Attention-deficit/hyperactivity disorder (ADHD) is a heritable neurodevelopmental condition, there is also considerable scientific and public interest in environmental modulators of its etiology. Exposure to neurotoxins is one potential source of perturbation of neural, and hence psychological, development. Exposure to lead in particular has been widely investigated and is correlated with neurodevelopmental outcomes, including ADHD. To investigate whether this effect is likely to be causal, we used a Mendelian randomization design with a functional gene variant. In a case-control study, we examined the association between ADHD symptoms in children and blood lead level as moderated by variants in the hemochromatosis (HFE) gene. The $HFE$ gene regulates iron uptake and secondarily modulates lead metabolism. Statistical moderation was observed: The magnitude of the association of blood lead with symptoms of ADHD was altered by functional $HFE$ genotype, which is consistent with a causal hypothesis.

Keywords
attention, childhood development, psychopathology

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Attention-deficit/hyperactivity disorder (ADHD) is a neurodevelopmental condition with complex etiology that includes effects of genetics, early environment, and their interplay. Because of the extensive literature on the neural effects of lead (Bellinger, 2011; Hu et al., 2011; Senut et al., 2014) and its established epidemiological associations with ADHD and other behavioral and cognitive outcomes, lead exposure provides an attractive “case study” of potential toxicological influences on neurodevelopmental conditions. Lead easily crosses the blood-brain barrier, and among the affected brain regions and tissues are those of long-standing interest in ADHD research, including prefrontal cortex, hippocampus, basal ganglia, cerebellum, and white matter in general (Costa, Aschner, VITALONE, SYVERSEN, & Soldin, 2004). Its effects therefore provide a biologically plausible route to ADHD via subtle environmental perturbation of neurodevelopment.
States, lead burden in the body is correlated with alterations in human neural development (Brubaker et al., 2009; Cecil et al., 2011) and with behavioral and cognitive outcomes in children, including ADHD, conduct problems, reductions in executive functioning, reductions in IQ, and learning problems (Canfield, Kreher, Cornwell, & Henderson, 2003; Goodlad, Marcus, & Fulton, 2013; Lanphear, 2015; Marcus, Fulton, & Clarke, 2010; Nigg et al., 2008). The effect sizes after controlling for covariates are medium to small (on the order of \( r = .15 \)) but are similar for ADHD, IQ, and conduct problems (a developmental outgrowth of some cases of ADHD). These correlations appear to be nonlinear, such that even at low doses, lead has clinically significant associations with outcomes (Lanphear, 2015). Although these effects are statistically modest, they may have substantial population importance because of the near-universal exposure of children to lead (see Lanphear, 2015).

Although many potential confounders have been statistically covaried in this research, and animal studies have demonstrated a causal effect of lead exposure on activity level (Luo et al., 2014), what appear to be causal effects of lead on ADHD in humans could still be due to unmeasured confounders. One approach to clarify causality is Mendelian randomization (Lewis, Relton, Zammit, & Smith, 2013), which is a type of natural experiment. This approach entails dividing groups of people by genotype for a functional gene that metabolically or physiologically influences the presumed causal input (e.g., lead). The genotype must vary in the population at random and must be independent of both measured confounders (i.e., lower socioeconomic status or parental IQ); therefore, it can be assumed to be independent of unmeasured confounders. In particular, the absence of a correlation between the genotype and the phenotype (in this case, ADHD) sharply reduces the likelihood of a reverse causality (i.e., the probability that increased hyperactivity is causing more lead exposure). Therefore, if this change in functional metabolism moderates the clinical endpoint, a causal pathway is supported.

Mendelian randomization has been a valuable strategy for other disorders, including alcoholism (Irons, McGue, Iacono, & Oetting, 2007; Ritchie et al., 2014); however, it is underused in research concerning most psychiatric conditions and has not been previously used to study ADHD in children. Figure 1 schematizes the predictive logic of this design as implemented in the current study.

Among the most widely studied functional gene variants thought to influence lead metabolism is the human hemochromatosis gene (HFE) located at 6p21.3. Two somewhat common missense mutations (i.e., mutations in which a change in one nucleotide results in a different amino acid) within the HFE gene—cysteine to tyrosine at position 82 (C282Y) and histidine to aspartic acid at position 63 (H63D)—cause a shortage of the HFE protein, resulting in up-regulation of transferrin receptors and divalent metal transporters and increased iron uptake in the gut. Homozygous mutation can cause adult-onset hemochromatosis, an autosomal recessive disease of iron overload (Hanson, Imperatore, & Burke, 2001; Santos, Krieger, & Pereira, 2012).

