ClinicalTrials.gov Name: The Effects of ADHD Medication (TEAM) Study

ClinicalTrials.gov Identifier: NCT02293655

NIH Title: Neurobehavioral Effects of Abrupt Methylphenidate Discontinuation

NIH Identifier: R01MH105425

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Specific Aims

The stimulant methylphenidate (MPH) is the most commonly prescribed psychoactive medication in children.[1] In 2010, U.S. retail pharmacies dispensed ~10 million MPH prescriptions to children.[2] MPH is predominantly prescribed to treat Attention-Deficit/Hyperactivity Disorder (ADHD) since it effectively and significantly attenuates inattentive, hyperactive, and impulsive symptoms and positively impacts several areas of functional impairment, including comportment and academic achievement in children with ADHD.[3, 4] Despite abundant evidence of its benefits, most children with ADHD who are prescribed MPH have poor continuity of treatment. Children experience frequent periods of inconsistent MPH use[5, 6] for a variety of reasons, including forgetting to administer the medication and difficulties obtaining refills.[7] In addition, it is an accepted clinical practice for physicians to omit MPH for periods of time, such as during the summer or on weekends (i.e., drug holidays).[8, 9] Since MPH discontinuation is considered to be benign, clinical guidelines do not recommend a gradual weaning process and little counseling is usually given to families regarding precautions when stopping MPH.[3, 10, 11] However, increasing evidence suggests that the pharmacological effects of MPH cause changes in brain neurochemistry that persist beyond the immediate medication discontinuation period.[12, 13] Moreover, these neurobiological effects of discontinuation appear to have neurobehavioral consequences. Our own preliminary data suggest that MPH discontinuation results in post-discontinuation behavioral, cognitive, and academic functioning that is compromised to the point of being significantly worse than prior to starting medication, and these effects persist as long as 2-3 weeks after the last dose of MPH.[14] Furthermore, the magnitude of discontinuation-related worsening is comparable to, or larger than, the magnitude of benefits seen from MPH treatment.[14] There is a critical need to better understand the breadth and magnitude of the neurobehavioral effects caused by MPH discontinuation as well as to better understand the temporal trajectory of these deleterious effects. Hence, the primary goal of the proposed research is to conduct the first randomized, double–blind, placebo-controlled trial designed to study the neurobehavioral effects of MPH discontinuation over multiple time points. Multi-informant (parents, teachers, study staff) and multi-modal (direct behavior observations, behavior/mood/affect rating scales, standardized cognitive and academic testing) methods will be used to assess a broad range of outcomes. We will address the following specific aims:

Aim #1: Examine the magnitude and time course of effects of MPH discontinuation on behavioral functioning and affect in children with ADHD.

Aim #2: Examine the magnitude and time course of effects of MPH discontinuation on cognitive and academic functioning in children with ADHD.

Aim #3 (Exploratory): Examine potential moderators (e.g., MPH dose, sex, baseline psychiatric comorbidity, genetic polymorphisms) of the adverse effects of MPH discontinuation on behavioral and cognitive functioning in children with ADHD.

Aim #4 (Exploratory): Examine whether social/financial hardships predict medication supply (i.e. number of days covered with medicine) and medication continuity (i.e. time to first 30-day gap in medication supply) over the 12 months following completion of the study medication trial as determined by the child's pharmacy/prescription records of medication dispensed.

Aim #5 (Exploratory): Test for an association between baseline (medication-naïve) whole blood DNA methylation at CpG sites [proximal to candidate genes (*CES1*, *ARSA*, *GRM7*, *SLC6A2*, *SLC6A3*, *SLC6A4, SLC6A6*, *DRD4*) as well as genome-wide] and MPH response (ADHD symptom reduction as well as methylphenidate side effects).

Aim #6 (Exploratory): In MPH responders, characterize changes in whole blood DNA methylation profiles at CpG sites proximal to candidate genes (*SLC6A3*, *SLC6A2*, *DRD4*) and genome-wide between the baseline (medication-naïve) and on-MPH time points, using a within-subjects analysis.

Aim #7 (Exploratory): In MPH responders, test for an association between epigenetics profiles and MPH plasma levels to determine if (a) methylation profiles at CpG sites proximal to *CES1* (the enzyme which

metabolizes MPH) will be linked to specific MPH pharmacokinetic [PK] profiles and (b) specific MPH PK profiles will mediate the association between *CES1* methylation profiles and MPH adverse effects

APPROACH

Participants. We will enroll 168 stimulant-naïve children (aged 7-11) with a diagnosis of ADHD. Cincinnati Children's Hospital Medical Center (CCHMC) will recruit 112 patients and Seattle Children's Hospital (SCH) will recruit 56 patients. Referrals through clinical care at these two Centers (see below) will be the primary referral source. However, if clinic patient flow is not sufficient, we will use established methods of recruitment at each site including referrals from other local doctors, mental health professionals, and schools, paper (including newsletter) and email advertisements, and social media postings. Participant consent will be documented on either paper consent forms or via the REDCap eConsent system.

CCHMC: ~400 patients with ADHD are seen at the CCHMC Center for ADHD each year. Further, we have 1025 patients within our ADHD patient registry who will be in the 7-11 year old age range during the course of this study who all have agreed to be contacted for participation in research studies. Using Center for ADHD patients, we recently concluded a research study (*R01MH074770*[14, 15]) in which we enrolled 90 *stimulant-naive patients with ADHD over three years using the same enrollment criteria as proposed for this study.*
SCH: At the Seattle Children's Hospital Psychiatry and Behavioral Medicine Center, 1167 patient visits were conducted for children age 7-11 years old in 2013, and 523 (45%) of these had a diagnosis of ADHD.

Inclusion Criteria. Participants for the study must meet all of the following criteria:
a. Age at Screening: 7.0 years to 11.9 years, inclusive. The targeted age range corresponds to the modal age range when children are diagnosed with ADHD, consistent with the majority of ADHD MPH trials.[16]
b. ADHD Diagnostic Status: Meets DSM-V criteria for ADHD (see 3.C.3), with Clinical Global Impression (CGI) rating corresponding to at least "moderately ill" (See 3.C.11).
c. Cognitive Functioning: Intelligence Quotient (IQ) Composite or Verbal IQ score of ≥70 as estimated by the Kaufman Brief Intelligence Tests-2. We exclude children with evidence suggesting cognitive disability) given that some studies have shown differences in MPH response for children with comorbid ADHD-Cognitive Disability versus those with ADHD but no cognitive disability.[17]
d. Physical Health: Physical exam findings are judged to be normal for age and sex by study physician and/or medical consultant, and there is no co-existing condition (including cardiac or cardiovascular condition, consistent with standard clinical practice and the current American Academy of Pediatrics policy statement[18]) for which MPH is contraindicated.

