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ARTICLE

A longitudinal study of cytochrome P450 2D6 (CYP2D6) activity during adolescence

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Abstract

CYP2D6 substrates are among the most highly prescribed medications in teenagers and also commonly associated with serious adverse events. To investigate the relative contributions of genetic variation, growth, and development on CYP2D6 activity during puberty, healthy children and adolescents 7–15 years of age at enrollment participated in a longitudinal phenotyping study involving administration of 0.3 mg/kg dextromethorphan (DM) and 4-h urine collection every 6 months for 3 years (7 total visits). At each visit, height, weight, and sexual maturity were recorded, and CYP2D6 activity was determined as the urinary molar ratio of DM to its metabolite dextrorphan (DX). A total of 188 participants completed at least one visit, and 102 completed all seven study visits. Following univariate analysis, only CYP2D6 activity score ($p < 0.001$), urinary pH ($p < 0.001$), weight ($p = 0.018$), and attention-deficit/hyperactivity disorder (ADHD) diagnosis ($p < 0.001$) were significantly correlated with log(DM/DX). Results of linear mixed model analysis with random intercept, random slope covariance structure revealed that CYP2D6 activity score had the strongest effect on log(DM/DX), with model-estimated average log(DM/DX) being 3.8 SDs higher for poor metabolizers than for patients with activity score 3. A moderate effect on log(DM/DX) was observed for sex, and smaller effects were observed for ADHD diagnosis and urinary pH. The log(DM/DX) did not change meaningfully with age or pubertal development. *CYP2D6* genotype remains the single, largest determinant of variability in CYP2D6 activity during puberty. Incorporation of genotype-based dosing guidelines should be considered for CYP2D6 substrates given the prevalent use of these agents in this pediatric age group.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

Several CYP2D6 substrates are used in the management of neurodevelopmental, behavioral, and psychiatric disorders in children and adolescents, often off-label, for indications that often differ from the adult conditions for which the medications originally were developed. Therefore, little information is available to guide

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dosing in pediatric patients, and even less regarding the influence of growth and development on the processes influencing drug disposition and response.

WHAT QUESTION DID THIS STUDY ADDRESS?

What is the relative contribution of genetic variation and the processes of growth and development on variability in CYP2D6 activity during puberty?

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?

CYP2D6 genotype remains the single, largest biological determinant of variability of CYP2D6 activity during puberty. Effects of factors associated with growth and sexual maturation are small and have limited ability to explain variability in CYP2D6 activity.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?

Incorporation of genotype-based dosing guidelines should be considered for CYP2D6 substrates, especially given the prevalent use of antipsychotic and antidepressant medications in children and adolescents. A challenge for the future remains to build on, and extend the accumulating database describing the relative contribution of ontogeny and genetic variation to observed variability in drug disposition and response across the continuum from birth to adulthood.

INTRODUCTION

The treatment of neurodevelopmental, behavioral, and psychiatric disorders in children, such as attention-deficit/hyperactivity disorder (ADHD), autism spectrum disorder (ASD), and mood disorders, represents a critically important opportunity to optimize the use of medications through precision therapeutics. ASD, for example, has a relatively high prevalence (2018 data) of 23 per 1000 (or 1 in 44) among 8-year-old children.[1] Medications used in the treatment of children with ASD primarily target symptoms of irritability, aggression, and anxiety, with the use of antipsychotic medications, such as risperidone, aripiprazole, and quetiapine,[2] and antidepressant medications, such as fluoxetine and sertraline. For many of these patients, dose adjustment and optimization rely on interpretation of behavioral response to assess efficacy and tolerance.

CYP2D6 pharmacogenetics has considerable potential to impact precision therapeutics in children,[3,4] and guidelines for *CYP2D6* genotype-dependent dose adjustments are becoming available.[5] Available data indicate that *CYP2D6* substrates risperidone, fluoxetine, aripiprazole, amitriptyline, and haloperidol,[6] are among the most highly prescribed central nervous system (CNS)-acting medications in children.[7] A survey of antipsychotics used in Medicaid-insured children and adolescents revealed that among youth who used a single atypical antipsychotic agent ($n = 8053$), the top three agents were risperidone (55.5%), aripiprazole (31.7%), and quetiapine (20.6%).[8] The majority of antidepressant and antipsychotic use in children and adolescents is off-label.[9] The primary indication for antipsychotics in children is irritability and aggression in neurodevelopmental disorders compared to psychosis in adults. The limited data from pediatric clinical trials have important safety implications as well. In an analysis of serious adverse events in children aged 0–17 years reported to the US Food and Drug Administration (FDA) from 2008–2012, 41% of all reports were accounted for by a total of 15 drugs, including several CYP2D6 substrates associated with psychiatric side effects (i.e., aripiprazole, quetiapine, risperidone, and atomoxetine).[10] Available data suggest that selective serotonin reuptake inhibitor adverse drug reactions (e.g., agitation, restlessness, hyperactivity, and irritability) are two to three times more prevalent in younger children than in adolescents, and lowest in adults.[11] Given that side effect profiles may be age-dependent and differ between children and adults, the need for a better understanding of the dose→exposure→response relationship for these medications in children is becoming increasingly apparent.

It has been proposed that children as young as 4 years of age can be considered as small adults such that scaling of pharmacokinetic parameters based on body weight alone allows available adult data to inform pediatric dosing.[12] However, the premise that maturational processes may be sufficiently complete to infer drug disposition in children and adolescents from adult data does not necessarily apply to expression and function of drug targets. Furthermore, the process of dose selection for use in a pediatric clinical trial intended to generate population data for regulatory purposes is different than the clinical situation faced by

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a pediatrician who must adequately manage the disorder for the specific child for whom a medication has been prescribed. In this context, *individual* children are *NOT* small *average* adults, and the challenge is to identify factors that make each child unique, and to develop dosing strategies that take these unique characteristics into consideration.

The impact of developmental changes in the processes governing drug absorption, distribution, metabolism, and excretion from birth through adolescence on drug dosing in children is well-recognized,[13] and new knowledge continues to accumulate.[14,15] In the current investigation, we conducted a longitudinal phenotyping study to assess the relative contribution of genetic variation and the processes of growth and development to observed variability in CYP2D6 activity during puberty.