Because lead interacts with iron metabolically, these mutations are believed to alter lead’s effects (Gundacker, Gencik, & Hengstschlager, 2010). There is no consensus on the mechanism of HFE’s effects on lead metabolism, but one hypothesis is that HFE variants alter lead’s health effects via increased oxidative stress (Park et al., 2009). That hypothesis is supported by laboratory work demonstrating increased iron-related lipid oxidation in the presence of lead (Adonaylo & Oteiza, 1999). Under that model, the HFE variants are not required to alter blood lead level per se; rather, the variants alter the biochemical reactions involving lead and iron at a given blood lead level and thus change the phenotype.

Accordingly, HFE variants appear to modulate lead’s association with several phenotypic outcomes. For example, in a series of studies of older men, Wright and his colleagues reported that the association between lead level and cognitive decline was stronger for men who were carriers of HFE mutations (Wang et al., 2007) and that the association between blood lead level and disturbed cardiac function was greater for those with the C282Y variant of the HFE gene (Park et al., 2009). The C282Y variant also altered the association between lead and amytrophic lateral sclerosis (Eum et al., 2014) and altered the rate of placental lead transfer (Karwowski et al., 2014); H63D variants altered lead’s association with low birth weight (Cantonwine et al., 2010). These findings are quite consistent, even though the strength of association among HFE variants and absolute blood lead level is modest and inconsistent in these studies.

Our governing hypothesis, therefore, was that carriers of specific HFE mutations (C282Y and H63D) are more susceptible than noncarriers to developmental damage as a result of low-level lead body burden. Thus, if lead influences ADHD symptoms, HFE variants will statistically moderate the magnitude of the association between blood lead level and ADHD.

**Method**

**Participants**

We recruited physically healthy children with normative lead exposure for a study with a case-control design. To ensure that effects on ADHD symptoms within the clinical range would be adequately represented, we
overselected youths with ADHD. Our participants were from Michigan, a U.S. region in which generalizability of allele frequencies could be compared with those found in a randomized population study published previously (Barry, Derhammer, & Elsea, 2005). All children were recruited via mailings to parents in regional school districts, public advertisements, and outreach to local clinics. Parents provided written informed consent, and children provided written informed assent. All procedures were approved by the university’s institutional review board and complied with all applicable guidelines for protection of human participants. Data collection proceeded until funding was exhausted and sample size was sufficient to meet power targets.

Families entered a multistage screening process that established diagnostic groupings for the case-control design. For each child, a parent and a teacher completed standardized clinical rating scales, and a trained clinician completed a semistructured clinical interview with the parent about the child (Kiddie Schedule for Affective Disorders and Schizophrenia—epidemiologic version, or K-SADS-E; Puig-Antich & Ryan, 1986). All interviewers had master’s degrees in clinical psychology or social work. To establish reliability of the coder KSAD diagnoses and fidelity to procedures, we video-recorded 20 interviews conducted by each interviewer. These interviews were then double-coded by the primary interviewer and by an expert criterion coder (for all disorders mentioned in this report, $\kappa > .80$).

The experienced clinicians’ agreement rates for diagnostic assignment were acceptable for ADHD, conduct disorder (CD), and oppositional defiant disorder (ODD), all $\kappa > .80$, as well as for all other disorders with a base rate of greater than 5% in the sample, all $\kappa > .75$. Disagreements were resolved by discussion. The clinicians assigned a primary diagnosis of ADHD when they saw evidence of impairment, cross-situational symptoms, and onset by the age of 7 years and concluded that a diagnosis of ADHD best accounted for symptoms; this practice was consistent with ADHD criteria from the Diagnostic and Statistical Manual of Mental Disorders (4th ed., text rev.; American Psychiatric Association, 2000), intrarater agreement $r > .80$.

Using all available data, two experienced clinicians (a board-certified child psychiatrist and a licensed clinical child psychologist) blind to the study’s hypotheses and to the children’s blood lead levels arrived independently at a best-estimate diagnostic decision for ADHD and comorbid disorders, for each child. Impairment was required, and so the two clinicians quantified their impairment ratings using the Global Assessment of Functioning Scale (American Psychiatric Association, 2000), intrarater agreement $r > .80$.