Exclusion Criteria. Potential subjects meeting any of the following will be excluded:
a. Psychiatric Medications: Current or prior use of any medication for psychological/psychiatric problems.
b. *Behavioral Interventions*: Current active participation in *new* ADHD-related behavioral interventions given that improvements due to these interventions may confound our group comparisons. However, children will *not* be excluded for participation in behavioral intervention that have been or will be ongoing for ≥ 3 months with the same therapist at the time of medication titration trial initiation.
c. Psychiatric or Neurobehavioral Conditions: Children with mania/hypomania, schizophrenia, or severe depressive disorder, as determined by the K-SADS, will be excluded since ADHD medications may not be an appropriate first line of treatment for children with these comorbid disorders.[19]
d. Organic Brain Injury: History of traumatic brain injury with loss of consciousness, neurological disorder (including epilepsy), or other disorder affecting brain function due to potential differences in neurophysiology of ADHD phenotype.[20, 21]
e. *Cardiovascular Risk Factors*: Children with a personal history or family history of cardiovascular risk factors will be excluded, or given the option of participating in the study after obtaining an EKG and verification from a pediatric cardiologist regarding the safety of their participation in a trial of methylphenidate. In this case, families will be responsible for the costs of EKG and any necessary cardiologist evaluation. If for any reason a family is unable to assume the cost of the EKG and cardiologist evaluations but still wishes for their child to participate, study staff will determine on a case-by-case basis whether the study budget allows the study to offer financial assistance to the families for these evaluations

f. *Pregnancy:* The safety of MPH use during pregnancy has not been established.

Diagnostic and Clinical Assessment. All children will undergo a comprehensive diagnostic battery, including a parent-report diagnostic interview (K-SADS[22]) and parent- and teacher- ADHD rating scales (Vanderbilt Scales[23]). A child will be considered to meet ADHD diagnostic criteria if s/he meets diagnostic criteria for any ADHD subtype on the K-SADS, as children are required to meet ADHD symptom, age of onset, pervasiveness, and impairment criteria on the K-SADS. However, based on the K-SADS alone, some children will have subthreshold symptom counts for ADHD diagnostic and/or subtype criteria (i.e., less than 6 symptoms in either or both the inattentive or hyperactive-impulsive symptom domains). For a portion of these children, thresholds may not be met because of inaccuracies in parent report. That is, teachers may provide a better account of some ADHD symptoms (e.g., trouble focusing, difficulty remaining seated) than parents. Therefore, following the MTA Cooperative Group Study diagnostic algorithms,[24] the K-SADS count of ADHD symptoms will be supplemented by up to two non-overlapping ADHD symptoms per symptom domain reported on the Vanderbilt teacher rating scale. For cases in which a child is homeschooled and no school teacher ratings are available, ratings by an adult who does not live in the home but has regular contact with the child may be substituted for the school teacher ratings (i.e., coach, religious education or music teacher, etc.).

Overview of proposed research: Figure 1 – Study Design

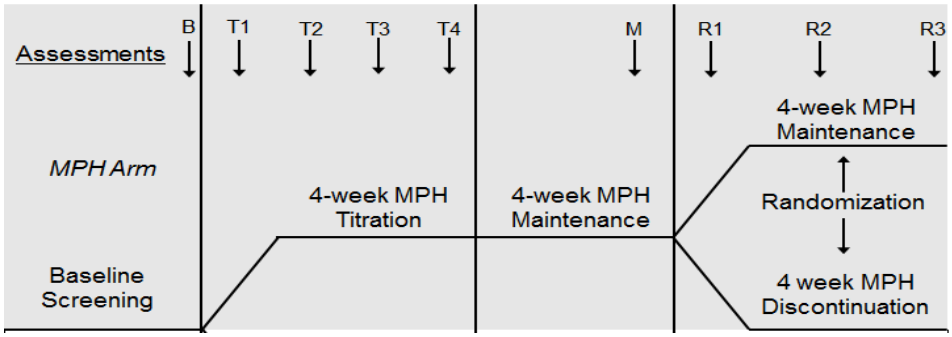

Figure 1 graphically depicts the study design, which is further explained in the subsequent text.

B = Baseline Assessment

T1, T2, T3, T4= Titration Phase Visits

M= Maintenance Assessment

R1, R2, R3= Randomized Phase Assessments

Baseline [B] Assessment (prior to randomization). After participants have satisfied all inclusion / exclusion criteria, they will complete the full battery of baseline measures (see Table).

MPH Titration, Maintenance, and Randomization Procedures.

Randomized Placebo-Controlled MPH Titration Trial. All children will receive a double-blind placebo-controlled forced-upward titration trial. Children will experience 3 active dosages (low, medium, high) of MPH (18mg, 27mg, 36mg for children ≤30kg or 18mg, 36mg, 54mg for children >30kg) as well as 1 random week of placebo. Children will begin on the lowest dosage (or a randomized placebo week) and proceed through all of the dosage conditions in an incremental fashion. However, children >30kg who are not tolerating the 18mg, 36mg, 54mg MPH dosing schedule during the titration trial will have the option of dropping down to the 18mg, 27mg, 36mg MPH dosing schedule. For more detailed information on changing medication doses during the titration trial see Appendix A. At the end of each week of titration, parents and teachers will complete the *Vanderbilt Parent and Teacher ADHD Rating Scales (VADPRS[23] and VADTRS[25])* and *Pittsburgh Side Effects Rating Scale*. Parents will be given options of methods to supply ratings: they will be allowed to enter these rating scales into a secure online web portal that allows online entry of ratings OR they will be given paper versions of these forms that they can complete and mail or fax back to the clinic OR they will be given Word documents that they can fill in and email OR the physician will go through the form with them via telephone call, recording their responses. Using the online web portal, the study physicians or research staff will register participants onto the web portal, a letter is automatically emailed to the family instructing them how to log on and access their child's page. Physicians or research staff will walk parents through this process at

the first clinic visit when medication is prescribed. Thereafter, parents will be sent a weekly (or monthly during maintenance) email with a link to the web portal that will prompt them to log in and complete the online rating scales. The online web portal is completely secure and resides behind a firewall at Cincinnati Children's Hospital Medical Center.

Parents and children will then attend a clinic visit where the study doctor/doctor equivalent (e.g., nurse practitioner) will interview the family about behavior and side effects, review the behavioral and side effect ratings, and derive a *Clinical Global Improvement (CGI)* rating[26] to gauge clinical improvement. The doctor/doctor equivalent will also measure weight and vital signs. At each visit, the doctor/doctor equivalent will decide whether the child is experiencing intolerable side effects (e.g., tics, abnormal cardiac function, etc.). If present, the doctor/doctor equivalent will direct the child to either stop taking the study medication or return to the previous dosage. If there are no intolerable side effects, the doctor/doctor equivalent will allow a dosage increase the following week. To ensure that physician/physician equivalent decisions and parent/teacher ratings are not biased by presumptions regarding dose, one random week of the titration trial will be placebo.

**Note on duration of taking each dosage during the titration trial*: Children will take each titration dosage (consisting of 3 dosages of MPH or placebo) for one week as standard protocol, but in some cases it may be necessary to extend the duration of taking each dosage for some additional days. Cases in which this may occur include: child misses too many days of school (due to illness, inclement weather, or school vacation days), or forgets to take the study pills, such that there is insufficient observation time for teachers or parents to provide valid behavior ratings.