METHODS

Study participants

This study was open to healthy children and adolescents 7–15 years of age, boys or girls, of any racial or ethnic background. A subset of participants were pediatric patients meeting the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) criteria for a primary diagnosis of ADHD or attention-deficit disorder (ADD) to facilitate a substudy assessing the effect of *CYP2D6* genotype on the atomoxetine dose–exposure relationship.[16] Participants were recruited through notices posted in the Developmental and Behavioral Health Clinics and directly by providers in those clinics. Study enrollment opportunity was also shared system-wide through a staff-facing website at Children's Mercy Kansas City, Kansas City, MO. Participants were deemed to be otherwise healthy as determined by physical and laboratory examination, including laboratory assessment of hepatic, renal, and hematopoietic function. Exclusion criteria included: an adverse reaction to dextromethorphan (DM); historical or physical evidence of any disorder associated with swallowing or gastrointestinal function that may influence absorption and/or gastric emptying, such as reflux, inflammatory bowel disease, or Crohn's disease; historical or physical evidence of neurological disease (excluding simple febrile seizures and/or ADHD); patients with known serious structural cardiac abnormalities, cardiomyopathy, serious heart rhythm abnormalities, or other serious cardiac problem; concurrent therapy with medications known to inhibit CYP2D6 (listed in Supplementary Methods). Additional exclusion criteria included treatment within the past 2 months with paroxetine or fluoxetine, or the past 6 months with terbinafine; clinically significant abnormal safety laboratory values as determined by the treating physician; and pregnancy. If during the 3-year longitudinal phase of the study, participants were prescribed medications known to be CYP2D6 inhibitors, they were allowed to continue in the study; the dose and start date of these medications were recorded and the effect of the inhibitor on CYP2D6 activity was assessed, but the data points were excluded from the ontogeny and genotype–phenotype correlation analysis, as described in more detail below. The study was approved by the University of Missouri-Kansas City Pediatric Institutional Review Board at Children's Mercy Hospitals and Clinics, Kansas City, MO, and registered as study NCT01118858 at ClinicalTrials.gov. Written, informed parental permission and child/adolescent assent was obtained for each participant.

Study protocol and procedures

During the pre-study screening visit, prospective participants (and their parents) were provided with a description of the proposed study, their questions were answered, and they were given a copy of the permission/assent form to review and sign. After review of participants' medical history and use of medications, including any non-prescription and herbal remedies, all participants underwent a complete physical examination, including vital signs (blood pressure, heart rate, respiration rate, and temperature), height, and weight. Body mass index (BMI) was calculated from height and weight, and z-scores/percentiles for height, weight, and BMI for age and sex were determined using Centers for Disease Control and Prevention (CDC) growth charts and an internally developed program.[17] Pubertal development was assessed by Tanner stage, breast development was assessed by both visual inspection and palpation, pubic hair was assessed by visual inspection, and testicular volume was measured by direct comparison to orchidometer beads. All Tanner staging was conducted by pediatric subspecialists and scored (stages 1 through 5) based on the methods of Marshall and Tanner[18,19] as described in Zitelli and Davis.[20] For male pubic hair development, a Tanner score of 6 is possible and scores of 6 were included with the Tanner 5 group. Blood was drawn for serum chemistries, liver function tests and a hematology panel, and *CYP2D6* genotyping. A urine pregnancy test was performed for any girls of childbearing potential.

Dextromethorphan phenotyping protocol

Participants were admitted to the study unit the morning after an overnight fast (only water was permitted).

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At each study visit, the medical history and use of medications were reviewed and recorded, and all participants underwent a physical examination and an assessment of pubertal development by Tanner stage, as described above. Prior to DM administration, the participants completely emptied their bladders and provided a blank urine sample. A single 0.3 mg/kg dose of DM as Robitussin Cough (7.5 mg/5 ml; alcohol-free, fruit punch flavor), as used in previous studies,[21,22] was administered orally. All urine produced over the 4-h study period was collected, pH measured using an Accumet model AB15 pH meter (Fisher Scientific, Pittsburgh, PA), and an aliquot retained for analysis of DM and its three metabolites. One hour after dosing, participants were provided with a small meal selected from the standard hospital menu. If participants were suffering from flu-like symptoms around the time of the scheduled study visit, the visit was re-scheduled for 1–2 weeks later. This phenotyping protocol was conducted as described above, every 6 months for a total of seven phenotyping visits over 3 years.

CYP2D6 genotyping

CYP2D6 genotyping was conducted as described in detail elsewhere.[23–27] Details of genotyping strategy and assays[24–29] are provided in Supplementary Methods and Table S1. Alleles were defined according to the Pharmacogene Variation Consortium (PharmVar) at https://www.pharmvar.org/gene/CYP2D6.[30]

CYP2D6 genotype data were translated into predicted phenotypes based on "activity score" (AS), a simplified system to infer predicted phenotype from diplotype calls.[29,31] Genotypes were translated into predicted phenotypes as recommended by the Clinical Pharmacogenetics Implementation Consortium (CPIC) and Dutch Pharmacogenetics Working Group (DPWG).[32] Normal function alleles (*CYP2D6*1*, **2*, **35*, etc.) were assigned a value of "1," decreased function alleles a value of "0.25" (**10*) or "0.5" (**9*, **17*, and **29*), and no function alleles (**3*, **4*, **5*, **6*, etc.) a value of "0"; for duplications/multiplication events, the value was multiplied by the number of copies detected (e.g., *CYP2D6*2* × 2 = 1 × 2 = "2"). The AS for an individual study participant was assigned as the sum of the scores for each allele, with poor metabolizers (PMs) defined by an AS of "0" and ultrarapid metabolizers (UMs) defined by an activity score ≥2.25. Participants with an AS of 0.25 ($n = 1$), 0.75 ($n = 1$), and 1.25 ($n = 3$) were grouped with the next higher AS group (0.5, 1, and 1.5, respectively) for analyses. *CYP2D6* genotypes and assigned phenotypes for study participants are provided in Table S2.

Phenotyping analysis

Urinary concentrations of DM and its metabolites dextrorphan (DX), 3-methoxymorphinan, and 3-hydroxymorphinan were quantified by high-performance liquid chromatography with fluorescence detection, as described previously.[22] Details are provided in Supplementary Methods.

Statistical analysis

Normality of variable distribution was assessed by visual inspection of frequency histograms and normal quantile plots. Normally distributed continuous variables are summarized with mean ± SD, and non-normally distributed variables are summarized with median and interquartile range. The urinary DM/DX ratio was log-transformed for analyses (log(DM/DX)). An initial univariate analysis was conducted to explore the effect sizes and explanatory power of the demographic, developmental, physiological, and genetic variables of interest on log(DM/DX); the relationship between log(DM/DX) and continuous variables was estimated by linear regression, whereas analysis of variance (ANOVA) followed by post hoc analysis using Tukey's Honestly Significant Difference was used for nominal and categorical variables. These preliminary analyses were primarily for the purposes of visualization and were not adjusted for other explanatory variables or clustering of visits within participants. Assessments of variable distribution, linear regression, and ANOVA were conducted using JMP Pro 14 (SAS) with a nominal threshold of significance set at $p < 0.05$.

Linear mixed modeling was conducted to assess the effect of age on log-transformed urinary DM/DX ratio (log(DM/DX)) with adjustment for dependence of repeated observations within participant. Linear mixed models were fit in SAS using the Mixed Procedure, with age at study visit, race, sex, ADHD diagnosis, *CYP2D6* AS, and urinary pH included as explanatory variables. An age X sex interaction was included in the model as pubertal growth spurts and sexual maturation occur earlier in girls than in boys. To choose a covariance structure, the model was fit three times: once with a random patient intercept and first-order autoregressive (AR (1)) structure specified, once with a random patient intercept and random patient age slope, and once with a random patient intercept, random patient slope, and AR (1) structure specified. Models were fit with maximum likelihood to allow comparison of covariance structures using the Bayesian Information Criterion (BIC), a likelihood-based measure of model fit.