Fig. 1. Diagram of the hypothesized interplay between blood lead level and hemochromatosis (HFE) genotype in influencing attention-deficit/hyperactivity disorder (ADHD) symptoms.
ancillary studies of neurocognitive function in ADHD (Martel, Nikolas, Jernigan, Friderici, & Nigg, 2012; Nikolas & Nigg, 2015). Children whose parents reported a history of seizure (except a single febrile seizure), neurological impairments, prior diagnosis of intellectual disability or autism spectrum disorder, head injury with loss of consciousness beyond 30 s, sensorimotor handicap, or other major medical conditions were excluded. If the research diagnostic evaluation revealed substance addiction, autism spectrum disorder, bipolar disorder, history of psychosis, sleep disorder, a medical or neurological condition, or an estimated IQ of less than 75 (evaluated by a short form of the Wechsler Intelligence Scale for Children–4th Edition; Wechsler, 2003), children were excluded. These criteria were intended to minimize diagnostic assignment errors for ADHD. After exclusion, the sample comprised 386 children, ages 6 to 17 years, from 267 families (148 singletons, 119 sibling pairs).

**Measures**

**Blood lead.** Researchers drew 2 ml of whole blood from each child’s arm into a 2-ml Vacutainer tube (BD Diagnostics, Franklin Lakes, NJ) containing ethylenediaminetetraacetic acid (tubes were lot checked for lead by lab before use). Blood samples were labeled with a study number, frozen, and stored at −20 °C before analysis. Samples were assayed using the process of inductively coupled plasma mass spectrometry. This method’s detection limit for lead is 0.3 µg/dl; interrun precision was 5.8% (coefficient of variation) at a lead value of 2.9 µg/dl. The process began with bringing each whole-blood sample to room temperature and vortexing it so that no particulate matter remained at the bottom. Samples were diluted 1:50 with a diluent composed of 1.0% tetramethylammonium hydroxide, internal standard (iridium), 1.0% isopropyl alcohol, 0.01% ammonium pyrrolidine dithiocarbamate, and 0.05% wetting solution (Triton X-100). Samples were then mixed by inverting them three or four times. The analysis then entailed quantitating total lead by summing the signal intensities for three isotopic masses of lead (206, 207, and 208), using three replicates per sample on an Elan DRC Plus inductively coupled plasma mass spectrometer (PerkinElmer, Waltham, MA).

**Genotyping.** Samples were obtained from buccal swabs (43%) and salivary DNA (57%). Buccal samples were purified using a method described previously (Meulenbelt, Droog, Trommelen, Boomsma, & Slagboom, 1995). Organe samples were processed according to the manufacturer’s instructions (DNAgenotek, Ottawa, Ontario, Canada). Polymerase chain reaction (PCR) was performed according to the method used by Barry et al. (2005), but the number of cycles was increased from 30 to 35 for a more robust amplification product. Genomic DNA (40–60 ng) was amplified using 0.5 units of Taq DNA polymerase (Invitrogen Corp., Carlsbad, CA) in standard PCR buffer consisting of 20 mmol/L tris(hydroxymethyl)aminomethane hydrochloride, 50 mmol/L potassium chloride, 1.5 mmol/L magnesium chloride, 0.2 mmol/L deoxynucleoside triphosphates, and 0.7 µmol/L concentrations of each primer— for C282Y: 5′-TCCCAAGGCTAAACGATCC-3′ (forward) and 5′-TACCTCTCAAGCAGCTCC-3′ (reverse); for H63D: 5′-CACTTGGGTGCTGAGGCTCG-3′ (forward) and 5′-CCCTTGCTGTGTTGTGATT-3′ (reverse).

Reaction conditions consisted of an initial denaturing step at 94 °C for 3 min, followed by 35 cycles at 94 °C for 30 s each, annealing at 63 °C for 30 s, and an extension at 72 °C for 30 s, followed by a final extension step at 72 °C for 5 min. Expected product sizes for C282Y and H63D were 393 and 243 base pairs, respectively. Digestion of the C282Y PCR product with the restriction endonuclease RsaI (New England Biolabs, Ipswich, MA) for 1 to 2 hr yielded the fragment sizes of 251 and 142 base pairs (bp) for the wild type and 251, 113, and 29 bp for the mutation. Digestion of the H63D PCR product with the restriction endonuclease DpnII (New England Biolabs) yielded fragment sizes of 100, 85, and 58 bp for the wild type and 185 and 58 bp for the mutation. Both were analyzed on a 3.0% agarose gel. Genotyping failed for 3 children, meaning that 383 were included in the final analyses.