**Between the end of the titration trial week 4 and the beginning of taking the Maintenance phase dose, study participants will take the lowest MPH dosage for at least three days, and then will start taking their "optimal dosage" for the rest of the Maintenance phase (see below for information on determining "optimal dosage."). This low dose lead-in to the Maintenance phase has the following benefits: 1) no participants will suddenly go from a period of placebo/not receiving MPH to medium or high dose MPH, which is more apt to be associated with an exacerbation of side effects than stepping up more gradually to the Maintenance phase dose, 2) if receipt of teacher ratings is delayed, leading to a delay in the study physicians/physician equivalents/investigators determining a child's optimal dosage (see below), the child will not have a break in receipt of medication while these ratings are being obtained.

Determination of optimal dosage. The CGI ratings and ratings of behavior and side effects by parents and teachers from the 4 titration trial weeks will be graphed. Two study physicians/physician equivalents/investigators will *blindly* and independently review the graphs and judge which week(s) was the optimal dose week. Any discrepancy in the "best week" ratings will be resolved by discussion between the physicians/investigators while still blind to dose conditions. The study physician/physician equivalent will also discuss with the titration results with the family and get the family's ratings of "best week" during the titration. Thus, "best week" will be agreed upon by clinicians and the family. In cases where the clinicians and family differ in their designation of '"best week," the designation of the family will take precedence. If it is decided that multiple weeks had equally optimal response, the child will be assigned to the lower of the optimal dosages. If a child responded best to the placebo dosage or placebo response was equal to that of other dosages, s/he will be designated as a placebo responder. Children who do not show palpable improvement (vs. their baseline) during any trial week based on CGI and parent and teacher behavior ratings will be designated as non-responders. These placebo- and non-responders will exit the trial at this point and will not progress to the Maintenance phase. Based on studies using similar methodology, we expect that ~25% of MPH trial patients will be placebo- or non-responders,[15, 24] and have accounted for this in sample size and power estimates.

MPH Maintenance Phase. During the Maintenance phase, participants will receive a lead-in of at least 3 days of the lowest MPH dosage (overencapsulated), after which they will receive their optimal dosage of MPH (overencapsulated). This phase will last 4 weeks as standard protocol, but in some cases it may be necessary to extend or truncate the phase by some days to suit the families' schedules and/or ensure that they can attend visits, and to ensure that teachers can provide behavior ratings when school is in session. If the child were to

need to decrease their medication doses during the MPH Maintenance Phase, the dosing algorithm in Appendix A would be used. Per clinical practice, if the participate were to revert back to baseline symptoms, the medication dose could be increased (NOTE: the increase would not exceed 2 mg/kg per day or 54 mg total; see Appendix A for further details).

MPH Randomization Phase. During the double blind Discontinuation phase, children will be randomized to receive placebo (MPH Discontinuation group, N=150) or to continue their optimal dose of MPH (Sustained MPH group, N=30). This phase will last 4 weeks as standard protocol, but in some cases it may be necessary to extend or truncate the phase by several days to suit the families' schedules and/or ensure that they can attend visits, and to ensure that teachers can provide behavior ratings when school is in session. *Comparing performance of the Discontinuation and Sustained MPH groups will provide a check of the study manipulation.* If the child were to need to decrease their medication doses during the MPH Randomization Phase, the dosing algorithm in Appendix A would be used.

COVID-19 MODIFICATIONS FOR TITRATION, MAINTENANCE, AND RANDOMIZATION PHASE VISITS. Some in-person Titration, Maintenance and Randomization phase visits may fall during periods when social distancing is recommended due to COVID-19 concerns, and so will be converted to remote/virtual visits. As a result, in-person measurements at these visits related to effects of methylphenidate treatment on health (such as measurement of participant weight, heart rate, and blood pressure, as well as cardiac auscultation during the medical exam) will not be performed. However, during the Maintenance and Randomization phases, participants will either be on their optimal methylphenidate dose or placebo, so this change should not present a safety concern for participants as weight, heart rate, blood pressure, and cardiac auscultation would have been performed between one to five other times on methylphenidate during prior in-person study visits. Converting in-person Titration trial visits to remote visits may lead to situations in which a participant has not previously had weight and cardiac parameter measurements performed while on methylphenidate during the course of the study, but this clinical scenario is not uncommon in routine clinical practice: over half (53%) of community providers do not have either an in-person visit *or* telephone contact with their patients in the first month after starting stimulant medications (Epstein JN et al, *Pediatrics,* 2014; 134:1136-1143). Despite the absence of the in-person weight and cardiac parameter assessments for the Titration, Maintenance, and Randomization visits affected by COVID-19 precautions, we will ensure participant safety by maintaining the clinical interview portion of these visits during the telephone visit. During the telephone visit, we will query families about the presence of 19 potential adverse effects of methylphenidate (including appetite suppression) using the Pittsburgh Side Effects Rating Scale, and we have added questions to the visit form which explicitly ask whether the participant is experiencing heart racing, heart palpitations, or chest pain. If participants or their families express any concerns about adverse effects that the study clinician judges as warranting an in-person evaluation, the family will be notified. For this in-person visit, the family will be offered the options to come in to see the study clinician (with visit conducted according to hospital procedures for limiting and preventing spread of COVID-19) *or* to see their primary care provider (in case the family is not comfortable coming to the medical center [CCHMC or SCH] due to COVID-19 concerns).

Study Blinding. With blinded randomization during MPH Titration Trial and Discontinuation Phase (through use of over-encapsulated medication and identical placebos), the entire study will be triple-blind (i.e., participant families, study staff, and teachers will be blinded to medication and dose). Please note that the Study Consent form does not specify the proportion of children who will be randomized to discontinue MPH (MPH Discontinuation group, N=140) versus continue MPH (Sustained MPH group, N=28). This is because the vast majority (80%) of children will be randomized to discontinue MPH, likely leading parents aware of this proportion to assume that their children will be randomized to MPH discontinuation and defeating the purpose of Study Blinding. Parents who presume their children have discontinued MPH may then be biased toward rating their child's behavior unfavorably during the MPH Discontinuation Phase. If parents ask the study personnel about the proportion of children who will be randomized to discontinue MPH during the study, the study personnel will reply that randomization to discontinue MPH is "more likely" than randomization to continue MPH but will not provide the specific proportion.

Compliance with Study Capsule Administration. A number of procedures will be undertaken to ensure that children take the study capsules on a consistent basis. At each study visit, the study physician/physician equivalents/investigators will ask each family if there are any problems with study capsule administration. A medication event monitoring system (MEMSCAP™) will also be used to electronically track time of medication administration. Lastly, families will be required to bring in their pill bottles with any remaining pills to each visit, which will be counted and recorded.

Saliva Samples for DNA. Participants will each provide two saliva samples for DNA extraction (collected at any point during the MPH titration visits) to minimize problems due to sample loss or insufficient DNA extraction from a single sample. Sample collection tubes are marked with a fill line that designates the amount of saliva that typically provides 20 mcg of DNA (our target amount); thus we expect our two samples to yield ≥40 mcg. To verify that a minimum of 20 mcg is collected per participant, the CCHMC Genetic Variation and Gene Discovery Core runs our samples approximately every 2 to 4 weeks and provides us with a report of the amount of DNA extracted from each sample. In rare cases where the total DNA yield from the *two* samples is less than <20 mcg for a given participant, we will contact the family and ask the child to provide a *third* sample.