To evaluate effects on log(DM/DX) of surrogates of age associated with growth (height and weight) or

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development (Tanner scores for pubic hair and testicular size/breast development), the model described above was re-fit with each surrogate substituted for age. For example, the height model included height and a height X sex interaction in place of age and an age X sex interaction, and a random patient height (rather than age) slope. Although height and weight are correlated, each was substituted for age in the model as there is a limit as to how tall an individual will grow, but there is no limit on how heavy they may become. Similarly, changes in pubic hair and increases in testicular size for boys or breast development in girls demonstrate different developmental trajectories,[18,19] and were also assessed individually. As with the age model, each of the four surrogate models was fit three times, once for each of the three covariance structures.

RESULTS

Demographic characteristics of the study population

A total of 188 children and adolescents, representing 116 families (41 families with 2 siblings, 10 families with 3 siblings, and 4 families with 4 siblings) completed at least one study day. Over the 3-year study period, a total of 1115 study days were completed, and 102 participants completed all seven study visits. The number of participants completing each study visit decreased at each visit. Loss to follow-up was primarily due to an inability to contact the family to schedule the following visit. No participants discontinued the study due to an adverse reaction related to DM administration.

Twelve postdose urine samples were excluded from the statistical analysis. Five samples were from a participant who was enrolled while taking a medication that was not included in the list of exclusions, in part, due to confusion between "bupropion," a CYP2D6 inhibitor, and "buspirone," a non-CYP2D6 inhibitor. This individual had urinary log(DM/DX) values concordant with genotype, albeit at the low end of the distribution for AS of 1.5, and five study visits were completed before the interaction was recognized. One urine sample from one participant was excluded due to an interfering peak, present in both the pre- and postdose visit four urine samples, that precluded accurate quantitation of DM in the postdose sample. Two participants reported fluoxetine as a concurrent medication at visits six and seven resulting in the exclusion of these samples, accounting for 10 of the excluded samples. For the remaining two excluded samples, fluoxetine use was not reported for either participant at visit five, but the urinary DM/DX ratio values for both participants were similar to the values obtained at visits six and seven, and markedly different from the values obtained at visits one through four (see footnote *a* in Table 1). As neither the participant or parent reported fluoxetine use at that visit, it was not possible to determine with certainty whether concurrent fluoxetine was present, or not. Therefore, the analysis was conduct with (n = 1105 evaluable samples) or without (n = 1103 evaluable samples) the visit five samples for these participants across the seven visits, with 714 samples contributed by the 102 participants who completed all seven study visits.

The number of participants completing each study visit and their demographic characteristics are presented in Table 1. Participants were primarily of European descent (47%) or African American (41%), 60% were boys, and ~40% of the cohort had a diagnosis of ADHD/ADD. There were no significant changes in any of these characteristics across the study visits due to dropouts, nor in the pattern of medication use within the ADHD/ADD subgroup.

At the first study visit, the mean age was 11.2 ± 2.5 years. For those participants completing all visits (n = 102), height increased by 16.8 ± 6.4 cm. Inspection of the distribution of height revealed a subset of 14 participants (5 boys and 9 girls) who grew significantly less (3.5 ± 2.1 cm, range: 0–7.7 cm) than the majority of the cohort (18.9 ± 3.8 cm, range: 11.1–26.7 cm; $p < 0.001$; Figure S1A), consistent with near complete maturity at the start of the study. Twelve of 14 participants were Tanner stage 4 or 5 at visit one, indicating that growth and development was nearly complete for this cohort. This subset was significantly older (13.7 ± 0.8 vs. 10.0 ± 1.9 years, $p < 0.001$) and gained less weight (9.5 ± 7.4 vs. 17.9 ± 8.4 kg, $p < 0.001$) than the other participants. Overall, weight increased by 16.7 ± 8.7 kg for the entire study group; for three participants, weight increased more than 40 kg (Figure S1B).

From a developmental perspective, the average Tanner score increased from ~2.4–3.7 over the 3-year study period ($p < 0.001$). The distribution of Tanner scores at each visit is presented separately for pubic hair and for breast development (girls) or testicular size (boys) in Table 1. Maturation of the cohort over the study period is presented in Figure 1a,b. Hierarchical clustering of the Tanner stage data for breast development/testicular size and pubic hair for the 102 participants completing all 7 study days revealed two distinct clusters that could be broadly defined as "not maturing" (n = 32) or "maturing/fully matured" (n = 70). The "not maturing group" was defined by Tanner stage (generally stage 1 for at least the first four visits and no progression beyond stage 3, except at the final visit) and younger age at entry into the study compared to the maturing/fully matured group. The younger, non-maturing group tended to have a greater height increase (18.3 ± 3.0 vs.

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TABLE 1 Characteristics of participants at each individual study visit included in the analysis ($n = 1103$ total visits[a])