**Measures of ADHD and comorbid externalizing symptoms.** For data-analysis purposes, ADHD symptoms were assessed by parental and teacher ratings on three widely used rating scales: (a) the ADHD Rating Scale (DuPaul, Power, Anastopoulos, & Reid, 1998) inattention and hyperactivity-impulsivity symptom scores; (b) Conners’ Rating Scale–Revised (Conners, 1997), specifically the subscales for cognitive problems (i.e., inattention) and hyperactivity problems; and (c) the Strengths and Weaknesses of ADHD Symptoms and Normal Behavior Scale (SWAN; Swanson et al., 2012) inattention and hyperactivity symptom scores (from parents but not teachers), on which ADHD symptoms are listed on a normalized scale and worded to reflect normal variation, to avoid floor effects often seen on symptom scales. Teachers also completed ratings of ODD and CD behaviors as part of the ADHD Rating Scale; parents’ ratings of ODD and CD symptoms were derived from their reports on the K-SADS-E. In this sample, all scales exhibited adequate internal consistency (αs from .83 to .94), and their manuals provide adequate test-retest reliabilities. For children taking prescribed medication, raters were asked to rate, to the extent possible, the children’s behavior as observed when they were not taking medication.
Data analysis

Statistical power. A review of prior studies of Mendelian designs, studies of Gene x Environment in ADHD, and HFE interaction studies suggested that we needed to be able to detect a small to medium interaction effect. Power analysis was conducted in G*Power (Faul, Erdfelder, Lang, & Buchner, 2007) using assumptions regarding allele frequencies and correlational structure. With more than 350 participants, as we were able to achieve, power was .80 to detect an interaction effect size (ΔR^2) of .037 at an α level of .05. When α was adjusted to .0125, power was .80 for an effect size of .04. These effect sizes are between small, R^2 = .01, and medium, R^2 = .09, by Cohen’s conventions.

Handling of covariates. Total gross annual income in the child’s primary household was reported by parents as a proxy for socioeconomic status and was included as a covariate. Because of the relatively wide age range of the children, we included age as a covariate even though it was not reliably correlated with blood lead level.

HFE mutations are more common among White participants (Hanson et al., 2001). In our sample, White youths were more likely to have one or two copies of the H63D mutation, p = .004, whereas the C282Y mutation was not significantly higher among White youths relative to non-White youths (p = .29).

Race is an important potential confound in any genetic study. Table 1 reports in detail the racial makeup of the sample. We controlled for race in two ways, following the method of Keller (2014). For our main results, we simply used a single race code (White vs. non-White) as a covariate. We then conducted secondary checks in which we entered all the interactions (one for each group listed in Table 1) simultaneously, so that all effects of race were controlled. Mixed-race individuals were coded separately as biracial and treated as one group.

To rule out artifact, we created a comparison variable that was not expected to yield an interaction with HFE genotype. Specifically, we obtained measures of parenting behavior using the Alabama Parenting Questionnaire (Shelton, Frick, & Wooton, 1996). Because there is no evidence or theory suggesting that HFE genotype moderates the association of parenting behavior with ADHD symptoms, we used this check to rule out the possibility that our analyses overidentified interactions with genotype.

Testing for ODD and CD is important to clarify whether any effects found are specific to ADHD or are related to externalizing problems. We report secondary data checks involving a composite ODD-CD outcome variable (based on parental reports on the K-SADS-E and teacher reports on the ODD and CD items added to their ADHD Rating Scale).

Iron hemoglobin level was assayed by standard methods from blood obtained by venipuncture and was a covariate in all analyses to protect against the possibility of attributing to lead an effect that was actually attributing to iron. The reference range for iron hemoglobin in children is 11 to 13 g/dl; in adolescents, the reference range is 12 to 16 g/dl for girls and 14 to 18 g/dl for boys. Values for the current sample were within the reference range (11.0–15.6 g/dl).

IQ was not covaried because reduction in IQ may be part of ADHD as a neurodevelopmental condition or may even be one of its consequences (Dennis et al., 2009; Miller & Chapman, 2001). Medication and treatment history were not covaried because they were substantively correlated with symptom severity; greater symptom severity was positively correlated with past or current medication or other treatment, rs ranging from .49 for lifetime treatment to .71 for current treatment, ps < .001.