Saliva is self-collected by spitting into an Oragene cup (DNA Genotek, Ottawa, Canada). The cup is sealed and transported to the CCHMC Genetic Variation and Gene Discovery Core for processing. The Oragene saliva sample is stable at room temperature for several months. DNA is extracted using the manufacturer's recommended procedure. Briefly, the sample is incubated at 50°C for an hour and a solution is added to precipitate contaminating proteins. DNA is then selectively precipitated by addition of isopropanol and re-suspended in a TE buffer overnight. DNA extraction yields are determined spectrometrically and entered in a database. *Using this procedure, the amount of DNA extracted from a saliva sample is comparable to that extracted through a blood draw,[27, 28] and is more abundant and of better quality than that provided by a buccal swab.[29] The advantage of using saliva, as opposed to blood, is that we are likely to increase participant compliance and willingness to participate in the study.*

Single nucleotide polymorphism (SNP) genotyping will be performed using theTaqMan allelic discrimination system (Applied Biosystem, Forest City, CA). We will use an ABI-7500 real-time PCR system for the post-PCR-read allelic discrimination. The custom assay-by-design kits, which include the amplification primers and hybridization probes, will be obtained from Applied Biosystem if the probes for our SNPs of interest have not already been designed. The fluorescence-labeled probes will be specifically formulated to detect wild type and variant alleles. For VNTR genotyping, we will use established assays (e.g., conditions and primers described by Stein et al[30] for the DAT 480bp VNTR).

Blood Samples for Epigenetic Analyses. Participants will have their blood drawn and placed in PAXgene Blood DNA and RNA tubes at two time points: 1) prior to the MPH titration trial for the baseline (MPH-naïve) time point [for Aim #5 and Aim #6 analyses], 2) after 4-7 weeks of MPH treatment for the on-MPH time point [for Aim #6 and Aim #7 analyses].

Additional banked samples. Thirty de-identified banked DNA samples (extracted from blood) are available from children ages 7-17 who completed a National Institute of Mental Health-funded R01 (MH070935) at Icahn School of Medicine at Mount Sinai (PI: Newcorn). These participants completed a randomized, placebo-controlled, crossover trial of long-acting methylphenidate using similar titration and data collection procedures as for currently enrolling R01MH105425. Therefore, the epigenetic data from the banked samples, as well as their de-identified demographic and outcome assessment data, will be combined with the data from the currently enrolling samples to increase sample size for Aim #5 analyses.

DNA extraction from the blood samples and methylation analysis will be performed by the CCHMC Genotyping Core. Genomic DNA will be digested with MspI restriction enzyme and ligated with an Illumina Adapter. The DNA will be treated with sodium bisulfite which converts unmethylated cytosine to uracil, while methylated cytosine will remain unaffected. Size selection will be performed to obtain the optimal fragments for genome

coverage and remove restriction fragments that failed to ligate with the adapter. Purified DNA will undergo the minimum number of cycles to produce an evenly represented library. 20 million SE75 reads will be generated with an Illumina HiSeq 2500. After sequencing, the reads will be trimmed from their adapters *in silico* using the analytical package Trim Galore. The trimmed sequences will be aligned to the genome using the Bowtie based aligner. Methylation levels will be determined using the package Bismark.

Methylphenidate Plasma Concentration Measurements. We will use a sparse optimal sampling design as in previous studies.[31, 32] Participants will self-administer MPH in the AM in their homes, with administration time tracked by log completion as well as time stamp on the eCAPs electronic pill bottle monitoring system. At the MPH Maintenance assessment visit, children will have 1-2 blood samples collected in EDTA tubes within the visit time frame. Samples will be centrifuged to separate plasma for -80 C storage until measurement of MPH and ritalinic acid (MPH metabolite) levels via High Performance Liquid Chromatography/Tandem Mass Spectrometry.[33]

Collection of Pharmacy Medication Records. In order to examine medication usage following the clinical trial, parents will be given the option to provide written permission for CCHMC staff to access their child's pharmacy and prescription records for medications dispensed. At study entry, interested parents will provide a contact information of their child's pharmacy and prescription card if applicable, and sign an authorization to disclose child protected health information specifying disclosure of claims records for medications dispensed (using the form specified/provided by the child's pharmacy company). Pharmacies are allowed or required to share patients' information for public health or health research purposes. They can do this as long as certain privacy rules are met. This allows for rapid access to data (i.e. medication, date prescribed, dosage instructions, number of pills dispensed, and cost). Twelve months after completing the clinic trial, the child's prescription record for medications dispensed will be retrieved. Participants may also consent to this information being retrieved through an Automated Rx Retrieval System (e.g, OARRS or analogous system; electronic medical record medication dispensing reconciliation listing).

Study Measures

Inclusion/Exclusion Measures

-Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS[22]): The K-SADS is a widely used semi-structured clinical interview used to assess psychopathology in youth according to DSM-IV criteria.[34] It is capable of generating 32 DSM-IV diagnoses, including *ADHD as well as mood, anxiety, disruptive behavior and psychotic disorders*. Psychometric properties for the K-SADS are well-established.[22] Study participants must meet full DSM-5 criteria for ADHD (any subtype). It should be noted that although the KSADS is based on the DSM-IV, changes made for diagnosing ADHD in children in DSM-5 are minor (e.g., age of onset changed from age <7 to <12). Similarly, adjustments to accommodate DSM-5 criteria for other disorders we will assess are relatively minor and will not preclude the KSADS' utility in assessing inclusion/exclusion criteria.

-*Vanderbilt ADHD Rating Scales*: The Vanderbilt ADHD Rating Scales are DSM-V-based scales with teacher- (VADTRS) and parent-report (VADPRS) forms.[35] The VADTRS and VADPRS include items that assess each ADHD symptom. Also, both scales allow parents and teachers to rate a variety of functional areas including school and social functioning. Internal consistency is good to excellent for both the VADTRS and VADPRS.[23, 36]

-*Clinical Global Impression (CGI)[26]:* The CGI is a clinician-rated instrument which yields an ordinal rating of the child's current level of impairment (1="Normal, not at all ill" to 7="very severely ill"). Ratings are derived from interviewing the child's caregiver, and can be used to generate both initial severity and improvement scores.

Kaufman Brief Intelligence Tests-2 (KBIT-2; Kaufman & Kaufman, 2004): The KBIT-2 is a culturally-sensitive standardized assessment that estimates verbal and non-verbal and overall intelligence. This test has good reliability and validity and will be administered to assess each child's intelligence and rule out possible intellectual disability.

- *Wechsler Individual Achievement Test—3rd edition (WIAT-III):* We will administer the Reading and Math subtests of the WIAT-III, which is a standardized academic achievement test for school-age children.[37]

-Physical Examination: The study physician/physician equivalent will query families about the child's past medical history and the family history of mental health, tics, cardiac risk factors, and screen the child for

hearing and vision impairment. A full physical examination, including measurement of height, weight, and vital signs, will be conducted.