	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
n	188	173	165	151	151	144	131
Age (year)[b]	11.2 ± 2.5	11.6 ± 2.4	12.1 ± 2.4	12.5 ± 2.4	13.1 ± 2.5	13.4 ± 2.5	13.6 ± 2.21
Boys (%)	115 (61.2)	105 (60.7)	102 (61.8)	95 (62.9)	92 (60.9)	89 (61.8)	81 (61.8)
Race							
European ancestry (%)	88 (46.8)	79 (45.7)	78 (47.3)	73 (48.3)	76 (50.3)	68 (47.2)	60 (45.8)
African American (%)	80 (42.5)	75 (43.3)	68 (41.2)	59 (39.1)	60 (39.7)	62 (43.1)	54 (41.2)
Mixed EA-AA (%)	15 (8.0)	14 (8.1)	14 (8.5)	15 (9.9)	13 (8.6)	11 (7.6)	14 (10.7)
Other (%)	5 (2.7)	5 (2.9)	5 (3.0)	4 (2.7)	2 (1.3)	3 (2.1)	3 (2.3)
Height (cm)[b]	146.8 ± 15.9	149.1 ± 15.5	152.1 ± 15.2	153.4 ± 14.8	156.5 ± 13.8	158.6 ± 13.5	160.2 ± 13.1
Weight (kg)[c]	41.6 (30.7, 57.9)	43.9 (32.1, 60.4)	47.5 (34.3, 64.5)	49.2 (36.5, 65.0)	53.9 (40.0, 69.1)	55.7 (41.9, 69.8)	55.7 (43.8, 68.6)
BMI (kg/m^2)[c]	19.2 (17.1, 23.0)	19.8 (17.1, 23.6)	20.2 (17.8, 24.2)	20.9 (17.6, 25.1)	21.5 (18.6, 25.1)	21.8 (18.8, 24.5)	21.4 (18.7, 24.4)
Height percentile[d]	55.6 ± 29.8	55.8 ± 29.6	55.8 ± 30.0	54.0 ± 29.8	55.1 ± 29.7	55.9 ± 28.7	56.9 ± 30.2
Weight percentile[c,d]	74.4 (43.9, 91.8)	76.2 (45.0, 93.8)	75.8 (48.7, 94.4)	77.0 (45.0, 94.2)	76.1 (52.6, 94.7)	78.6 (51.2, 93.9)	78.2 (47.4, 95.0)
BMI percentile[c,d]	77.7 (53.6, 92.0)	76.4 (51.9, 92.7)	79.0 (52.1, 92.9)	79.9 (48.3, 94.0)	78.0 (53.5, 93.3)	78.2 (55.0, 92.6)	77.2 (53.7, 93.8)
Testicular/breast Tanner stage[e]	2.39	2.61	2.78	2.99	3.36	3.51	3.73
Pubic hair Tanner stage[f]	2.37	2.57	2.79	2.91	3.31	3.45	3.66
CYP2D6 genotype[g]							
PM (%)	10 (5.3)	10 (5.8)	7 (4.3)	10 (6.6)	9 (6.0)	7 (4.9)	5 (3.8)
IM (%)	58 (30.9)	58 (33.5)	54 (32.7)	48 (31.8)	45 (29.8)	46 (31.9)	43 (32.8)
NM (%)	160 (60.6)	99 (57.2)	98 (59.4)	88 (52.3)	90.2 (60.9)	86 (59.7)	80 (61.1)
UM (%)	6 (3.2)	6 (3.5)	6 (3.6)	5 (3.3)	5 (3.3)	5 (3.5)	3 (2.3)
CYP2D6 AS							
0	10 (5.3)	10 (5.8)	7 (4.2)	10 (6.6)	9 (6.0)	7 (4.8)	5 (3.8)
0.25–0.5	12 (6.4)	12 (6.9)	10 (6.1)	8 (5.3)	9 (6.0)	7 (4.8)	6 (4.6)
0.75–1	46 (24.5)	46 (26.6)	44 (26.7)	40 (26.5)	35 (23.2)	38 (26.4)	35 (26.7)
1.25–1.5	56 (29.8)	45 (26.0)	46 (27.9)	37 (24.5)	42 (27.8)	42 (29.2)	37 (28.2)
2	58 (30.9)	54 (31.2)	52 (31.5)	51 (33.8)	51 (33.8)	45 (31.3)	45 (34.4)
>2.25	6 (3.2)	6 (3.5)	6 (3.6)	5 (3.3)	5 (3.3)	5 (3.5)	3 (2.3)
Urinary pH	6.71 ± 0.57	6.37 ± 0.60	6.45 ± 0.60	6.55 ± 0.58	6.69 ± 0.54	6.77 ± 0.59	6.71 ± 0.62

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TABLE 1 (Continued)

	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7
ADHD (%)	73 (38.8)	67 (38.7)	65 (39.4)	59 (39.1)	57 (37.7)	54 (37.5)	49 (37.4)
Stimulant – Any[h] (%)	55/73 (75.3)	42/67 (62.7)	38/65 (58.5)	35/59 (59.3)	28/57 (49.1)	25/54 (46.3)	19/49 (38.8)

Abbreviations: ADHD, attention-deficit/hyperactivity disorder; BMI, body mass index; CDC, Centers for Disease Control and Prevention; DM, dextromethorphan; DX, dextrorphan; EA-AA, European American–African American.

[a]The visit 4 sample from participant K198 was not included in the dataset due to the presence of an interfering peak that precluded accurate quantitation of DM; the interfering peak was also present in the pre-dose sample. For participants K196 and K198, fluoxetine was reported as a concurrent medication at study visits 6 and 7; thus, samples from those visits were excluded from statistical analysis. Although fluoxetine was not reported at the study 5 visits, the urinary DM/DX ratio values at that visit for each participant (DM/DX = 2.000 for K196 and 0.381 for K198) were similar to the values obtained at visits 6 and 7 (0.335 and 0.597 for K196; 0.869 and 0.314 for K198), and markedly different from the values obtained at visits 1 to 4 (0.028–0.043 for K196, and 0.010–0.021 for K198). As described in Results, the linear mixed model analysis was conducted with and without the visit 5 data for these two subjects.

[b]Mean ± SD.

[c]Median (interquartile range); comparison using Wilcoxon/Kruskal–Wallis rank sum test.

[d]Tanner staging for testicular size (boys) and breast development (girls); weighted average score followed by sex distribution for each stage.

[e]Tanner staging for pubic hair; weighted average score followed by sex distribution for each stage (Tanner score of 6 for pubic hair is possible for males only; a score of 6 was reported together with scores of 5).

[f]Percentile for age and sex based on CDC growth charts.

[g]Genotype-based predicted phenotype updated according to the Clinical Pharmacogenetics Implementation Consortium guidelines: poor metabolizer (PM), activity score = 0; intermediate metabolizer (IM), 0 < AS < 1.25; normal metabolizer (NM), 1.25 ≤ AS ≤ 2.25; ultrarapid metabolizer (UM), AS > 2.25. Phenotype was assigned as described in Table S2.

[h]Stimulant medication use for ADHD patients only, expressed as number of participants prescribed any stimulant (amphetamine-related or methylphenidate formulation)/number of ADHD participants (and as a percentage).

15.5 ± 7.2 cm, $p = 0.106$) and comparable weight gain (16.1 ± 7.4 vs. 17.3 ± 9.3 kg, $p = 0.349$) over the 3-year period, clearly differentiating between growth and development within this group.

Approximately 5% of the cohort (range: 3.8%–6.6% per visit) were *CYP2D6* PMs, and 3.3% were UMs (range: 2.3%–3.6% per visit). The distribution of genotypes classified as PM, intermediate metabolizer, normal metabolizer, and UM by AS did not vary markedly across study visits (Table 1).

Univariate effects on CYP2D6 phenotype (log(DM/DX))

The univariate effect of CYP2D6 AS, age, height, weight, Tanner score (pubic hair and breast development/testicular size), ADHD status, and urine pH on log(DM/DX) for all study visits (1103 total visits) was assessed. Only CYP2D6 AS ($p < 0.001$), urinary pH ($p < 0.001$), weight ($p = 0.018$), and ADHD status ($p < 0.001$) were significant ($p < 0.05$ without adjustment for clustering of visits within participant). Compared to the effect of CYP2D6 AS ($r^2 = 0.716$; Figure 2) and urinary pH (slope = 0.428, $r^2 = 0.116$; Figure 3), the effect sizes for age (slope = 0.016, $r^2 = 0.003$, $p = 0.063$; Figure 4a) and weight (slope = 0.003, $r^2 = 0.005$; Figure 4b) were considerably smaller with limited explanatory power. The distribution of log(DM/DX) values across Tanner stages for pubic hair and breast development/testicular size are presented in Figure 5a,b, respectively.

