 up to 1.8 mg/kg in a 24-hour span, so that findings may not apply to the more gradual dose titrations used in clinical practice that might promote greater habituation to drug-induced adverse events like anorexia. Future studies should control the types and amount of food provided to children and measure caloric and macronutrient intake. Last, a sizable number of our subjects met criteria for ODD or CD, and it is possible that these comorbidities impacted food intake. We think, however, that this is unlikely as several other studies (Spencer et al. 1998; Kramer et al. 2000) have found no difference in growth patterns in ADHD children with and without ODD/CD.

In summary, this study found that among children with ADHD, those with DA-related genotypes associated with greater brain DA signaling, DAT SLC6A3 9/9, and DRD2 A2/A2, showed a greater suppression of lunch meal intake as MPH dose increased in comparison to children with DA genotypes associated with lower brain DA signaling. Our results suggest that individual differences in dopaminergic activity, as indexed by genetic variation in the brain DAT and D2 receptor, may be related to MPH-influenced energy intake and could be predictive of which children with ADHD are at risk for significant stimulant-induced anorexia. If these results are confirmed and, if these genotypes are found to be associated with suppression of growth velocity, then they may have clinical utility as markers of greater suppression of energy intake. ADHD children with these genotypes may then warrant consideration for treatments with reduced risk for appetite suppression, such as behavior therapy, low-dose stimulants, or nonstimulants like atomoxetine.

Disclosures

In the past 3 years, Dr. Waxmonsky has served on the speaker’s board for Novartis, received an honorarium from Shire, and received research support from Shire and Eli Lilly. Dr. Epstein is a consultant for Kraft Foods and Griffin Hospital, Yale University. Dr. Erbe has received educational and research support from Genzyme Corporation and has a consulting relationship with the National Institute of General Medical Sciences, National Institutes of Health. Dr Pelham was paid an honorarium by Shire Pharmaceuticals in 2005 for his participation in a “Safety Consultants’ Meeting.” Drs. Leddy and Salis, and Mr. Mahaney, Ms. Gnagy, and Mr. Paluch have no conflicts of interest or financial ties to disclose.

Acknowledgments

We gratefully acknowledge Jennifer Trillizio, Amy Castle, and Lisa Burrows-MacLean for their assistance in collecting the data for this study.

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