Outcome Measures. Targeted study outcomes will be assessed at baseline (BL) and 4 assessment time points (M, R1, R2, R3). The M assessment point will occur once during the Maintenance phase while children are on their optimal MPH dose: the purpose of this assessment is to verify improvement from baseline with MPH. The assessment battery will again be given on/around days 1, 14, and 28 (assessment points R1, R2, and R3 respectively) of the Randomization phase. These time points were selected to assess the immediate- (i.e., 1 day post-discontinuation), intermediate- (i.e.,14 days post-discontinuation), and long-term (i.e., 28 days post-discontinuation) effects of MPH discontinuation. A 1-day period was selected for measuring immediate effects as we previously documented neurobehavioral worsening after MPH discontinuation at 24 hours after the last dose (see 3.C.1.c).[38] The 14-day point was selected to measure intermediate effects because we demonstrated detrimental effects of MPH discontinuation at this point in our preliminary data (see 3.C.1.a).[14] Finally, the 28-day point is outside the "several weeks" suggested for persistence of discontinuation symptoms by the amphetamine illicit use literature[39, 40] and in our estimation is the longest tolerable time off MPH for MPH responders from an ethical and practical subject retention standpoint. Of note, we will also collect daily behavioral and mood ratings from parents during the 4-week Randomization phase (see EMA measure, below) and we will use these data to more finely map the time course of MPH discontinuation effects.

Behavior/Emotional Outcome Measures

-Vanderbilt ADHD Rating Scales-Parent and Teacher Forms. See above.
-Revised Child Anxiety and Depression Scales – Short Version (RCADS-S; Chorpita et al., 2000) – Child Completed: -The RCADS is a 25-item measure that assesses *DSM-IV*-based anxiety disorder symptoms, as well as symptoms for depression, on a 4-point Likert scale (1 = *never*, 4 = *always*). Designed for child self-report, the RCADS has demonstrated excellent reliability and validity in clinical samples (Chorpita et al., 2005).
-*Columbia Suicide Severity Rating Scale (CSSRS)*[41]. The CSSRS is a validated tool for assessing and monitoring suicidal ideation/behavior.[41] It is widely used in clinical trials of children as young as 6 years old.[42] It will be utilized as needed if risk is determined by the participants response on the RCADS.
-*Patient Health Questionnaire (PHQ-9; Kroenke et al 2001)*: The PHQ-9 is rating scale utilized to screen for parental depression with good sensitivity and specificity (i.e., 88% for each). This form was added to assess whether parental depression moderates treatment outcomes.
-*Sluggish Cognitive Tempo Scale (SCTS)(Barkley 2013).* This parent- and teacher-completed measure has demonstrated good validity and strong reliability for assessing the frequency of sleepy/daydreaming and slowed behaviors that a child displays (Barkley 2013).
-*Behavior Rating Inventory of Executive Function (BRIEF)*[43]. The parent and teacher BRIEF scales have been validated for the assessment of everyday skills measuring executive functioning, including inhibition, shifting attention, emotional control, initiating tasks, problem solving, working memory, and monitoring activities.[43, 44]
-*Emotion Regulation Checklist (ERC)*. The 24-item ERC, a parent-report measure used to assess children's global emotion dysregulation, has demonstrated substantial validity and reliability.[45] The ERC yields two subscales: Emotional Lability/Negativity and Emotion Dysregulation.
-Pittsburgh Side Effects Rating Scale (PSERS). The PSERS[46, 47] is completed separately by parents and teachers, and allows report of whether side effects were not present, or were mild, moderate, or severe.
-Children's Sleep Habits Questionnaire (CSHQ). The CSHQ is a comprehensive sleep screening instrument for children with demonstrated reliability and validity.[48] The CSHQ yields both a total score and eight subscale scores reflecting key sleep domains (bedtime resistance, sleep onset delay, sleep duration, parasomnias, sleep-disordered breathing, night awakenings, daytime sleepiness, sleep anxiety).[48]
-*Ecological Momentary Assessment (EMA)*. EMA is used to collect real-time information about mood states and behavior directly on smart phone apps or personal data assistants within the context of families' typical daily lives. EMA provides more accurate response data, is less susceptible to recall bias, and is better suited to address how behavior changes over time and across contexts than retrospective or summary report.[49] The EMA device will alert the parent to complete ratings every day (at a time requested by parents to be compatible with the family's schedule). Ratings will include the parent *Vanderbilt ADHD Rating Scale* (see above) and a single rating of the child's current mood,[50] given the demonstrated utility, feasibility, and validity of collecting

daily ADHD symptoms ratings to assess MPH response[47, 51] daily mood ratings to assess emotional dysregulation in ADHD.[50], and 2 sleep questions (Stein et al., 2001). Parents will be instructed to return the EMA device to the study laboratory once per week to upload data.

-Direct Observation of Time on Task. Participants will be videotaped while completing the 20-minute Analogue Math task (see below). Behavior will be coded for on-task, distracted, fidgeting, and out of seat behavior at 10-second intervals.[52] As with our prior work using this observational task,[53, 54] we will train and calibrate coders on the coding scheme and Noldus® software until they reach 90% reliability. Once coding has begun, coders will meet weekly to code one video together to address coder drift. We will double code 33% of all videos and will compute intraclass correlation coefficients (ICC) to determine reliability for each behavioral code. In our prior studies, interrater agreement for the coded behaviors has been high (ICC range =.84-.98).

-Conners Adult ADHD Ratings Scales (CAARS): Caregivers will be asked to fill out the CAARS at Randomization 1 Visits. The self-report CAARS will allow us to gather information on the extent to which caregivers experience ADHD symptoms (Macey 2003). Responses are measured on a scale of 0 ("not at all, never") to 3 ("very much, frequently"). The CARRS has a four factor structure, with factor test-retest reliability ranging from good to excellent (coefficient alphas ranged from .86-.92). CAARS sensitivity and specificity were high, with an overall diagnostic efficiency rate of 85%. (Erdhart, Epstein, Conners, Parker & Sitarenios, 1999).

Cognitive and Academic Outcome Measures

-Go/No-go task (GNG). The GNG[55] assesses inhibitory control and inhibitory control reaction time variability. We include this measure given that deficits in inhibitory control[56] and increased reaction time variability[57] are among the neuropsychological impairments that are most strongly and consistently associated with ADHD and are responsive to MPH .[15] Moreover, our preliminary data using GNG outcomes documents neurobehavioral worsening compared to their baseline for children who had discontinued MPH[14] (see 3.C.1.a).

-Computerized Spatial Span Task (SST). The SST[58] measures spatial working memory. We include this measure given that spatial working memory deficits are among the neuropsychological impairments that are most strongly and consistently associated with ADHD[56] and are responsive to MPH.[59, 60]

-Curriculum-Based Measurement (CBM) - AIMSWEB). The AIMSWEB includes tests of math computation, math concepts and applications, reading fluency and comprehension, written expression, and spelling. Such CBM have high validity and reliability for measurement of student progress, including high concurrent validity with standardized measures.[61-63] National norms for AIMSWEB results on single test administrations as well as progress over time are available.[64, 65] We include the AIMSWEB math computation measure given its similarity to math computation CBM used to obtain our preliminary data,[14] and have included the other AIMSWEB tests to broaden our examination to additional academic domains. In addition to AIMSWEB, study staff will administer the Analogue Math Task, which is a naturalistic task modeled after math work undertaken in a typical classroom setting. We will use the timed math worksheet (with problems tailored to each individual's level of proficiency) for 20 minutes in which child will be videotaped doing this task. Math performance on this task will be measured by math productivity (total number of problems completed) and accuracy (number of math problems completed correctly divided by the total number completed).