The effect of CYP2D6 AS was present at each study visit (Figure S2), and was similar for African Americans ($p < 0.001$) and those of European ancestry ($p < 0.001$), although only one African American participant was genotyped as a PM. The effects of genotype were similar in boys and girls. Decreasing log(DM/DX) values with increasing urine pH was observed for all AS groups except AS greater than 2.25 (Figure 3), whereas the effects of age and weight were uniformly small with limited explanatory power within AS groups (Figure 4a,b, respectively). The differences in log(DM/DX) between patients with ADHD and non-ADHD patients were limited to PMs and the AS = 2 group (data not shown).

Linear mixed model analysis

The random intercept, random slope covariance structure was selected as the most appropriate covariance structure based on the lowest (best) BIC score across all five analyses (age, height, weight, Tanner stage for breast development/testicular size, and Tanner stage for pubic hair stage; see Table S3). Among the five models evaluated with this

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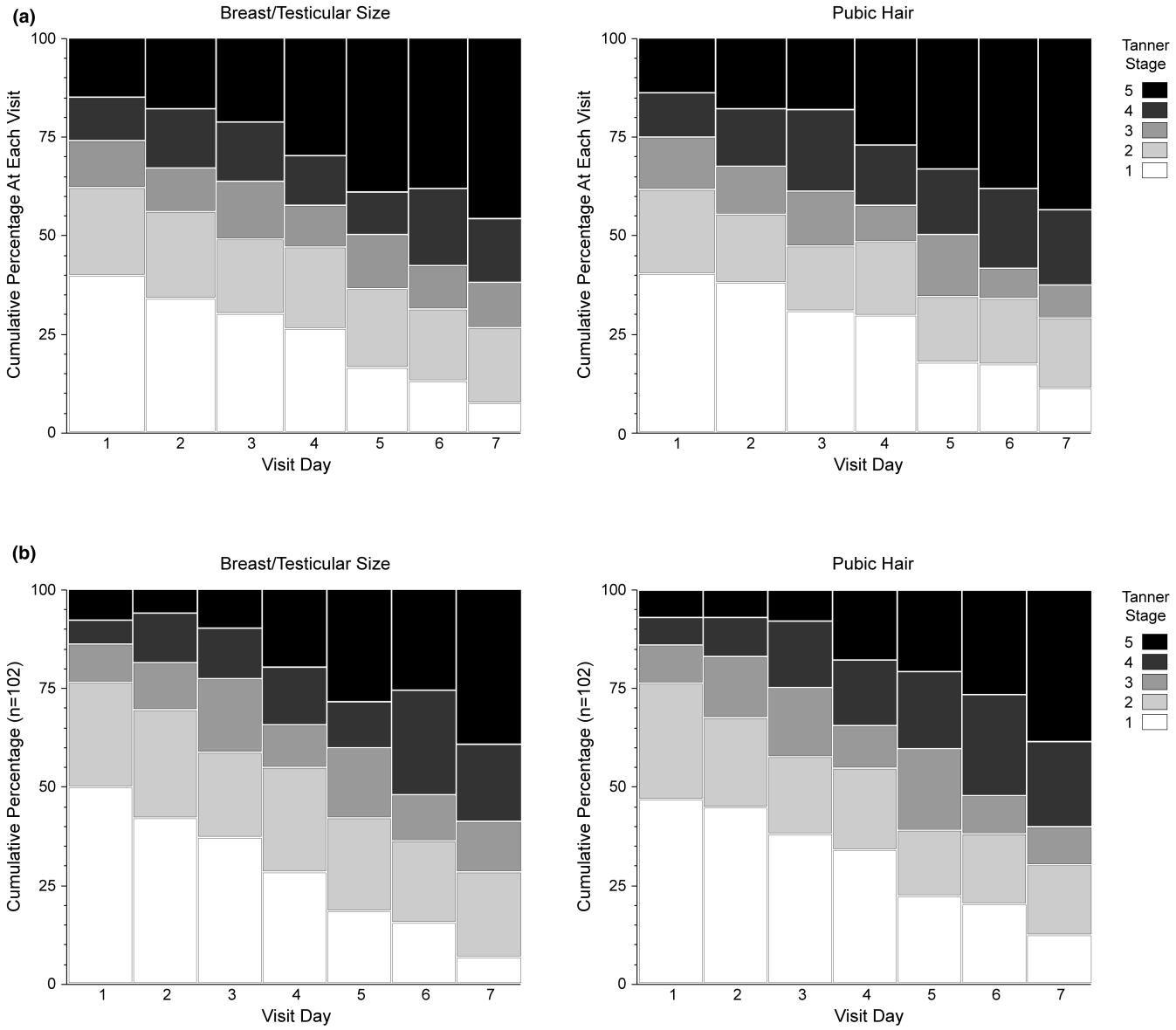


FIGURE 1 Mosaic plots describing the maturation of study participants over the 3-year study period for all participants and the subset of participants that completed all seven visits. Participants were assessed by Tanner staging for breast development and pubic hair for girls, testicular size and pubic hair for boys, and are presented for all study visits by 188 participants (panel a) and the 714 visits for the 102 participants completing all seven visits (panel b). The proportion of participants at a particular Tanner stage at each study visit is represented by shading gradation from white (stage 1), light gray (stage 2), medium gray (stage 3), dark gray (stage 4), to black (stage 5). Tanner stage 6 for pubic hair in boys were included in the Tanner stage 5 group. The width of the columns of the mosaic plot is proportional to the number of participants at each visit; in panel a, the width of the columns decreases as the number of participants decreases from 188 at visits 1–131 at visit 7, whereas the columns are equal in size for panel b as there were 102 participants for each study visit.

covariance structure, the age model had slightly better fit than the surrogate models, and the regression coefficients for the four surrogate models were almost identical to those for the age model (Table 2).

The strongest effects on log(DM/DX) were observed for CYP2D6 AS, with model-estimated average log(DM/DX) being 3.8 SDs higher for PMs than for patients with AS 3, adjusting for other explanatory variables (e.g., age, developmental factors, race, sex, and urinary pH). A moderate effect was observed for sex, with girls averaging an estimated 0.4 SDs lower than boys on log(DM/DX), and smaller effects were observed for ADHD diagnosis and urinary pH. There was little evidence that log(DM/DX) changes meaningfully with age or pubertal development.

DISCUSSION

Optimal use of medications in children requires consideration of several issues not commonly encountered in

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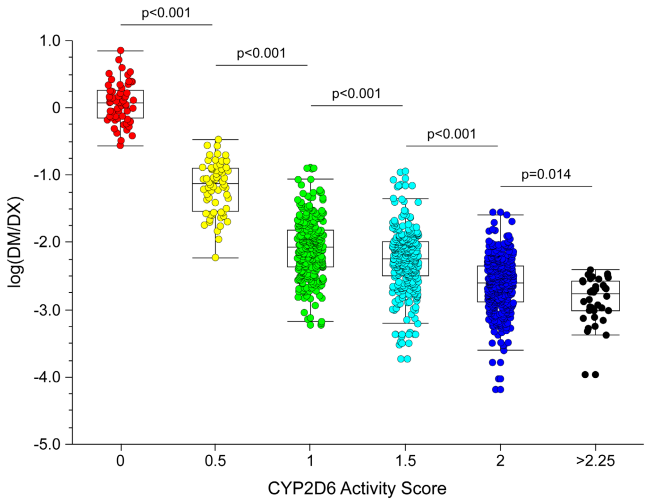


FIGURE 2 Relationship between urinary log(DM/DX) ratio and *CYP2D6* genotype as expressed by activity score (AS). Data from all seven study visits are presented together. All groups were statistically significantly different by ANOVA followed by Tukey's Honestly Significant Difference ($p < 0.001$; $p = 0.014$ for AS = 2 vs. AS > 2.25); p values were not adjusted for clustering of visits within participants. ANOVA, analysis of variance; DM/DX, dextromethorphan/dextrorphan.

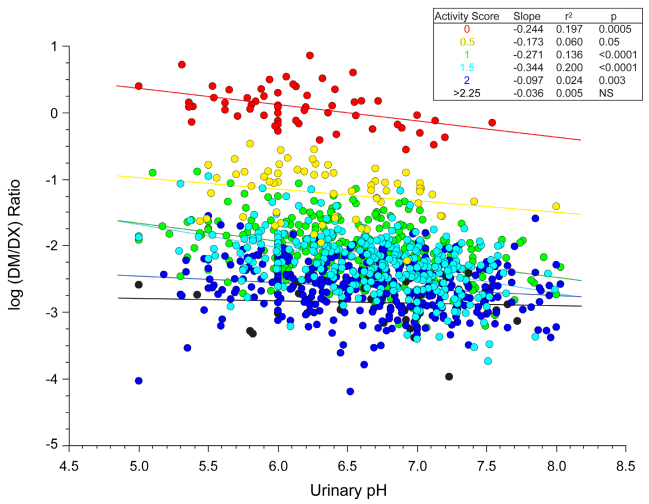


FIGURE 3 Relationship between CYP2D6 activity expressed as the log(DM/DX) ratio and urinary pH. Data from all seven study visits are presented together. Data are color-coded by *CYP2D6* activity score (AS). Linear regression was conducted for each AS group: 0 (red), 0.5 (yellow), 1 (green), 1.5 (turquoise), 2 (blue), and greater than or equal to 2.25 (black). Values for slope, coefficient of determination (r^2), and p values are presented in the inset; p values were not adjusted for clustering of visits within participants. DM/DX, dextromethorphan/dextrorphan; NS, not significant.

adults. Specifically, growth and development should be considered in addition to genetic variation and environmental influences as factors contributing to variability in drug disposition and response.

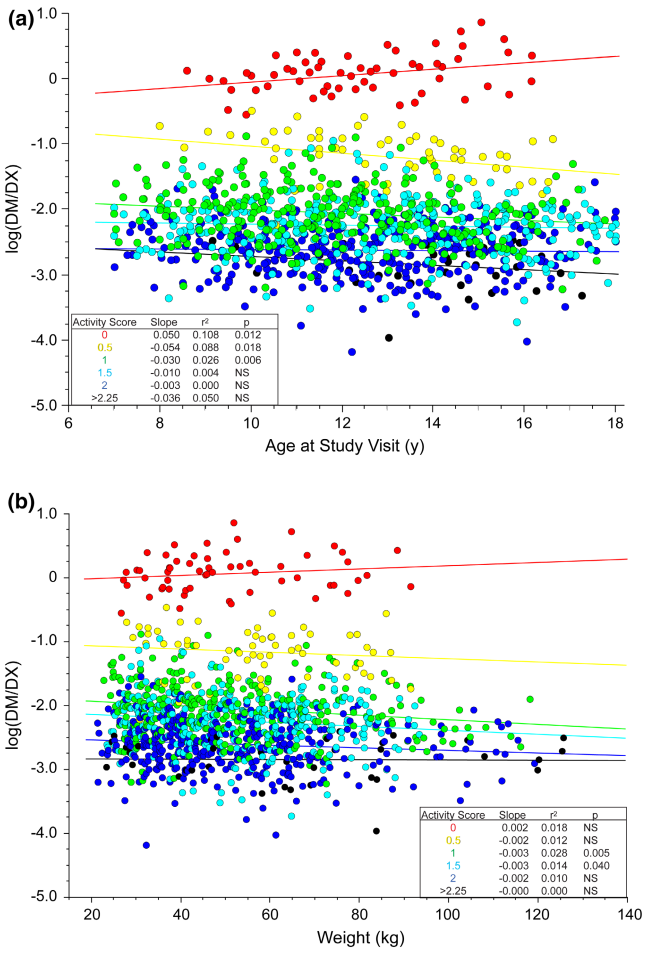


FIGURE 4 Relationship between CYP2D6 activity expressed as the log(DM/DX) ratio and age (panel a) or weight (panel b). Data from all seven study visits are presented together. Data are color-coded by *CYP2D6* activity score (AS). Linear regression was conducted for each AS group: 0 (red), 0.5 (yellow), 1 (green), 1.5 (turquoise), 2 (blue), and greater than or equal to 2.25 (black). Values for slope, coefficient of determination (r^2) and p values are presented in the inset of each panel; p values were not adjusted for clustering of visits within participants. DM/DX, dextromethorphan/dextrorphan; NS, not significant.

Given the prevalent use of CNS-acting medications in adolescents, many of which are CYP2D6 substrates,[2] we conducted a longitudinal phenotyping study to assess factors contributing to variability in CYP2D6 activity during puberty. Among the factors assessed, *CYP2D6* genetic variation (as reflected by activity score) had the greatest influence on the log-transformed urinary DM/DX ratio. In contrast, the effect of age, per se, or surrogates of age, such as weight or height representing growth, or Tanner stages representing sexual maturation were small and had limited explanatory power.

The effect of urinary pH on log(DM/DX) was first reported by Labbé et al.,[33] who reported three- to 20-fold intra-individual variability in the urinary DM/DX ratio