Documentation of Other Service Utilization

-Services for Children & Adolescents - Parent Interview (SCA-PI; Jensen et al., 2004). Developed for the MTA Study, this structured brief interview of the parent captures child services use across mental health, primary care, school, and community settings.

Summary of Study Measures and Procedures

NOTE: * Indicates an inclusion/exclusion measure that also serves as an outcome measure

Measure	Done By	Time to complete (mins)	Assess for Study Inclusion	Baseline		Titration Trial	Maintenance		Randomization	
			Single Visit	Single Visit (BL)	Daily for one week	Weekly visits	Single Visit (M)	Daily for week 4	Visits on / around Day 1,14,28 (R1, R2, R3)	Daily
Inclusion / Exclusion Measures										
KSADS	P	90	+							
Teacher Vanderbilt*	T	10	+			+	+		+	
CGI	P,C, M	20	+			+	+		+	
KBIT-2	C	30	+							
WIAT-III	C	30	+							
Physical Examination	C, M	30	+							
Weight, Vital Sign, Adverse Effect Measurement	C, M	30				+	+		+	
Cardiac Screening	C, M	5	+							
Behavioral/Emotional Outcome Measures										
Parent Vanderbilt	P	10	+		+	+	+	+	+[2]	+
PHQ-9	P	5		+						
RCADS	C	20		+			+		+	
SCTS	P, T	5		+		+	+		+[2]	
BRIEF	P,T	10		+			+		+[2]	
ERC	P	5		+			+		+[2]	
PSERS	P, T	3		+		+	+		+[2]	
CSHQ	P	15		+			+		+[2]	
EMA	P	5			+			+		+
SCA-PI	P, S	20							+[3]	
CAARS	P	20							+[1]	
Lab Observations	C, S	8		+			+		+	
Cognitive (Neuropsychological and Academic) Outcome Measures										
GNG	C	12		+			+		+	
SST	C	12		+			+		+	
AIMSWEB	C	30		+			+		+	
Biologic Samples										
DNA Saliva Samples	C, S	5				+				
Blood Samples for Epigenetic and Pharmacokinetic Assays	C, S	5		+			+			

Notes: P=parent; T=teacher; C=child; M=medical provider, S=study staff

[1]The CAARS will only be administered at the day 1 (R1) visit.[2]The teachers will complete the Vanderbilt form, PSERS, BRIEF, and SCT on/around R1, R2, and R3. However, the parent completion pattern differs. On R2 and R3, parents will complete all measures marked +[2]. On R1, parents will not complete any measures marked +[2]. Instead, several days to a week after starting the Randomization phase, parents will complete the SCT and PSERS.

[3]The SCA-PI will only be administered at the day 28 (R3) visit to determine what additional services may have been obtained by the families during the study.

Analyses

Aim 1*:* Examine the magnitude and time course of the effects of MPH discontinuation on behavioral functioning in children with ADHD.

-Hypothesis 1A: Children in the MPH Discontinuation group will experience a pattern of worsened behavior after discontinuation compared to their baseline.

-Hypothesis 1B: The behavioral worsening from baseline to discontinuation among children in the MPH Discontinuation group will dissipate over time.

Aim #2: Examine the magnitude and time course of the effects of MPH discontinuation on cognitive and academic functioning in children with ADHD.

- Hypothesis 2A: Children in the MPH Discontinuation group will experience a pattern of worsened cognitive and academic performance after discontinuation compared to their performance at baseline.

-Hypothesis 2B: The cognitive and academic worsening from baseline to discontinuation among children in the MPH Discontinuation group will dissipate over time.

Primary Data Analyses to Address Hypotheses for Aims 1 & 2: All Aim 1 & 2 hypothesis testing will use identical mixed effects models. Omnibus tests will be conducted using models including a variable for Group and for Assessment Point. The Group variable will be a 2-level variable indicating group status (i.e., Sustained MPH, MPH Discontinuation). The Assessment Point variable will indicate when the data were collected (i.e., BL, M, R1, R2, R3). In addition to the main effects for Group and Assessment Point, we will model the Group x Assessment Point interaction. The dependent variables (DVs) used in each statistical model will depend on the hypothesis being tested. For Aim #1 hypothesis testing, the primary DVs will be the Vanderbilt Parent Rating Scale ADHD symptom score and off-task behavior during direct laboratory observations. For Aim #2 hypothesis testing, the primary DVs will be GNG task reaction time variability to indicate cognitive performance and math computation on the AIMSWEB to indicate academic performance. In case demographic (e.g., age, race/ethnicity, and sex) or clinical measures (e.g. MPH dose, ADHD subtype, etc.) are related to outcomes in each model, we will consider adding these variables as covariates if they are significantly correlated with the outcome being modeled. Due to our medication manipulation and the established large effects of MPH on the primary dependent variables,[14, 15] we are confident that all of the model terms (i.e., main effects of Group and Assessment Point, the Group x Assessment Point interaction) will be significant in each omnibus test. Thus, our hypothesis testing will rely on deconstruction of these significant omnibus main effects and interaction effects as described below.

Namely, hypotheses 1A and 2A will examine neurobehavioral worsening with targeted Assessment Point comparisons *only among children in the MPH Discontinuation group.* Specifically, we will compare performance during each assessment point during the MPH Discontinuation period (R1, R2, R3) to baseline (BL) scores to test for statistically significant worsening of performance. We will also compute effect sizes for each of these comparisons. By determining the statistical significance and effect sizes for comparisons between each MPH Discontinuation Assessment Point (R1, R2, R3) and baseline scores, we will be able to document behavioral/cognitive/academic worsening during MPH discontinuation as well as the magnitude of this worsening at the various post-discontinuation time points.

For addressing hypotheses 1B and 2B which examine the temporal course of discontinuation effects, we will again perform post-hoc comparisons *using only the MPH Discontinuation group*. While the pattern of statistical significance and relative effect sizes across the post-discontinuation testing points from the hypothesis testing for 1A and 2A may suggest a temporal pattern of discontinuation effects, we will attempt to more specifically determine the temporal trajectory of the discontinuation effects by modeling performance during the MPH Discontinuation period using linear as well as curvilinear terms (e.g., quadratic). For these models, we will run initial models using a linear Assessment Point term to model the effects of discontinuation across the MPH Discontinuation period. We will then conduct additional models with a quadratic Assessment Point term to see if there is an incremental improvement in model fit indicating that the model that best describes the effects of discontinuation is a quadratic line (i.e., an initial deficit with rapid improvement and a performance plateau). Of note, we will test linear and curvilinear fit separately for each of our primary outcomes since it is possible that the temporal course of discontinuation effects may differ by the domain being assessed (e.g., behavior may

show a linear effect while cognitive outcomes may demonstrate a quadratic effect). Also, note that for some of our primary outcomes (e.g., parent ratings of ADHD symptoms), we will have a more comprehensive set of assessment points during the Discontinuation phase since parents will be completing daily ADHD symptom ratings (consistent with MTA Study titration trial methodology[47, 51]) during this phase using EMA technology (see 3.C.10.b). Where these data are available, we will use the more comprehensive set of assessment points in our statistical models to more accurately model the temporal course of discontinuation effects and to test higher order effects of time (e.g., cubic effects).