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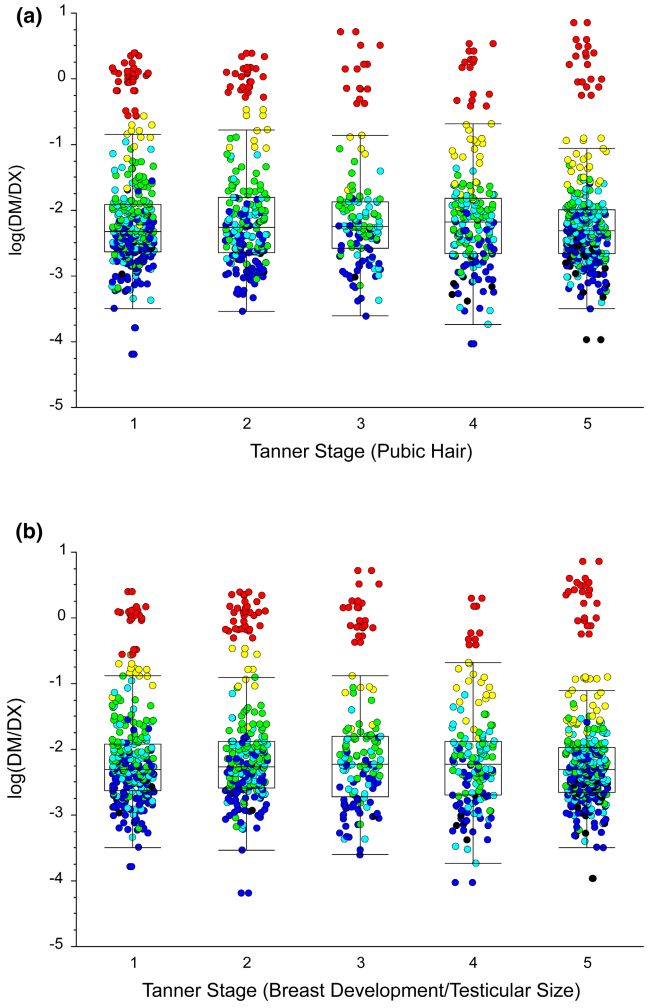


FIGURE 5 Relationship between CYP2D6 activity expressed as the log(DM/DX) ratio and Tanner score for pubic hair (panel a) and for breast development in girls and testicular size in boys (panel b). Data from all seven study visits are presented together. For pubic hair, a Tanner score of 6 is possible for boys, but not girls, and all scores of 6 were included in the Tanner score 5 group. Data are color-coded by *CYP2D6* activity score (AS): 0 (red), 0.5 (yellow), 1 (green), 1.5 (turquoise), 2 (blue), and greater than or equal to 2.25 (black). DM/DX, dextromethorphan/dextrorphan.

as a function of urinary pH and estimated that day-to-day variation in urinary pH may explain between 20% and 80% of the observed intra-individual variability in urinary DM/DX ratios. Given that urinary pH can affect the assessment of CYP2D6 phenotype using DM as a probe, Özdemir et al. further assessed the sensitivity of urinary metabolite ratios for DM and two other CYP2D6 phenotyping probes to changes in urinary pH produced by acidification and alkalinization with orally administered ammonium chloride and sodium bicarbonate, respectively.[34] Using this protocol, the urinary DM/DX ratio for 12 participants were 0.0017, 0.0120, and 0.0003 under control conditions (average urinary pH 6.5), acidification (pH 4.9), and alkalinization (pH 8.4), respectively—a 40-fold difference in urinary DM/DX ratio at the extremes of urinary pH generated by the study conditions. As the individual urinary pH values determined in our study (6.60 ± 0.60, range: 5.00–8.03) fell within the range reported by Özdemir et al.,[34] it is reasonable to expect that pH will have a similar influence on the observed log(DM/DX) values. Therefore, variability in urinary pH can be expected to confound the assessment of CYP2D6 activity and phenotype classification and obscure subtle effects of other covariates on CYP2D6 activity in our patient population. For the 102 participants completing all seven visits, the difference between minimum and maximum pH value averaged 1.44 ± 0.47 units per participant (range: 0.45–2.65), corresponding to a 27.5-fold range in hydrogen ion concentrations. In our dataset of 1103 samples, urinary pH preferentially affected the observed DM concentration (log[DM] vs. pH: slope = −0.407, $r^2 = 0.178$, $p < 0.001$) relative to DX (log[DX] vs. pH: slope = 0.021, $r^2 < 0.001$, $p = 0.337$, data not shown). Given that the effect of urinary pH may confound the magnitude of the *CYP2D6* genotype-dependent effect in individuals with functional CYP2D6 activity, development of a correction factor to minimize the influence of urinary pH on the estimation of CYP2D6 activity from urinary metabolite ratio data would be valuable for further studies investigating non-genetic sources of variability in CYP2D6 activity.

Beyond concerns regarding the potential for urinary pH to confound data interpretation, it is reasonable to consider whether CYP2D6 activity may be expected to demonstrate growth- or development-related changes during puberty. Physical changes occurring during puberty in boys and girls are the result of rising levels of testosterone and estradiol in response to rising concentrations of luteinizing hormone and follicle stimulating hormone secreted by the anterior pituitary. We observed a moderate effect for sex, with girls averaging an estimated 0.4 SDs lower log(DM/DX) values (higher activity) than boys. In adults, differences in urinary DM/DX ratios have been difficult to detect due to high interindividual variability in urinary DM/DX ratios and issues, such as the sample size required to detect small effects. For example, studies involving larger numbers of participants[33,35–37] have observed differences between males and females, whereas smaller studies involving 10–12 participants of each gender[38,39] did not. Thus, observed effects generally are small or inconsistent such that the effect of gender is likely of limited clinical relevance, especially given that the urinary DM/DX ratio has been reported to vary up to 20-fold in the same individual assessed on multiple occasions.[33,38,40]

More relevant to the role of hormones in the physical changes associated with puberty, hormonal changes occurring during the menstrual cycle or between oral

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TABLE 2 Results of the linear mixed model with random intercept and random slope covariance structure for age or surrogate variables on log(DM/DX)

	Age or surrogate variable									
	Age (year)		Height (10 cm)		Weight (10 kg)		Tanner stage: breast/testicular size		Tanner stage: pubic hair	
Effect on log (DM/DX)	β (95% CI)	*p* value	β (95% CI)	*p* value	β (95% CI)	*p* value	β (95% CI)	*p* value	β (95% CI)	*p* value
Base model	0.0 (0.0, 0.0)	0.121	0.0 (−0.1, 0.0)	**0.013**	0.0 (−0.1, 0.0)	**0.021**	0.0 (−0.1, 0.0)	0.104	0.0 (0.0, 0.0)	0.421
Girls (vs. boys)	−0.4 (−0.9, −0.1)	**0.036**	−1.3 (−2.3, −0.4)	**0.006**	−0.1 (−0.4, 0.2)	0.422	0.0 (−0.2, 0.2)	0.853	0.0 (−0.2, 0.2)	0.847
Age or surrogate variable X girls	0.0 (0.0, 0.1)	**0.003**	0.1 (0.0, 0.2)	**0.002**	0.1 (0.0, 0.1)	**0.025**	0.1 (0.0, 0.1)	**0.017**	0.1 (0.0, 0.1)	**0.023**
Race										
EA_AA	0.0 (−0.2, 0.2)	0.771	0.0 (−0.1, 0.2)	0.630	0.0 (−0.2, 0.2)	0.667	0.0 (−0.2, 0.2)	0.709	0.0 (−0.2, 0.2)	0.707
Other	0.2 (−0.1, 0.5)	0.219	0.2 (−0.1, 0.6)	0.173	0.2 (−0.1, 0.5)	0.229	0.2 (−0.1, 0.5)	0.224	0.2 (−0.1, 0.5)	0.221
EA	0.0 (−0.1, 0.1)	0.792	0.0 (−0.1, 0.1)	0.768	0.0 (−0.1, 0.1)	0.853	0.0 (−0.1, 0.1)	0.770	0.0 (−0.1, 0.1)	0.766
AA	Referent									
ADHD (patient vs. control)	0.2 (0.1, 0.3)	**<0.001**	0.2 (0.1, 0.3)	**<0.001**	0.2 (0.1, 0.3)	**<0.001**	0.2 (0.1, 0.3)	**<0.001**	0.2 (0.1, 0.3)	**<0.001**
AS[a]										
0	3.8 (3.4, 4.2)	**<0.001**	3.8 (3.5, 4.2)	**<0.001**	3.8 (3.5, 4.2)	**<0.001**	3.8 (3.4, 4.2)	**<0.001**	3.8 (3.4, 4.2)	**<0.001**
0.5	2.2 (1.8, 2.5)	**<0.001**	2.2 (1.8, 2.5)	**<0.001**	2.2 (1.9, 2.6)	**<0.001**	2.2 (1.8, 2.5)	**<0.001**	2.2 (1.8, 2.5)	**<0.001**
1	1.1 (0.8, 1.4)	**<0.001**	1.0 (0.7, 1.4)	**<0.001**	1.1 (0.8, 1.4)	**<0.001**	1.1 (0.7, 1.4)	**<0.001**	1.1 (0.8, 1.4)	**<0.001**
1.5	0.9 (0.6, 1.2)	**<0.001**	0.9 (0.6, 1.2)	**<0.001**	0.9 (0.6, 1.2)	**<0.001**	0.9 (0.6, 1.2)	**<0.001**	0.9 (0.6, 1.2)	**<0.001**
2	0.4 (0.1, 0.7)	**0.009**	0.4 (0.1, 0.7)	**0.009**	0.4 (0.1, 0.7)	**0.007**	0.4 (0.1, 0.7)	**0.010**	0.4 (0.1, 0.7)	**0.009**
3	Reference		Reference		Reference		Reference		Reference	
Urinary pH	−0.2 (−0.2, −0.2)	**<0.001**	−0.2 (−0.2, −0.2)	**<0.001**	−0.2 (−0.2, −0.2)	**<0.001**	−0.2 (−0.2, −0.2)	**<0.001**	−0.2 (−0.2, −0.2)	**<0.001**

Note: Log(DM/DX) and urinary pH were standardized for modeling. Values in bold are statistically significant at the $p < 0.05$ level.

Abbreviations: AA, African American; ADHD, attention-deficit/hyperactivity disorder; AS, activity score; CI, confidence interval; DM/DX, dextromethorphan/dextrorphan ratio; EA, European Ancestry; EA_AA, mixed European and African American ancestry.

[a]Participants with an AS of 0.25 ($n = 1$), 0.75 ($n = 1$), and 1.25 ($n = 3$) were grouped with the next higher AS group (0.5, 1 and 1.5, respectively) for analyses.

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contraceptive users and nonusers have not been associated with changes in CYP2D6 phenotype based on urinary DM/DX ratios.[33,35–39] In contrast, DM phenotyping studies during pregnancy have detected changes in CYP2D6 activity,[41,42] with the urinary DM/DX ratio reported to be 25.6%, 34.8%, and 47.8% lower at 14–18, 24–28, and 36–40 weeks gestation, respectively, indicating increased CYP2D6 activity compared to the postpartum period.[42] The mechanism of CYP2D6 regulation during pregnancy appears to involve complex interactions among retinoic acid, small heterodimer partner, and Kruppel-like factor-9, and continues to be elucidated,[43–45] but little is known concerning analogous regulatory factors during puberty.

Somewhat surprising was the difference in log(DM/DX) values between patients with ADHD and similarly aged children and adolescents without a diagnosis of ADHD. We are not aware of any influence of ADHD itself on CYP2D6 activity, but one notable difference between patients with ADHD and non-ADHD patients in our study was the use of medications to manage the disorder, such as stimulants (amphetamine and methylphenidate formulations) and α-agonists (clonidine and guanfacine). In fact, it appears that the "ADHD effect" is driven entirely by concurrent administration of amphetamine formulations, which was reported for 118 of the 1105 total study visits (35.6% of patients with ADHD at visit 1, declining to 18.4% at visit 7). Amphetamine has been reported to be an inhibitor of CYP2D6 in vitro, with a K_i value of 26.5 uM in human liver microsomes, greater than 100-fold less potent than the di-methoxy analogue, MMDA—a known CYP2D6 inhibitor with a K_i of 0.17 μM under the same experimental conditions.[46] However, a review of stimulant drug–drug interactions found little evidence of clinical interactions between amphetamines and CYP2D6 substrates, albeit there few reports to assess,[47] and amphetamines are not included in the FDA lists of CYP2D6 inhibitors (https://www.fda.gov/drugs/drug-interactions-labeling/drug-development-and-drug-interactions-table-substrates-inhibitors-and-inducers; https://www.fda.gov/regulatory-information/search-fda-guidance-documents/clinical-drug-interaction-studies-cytochrome-p450-enzyme-and-transporter-mediated-drug-interactions; both accessed June 11, 2022). Among patients with ADHD, treatment with amphetamine formulations was associated with lower urinary pH values ($p = 0.008$), whereas no differences in urinary pH were observed with use of methylphenidate or α-agonists. Thus, we cannot exclude the possibility that the ADHD effect is a false-positive result due to amphetamine effects on urinary pH, with subsequent effects on DM/DX values.

The primary conclusion of this study is that *CYP2D6* genotype remains the single, largest determinant of variability of CYP2D6 enzyme activity during adolescence, and incorporation of genotype-based dosing guidelines should be considered for CYP2D6 substrates, especially given the prevalence of use of these agents in this pediatric age group. To improve the development of medication dosing guidelines for children, ontogeny functions for drug metabolizing enzymes have been incorporated into several physiologically-based pharmacokinetic platforms, and their application to simulating dose–exposure relationships in pediatric patients at a population level is gaining acceptance among researchers, pharmaceutical companies, and regulatory agencies.[48–50] Implementation of precision therapeutics at the level of individual patients requires knowledge of patient genotype, but also requires knowledge of the effect of *CYP2D6* genotype on the dose–exposure relationship for a given drug. The reality is that genotype, per se, is not sufficient to truly individualize dosing as three- to five-fold differences in exposure can be observed within a CYP2D6 AS group, as demonstrated for atomoxetine.[16] A challenge for the future remains to build on, and extend, the accumulating database describing the relative contribution of ontogeny and genetic variation to observed variability in drug disposition and response across the continuum from birth to adulthood.

AUTHOR CONTRIBUTIONS

J.S.L., A.G., V.S.S., and R.E.P. wrote the manuscript. J.S.L., A.G., V.S.S., and Y.S.L. designed the research. A.G., K.J.W., S.E.S., and R.E.P. performed the research. J.S.L., A.G., V.S.S., and R.E.P. analyzed the data.

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CONFLICT OF INTEREST

The authors declared no competing interests for this work.

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