Aims 1 & 2 Power Analyses. Power for testing Assessment Point x Group interaction effects on performance in the omnibus mixed effects models was calculated using these parameters: standardized means, ranges of covariances between independent variables of r=0.0-0.20, a range of 1-4 possible individual level control variables, predictor variables (i.e., Assessment Point [5 levels], Group [2 levels] and the Group x Assessment Point interactions), a conservative estimated level of power of .80, alpha=.05, and an estimated effect size of d =.25 for the interaction effect. Although the preliminary study found effect sizes for similar interactions that were larger (all ds>.72), a conservative effect size was considered appropriate since the unequal N's across the treatment groups can have a deleterious effect on power.[66] Across the range of these parameters, the power analyses suggested a sample size from 88-103 participants for sufficient power to detect the estimated interaction effect. Therefore, to ensure proper power, there is a goal of 95 study-completers. To meet this goal, 140 participants will be randomly assigned to MPH Discontinuation and 28 to Sustained MPH, with the expectation that 25% of both groups will exit the study due to placebo or MPH non-response during the titration trial, and an additional 10% in the Sustained MPH and 20% in the MPH Discontinuation group will exit the study due to attrition (attrition estimate derived from a prior study by Gilberg et al[67] in which children received stimulant medication for three months, then were randomized to discontinue stimulants for 4-5 weeks). In total, 167 participants will start the study with 95 participants estimated to complete the study (MPH Discontinuation N=76, Sustained MPH N=19). This would provide sufficient power to detect the estimated effect size (i.e., d) of $\geq$.25 to evaluate the interaction effect in the omnibus tests.

For testing Hypotheses 1A and 2A which analyze the magnitude of MPH cessation effects in the MPH Discontinuation group, we calculated power using these multilevel analyses parameters: standardized means, ranges of covariances between independent variables of r=0.0-.20, a 2-level Assessment Point variable (BL vs. R1, BL vs. R2, or BL vs. R3), a range of 1-5 possible covariates, a conservative estimated level of power of .80, N=83 (based on the omnibus analyses), and alpha=.017 (adjustment made to control for multiple sets of analyses for each outcome). Using these parameters, the power analyses suggested 80% power to detect effect sizes (i.e., d) of $\geq$.20. The preliminary study found effect sizes (Cohen's d) of .67-.80.

For testing Hypotheses 1B and 2B analyzing the temporal course of MPH discontinuation effects in the MPH Discontinuation group, we calculated power using the following multilevel analyses parameters: standardized means, ranges of covariances between independent variables of r=0.0-.20, a 3-level Assessment Point ordinal variable (R1, R2, R3), linear and curvilinear effect variables, a range of 1-5 possible covariates, a conservative estimated level of power of .80, N=83 (based on the omnibus analyses), and alpha of .05. Using these parameters, the power analyses suggested 80% power to detect effect sizes (i.e., Cohen's d) of $\geq$.15. While there are no data or prior studies that can provide an effect size for a curvilinear effects as a comparison, we believe that a curvilinear effect <.15 would not be clinically meaningful.

Aims 1 & 2 Secondary Analyses: A first set of secondary analyses will focus on verifying that MPH did improve performance across our various outcomes, as has been shown in hundreds of prior studies testing MPH efficacy.[16, 68] To test MPH effects, we will again decompose our omnibus model. Specifically, we expect that a post-hoc model comparing the BL and R1 performance of the Sustained Placebo group to the combined Sustained MPH and MPH Discontinuation groups will yield a significant Group x Assessment Point interaction indicating improved performance for those receiving a maintenance dose of MPH vs. those on placebo.

Additional secondary analyses for the omnibus and all post-hoc models described above will be performed using the non-primary dependent variables measuring behavioral, cognitive, and academic functioning.

Aim #3: Explore potential moderators (e.g., MPH dose during the Maintenance phase, sex, baseline psychiatric comorbidity, genetic polymorphisms) of MPH discontinuation effects *in the MPH Discontinuation group*.
-Hypothesis 3: Given the paucity of pediatric studies on MPH discontinuation effects, it is difficult to know if there might be any patient variables moderating these effects. Hence, these analyses are largely exploratory.

***Data Analysis to Address Aim #3*:** It is expected that there will be variability across individuals in the size of the MPH discontinuation effects. To identify factors which explain variability in the discontinuation effects (i.e., which predict individuals who have stronger or a weaker declines in performance), various variables, such as MPH dose, sex, and baseline psychiatric comorbidity, will be tested as moderators. For each set of analyses described above for the omnibus mixed effects models, the same models will be used with three modifications to the models: 1) the Group variable will be removed, 2) the moderator variable being tested will be added, 3) an interaction between the moderator variable and Assessment Point will be added. The interaction will test whether the change from Baseline to Discontinuation is different depending on the level of the moderator variable. Significant interaction effects will be graphed to interpret the findings.

Aim 3 Power Analysis. The parameters for the Aim 3 power analyses are similar as those for Aims 1 & 2 since the Group variable will be replaced by the moderator variable. The only other difference between these power analyses and the analyses described above is that only the MPH Discontinuation group will be used (expected N= 83, see above). For these analyses, we will have 80% power to detect a cross-level interaction effect (i.e., d) of ≥.29, which translates to being able to detect a small effect size.

Aim #4 (Exploratory): Examine whether social/financial hardships predict medication supply (i.e. number of days covered with medicine) and medication continuity (i.e. time to first 30-day gap in medication supply) over the 12 months following completion of the study medication trial as determined by the child's pharmacy/prescription records of medication dispensed. Participants may be contacted, as needed, after finishing the study to sign additional forms for pharmacy companies and CCHMC release information. Participants may also consent to this information being retrieved through an Automated Rx Retrieval System (e.g., OARRS; electronic medical record medication dispensing reconciliation listing). The information will be given by the parent at the beginning of their study entry and will be verified at the end to make sure it has not changed. This information will include the pharmacy name, address, phone number, prescription plan (if known), and if any medications are obtained elsewhere.

Data Analysis to Address Aim #4: Based on pharmacy/prescription records for medications dispensed, we will calculate the medication supply, as defined by the number of days covered with ADHD medication over 12 months following completion of the study medication trial, and medication continuity, as defined by the time from starting medicine to the first 30-day gap in medication supply. Social and financial hardship will be characterized by using previously validated questions. These hardships will be assessed via difficulty making ends meet, being unable to pay rent or utilities for financial reasons, household's inability to borrow money during times of need. Those answering "yes" to any of these questions will be considered at risk. We will use linear regression models to assess the association between presence of social/financial hardships and the number of days covered with medicine. We will use Cox proportional hazards regression models to assess the association between presence of social/financial hardships and the number of days to the first 30-day gap.

Families who previously completed the study will be re-contacted via phone, mail, and/or email, to gain their medication record information. Families who consented prior to 10/27/16 will be asked to sign an addendum to the consent form for this portion of the study and a food/beverage incentive (worth approximately $5) will be offered. Participants who consented after 10/27/16 will just have consent via acknowledgement that the family accepts in the form of a signed HIPAA authorization release form. All participants will sign the HIPAA form to be submitted by research staff to the pharmacy or other entity. If upon contact, they do not wish to be a part of this aim, we will note that they should not be contacted in the future and their information will not be included. Participants may sign this consent addendum and HIPAA via phone consent, email, or mail. Participants can scan and email, mail or fax the HIPAA release to CCHMC staff. The signed consent form will be reviewed over the phone once the family has it in their possession and returned via postal mail, email, fax, or a CCHMC staff

will pick it up. Participants may also review and sign the consent addendum via REDCap eConsent supported by the CCHMC Division of Biomedical Informatics in compliance with HIPAA designed to protect PHI in the electronic transfer and storage of the consent form. Participants who choose to sign the addendum electronically will have the consent form and addendum emailed them through a REDCap link. Study staff will review the addendum with the participant. The participant will then record their consent on REDCap using a study-specific consent form modeled after the eConsent demo on the REDCap Resource Center. If the participant does not have enough time to review the addendum, study staff will schedule a time to call back and do so. Once the electronic form has been submitted, a copy of the signed form will be provided to the participant.

Aim #5 (Exploratory): *We hypothesize that variation in methylation at CpG sites proximal to CES1, ARSA, GRM7, SLC6A2, SLC6A3, and DRD4 will be associated with and predict ADHD symptom reduction after MPH treatment.* To test this hypothesis, 45 medication naïve children (n=15 from a currently enrolling R01MH105425 and n=30 from banked samples) with parent and teacher ratings of their ADHD symptoms at pre-MPH baseline and weekly thereafter during the 4-week MPH trial will have their pre-medication baseline methylome determined as described above and a repeated measures mixed model will be computed (child as the random effect). Specifically, at each of the methylation sites (predictor) for a candidate gene, the % change between each baseline and follow-up ADHD symptom reduction measure will be tested with standard longitudinal contrast statements (e.g., baseline vs. average of other timepoints: -3, 1, 1, 1; linear decline from baseline -3, -1, 1, 3). The analysis will assume a 1st-order autoregressive correlation in the mixed model to account for the repeated observations. To adjust for the number of comparisons, a Benjamini-Hochberg false discovery rate adjusted p-value will be computed. Age, gender, rater indicator (parent vs. teacher), and presence of mental health or developmental comorbidities will be included as covariates in the modeling if predictive of symptom reduction with ($p<0.10$).

We also hypothesize that MPH-naïve whole-blood methylation proximal to candidate genes will be associated with severity of MPH adverse effects of appetite suppression (CES1, SLC6A3, SLC6A4), sleep problems (CES1, SLC6A3, SLC6A4), and irritability (SLC6A6, DRD4, SLC6A3) measured at the four time points (pre-MPH baseline, placebo, and on each of 3 MPH doses). The primary outcome will be parent ratings of sleep problems, decreased appetite, and irritability (0=none, 1=mild, 2=moderate, 3=severe) on the Pittsburgh Side Effects Rating Scale (PSERS),[46] with which we have both clinical and research experience.[15, 69] To test these hypotheses, 45 medication naïve children (n=15 from a currently enrolling R01MH105425 and n=30 from banked samples) with PSERS ratings at pre-MPH baseline and weekly thereafter during the 4-week MPH trial will have their pre-medication baseline methylome sequenced as described above and a separate repeated measures cumulative logit (logistic regression) mixed model will be computed for each adverse effect (child as the random effect); the cumulative logit model accounts for the ordinal nature of the PAERS ratings. At each of the methylation sites (predictor), the MPH adverse effect score will be tested with standard longitudinal contrast statements (e.g., baseline vs. average of non-baseline timepoints: -3, 1, 1, 1; linear decline from baseline -3, -1, 1, 3), assuming a 1st-order autoregressive correlation to account for the repeated observations. To adjust for the number of comparisons, a Benjamini-Hochberg false discovery rate adjusted p-value will be computed. Age, gender, presence of mental health or developmental comorbidities, and *CES1* SNPs [rs2307240 (S75N), rs2307227 (D203E), rs71647871 (G143E), rs3815583, rs 200464425] will be included as covariates in the modeling if associated with the MPH adverse effect of interest ($p<0.05$). We will also conduct an exploratory genome-wide methylation association study using a genome-wide threshold of $P<1x10^{-6}$.

Aim #6 (Exploratory): *We hypothesize that treatment with MPH will alter methylation patterns, both specific to the candidate genes SLC6A3, SLC6A2, and DRD4 and at other CpG sites throughout the genome.* To test this hypothesis, MPH responders will have their baseline (medication-naïve) and 4+ weeks post initiation of MPH treatment methylation levels determined. The within-person difference between the medication-naïve and on-MPH methylation at CpG sites will be computed and tested against the null hypothesis of no change. The primary analysis will focus on the CpG sites proximal to the candidate genes and will have 0.80 power to detect differences of 1.54 standard deviation of the mean methylation difference in a sample size of 12 participants. Although these are large changes in methylation, in our experience such changes are regularly

observed in methylation studies we have completed [e.g., monozygotic twins discordant for lupus (under review), twins discordant for scleroderma (manuscript in preparation), twins discordant for Eosinophilic Esophagitis (manuscript in preparation)] and in the literature from genome-wide scans of methylation in autoimmune disease.[70-74]

A secondary exploratory analysis designed for hypothesis generation will identify other sites in the genome that differ between medication-naïve and on-MPH methylation time points. Co-methylation (correlated methylations patterns) and pathway analyses (e.g., ingenuity pathway analysis, STRING, MCODE, LINC) will be computed to aid in the interpretation of the results. We will use genome-wide thresholds for significance of $1x10^{-6}$.

Aim #7 (Exploratory): *We hypothesize that methylation profiles at CpG sites in CES1 (enzyme which breaks down MPH) are associated with specific MPH pharmacokinetic (PK) profiles from plasma concentration measurements and these profiles will mediate the association between CES1 methylation and MPH adverse effects.* We will use a pediatric population model-based approach and Bayesian estimation to generate the individual PK profiles from and methylphenidate exposure estimates.[75, 76] Population model parameters as observed in children with ADHD will be used as the prior information.[77] The PK model together with patient demographics, dosing, and concentration–time data will be entered into the PK modeling clinical software (MW/Pharm, Mediware, Prague, Czech Republic) and individual PK profiles will be generated based on the actual measured MPH concentration(s) using the Bayesian estimator. These Bayesian estimated PKs will be tested for association with the methylation at CpG proximal to *CES1*, using regression models (PK as outcome, methylation as predictor). A mediation analysis will be completed to test whether the PK profiles mediate the CES1 methylation association with MPH adverse effects.[78] Secondary modeling will adjust for *CES1* nonsynonymous variants rs2307240 (S75N), rs2307227 (D203E), rs71647871 (G143E).

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