A substantial literature suggests that boys are more vulnerable than girls to early-life neurodevelopmental perturbations (Jacquemont et al., 2014), which perhaps accounts for the fact that nearly all neurodevelopmental conditions affect more males than females (Martel, 2013). We therefore handled the sex variable in two ways. First, our primary analyses included sex as a covariate to preserve statistical power for detecting our primary interaction of interest (Blood Lead x Genotype). Because of boys’ greater vulnerability, we also conducted secondary analyses to explore the lower-powered but potentially important interactions between sex and lead exposure in predicting ADHD symptoms as reported by parents and teachers.

Data reduction

ADHD symptom composite scores as dependent variables. To maximize the reliability and validity of our outcome measures while minimizing the number of statistical tests required, we used structural equation modeling to create latent factors for inattention and hyperactivity-impulsivity for each informant (i.e., each parent and teacher). Thus, two separate two-factor models were fitted to the data for each informant. The models were based on reports from each informant for (a) composite inattention-disorganization, and (b) composite hyperactivity-impulsivity. Each composite was created from the relevant scales on the ADHD Rating Scale, the Conners’ Rating Scale–Revised, and the parent-reported SWAN. Each model provided a good fit to the data—parental reports: χ²(7, N = 383) = 51.3, comparative fit index = .97, Tucker-Lewis index = .94, root-mean-square error of approximation = 0.04; teacher reports: χ²(3, N = 369) = 12.5, comparative fit index = .98, Tucker-Lewis index = .97, root-mean-
square error of approximation = 0.08. To ease replication by other researchers, we created composite scores from the factor indicators for further analysis (results were unchanged using factor scores). These composite scores had satisfactory internal reliability—inattention: \( \alpha = .89 \) for parental reports, \( \alpha = .92 \) for teacher reports; hyperactivity-impulsivity: \( \alpha = .91 \) for parental reports, \( \alpha = .93 \) for teacher reports.

**ODD and CD.** A sum total of ODD symptoms reported by parents was computed for each child. To create a parallel sum score for teacher ratings, we counted the number of teacher-rated ODD and CD symptoms present (i.e., items rated as occurring “often” or “very often”). These totals were transformed (to correct skewness) and mean-centered.

**Lead nondetection.** Three children’s lead levels were below the limit of detection of 0.3 µg/dl. Following convention, those levels were scored as 0.2 (i.e., \( 0.3/2 \)). Also following a frequent convention in the literature, we \( \log_{10} \)-transformed the blood lead score to reduce the influence of outliers (posttransformation skew < 1.0).

**Handling gene-environment correlation effects.** To account for potential gene-environment correlation effects, we regressed blood lead level on \( \text{HFE} \) genotype to remove variance in lead scores associated with genotype. These residuals were standardized and used for all analyses.

**Multiple testing.** We provide 95% confidence intervals (CIs), but we also provide modified Bonferroni-corrected

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**Table 1. Demographic and Clinical Differences Between the Attention-Deficit/Hyperactivity Disorder (ADHD) and Non-ADHD Groups**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Non-ADHD group (( n = 147 ))</th>
<th>ADHD group (( n = 122 ))</th>
<th>Difference between groups: ( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male (%)</td>
<td>49.7</td>
<td>68.4</td>
<td>(&lt; .001 )</td>
</tr>
<tr>
<td>Age (years)</td>
<td>12.5 (3.1)</td>
<td>11.5 (2.8)</td>
<td>(.003 )</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White (non-Hispanic)</td>
<td>80.3%, ( n = 118 )</td>
<td>75.0%, ( n = 159 )</td>
<td>(.51 )</td>
</tr>
<tr>
<td>African American (non-White)</td>
<td>8.2%, ( n = 14 )</td>
<td>8.5%, ( n = 18 )</td>
<td>(.91 )</td>
</tr>
<tr>
<td>Latino-Hispanic (non-White)</td>
<td>1.4%, ( n = 2 )</td>
<td>3.8%, ( n = 8 )</td>
<td>(.17 )</td>
</tr>
<tr>
<td>Asian American (non-White)</td>
<td>1.3%, ( n = 2 )</td>
<td>0%, ( n = 0 )</td>
<td>(.28 )</td>
</tr>
<tr>
<td>Native American (non-White)</td>
<td>1.4%, ( n = 2 )</td>
<td>1.9%, ( n = 4 )</td>
<td>(.66 )</td>
</tr>
<tr>
<td>Mixed race</td>
<td>5.4%, ( n = 8 )</td>
<td>10.3%, ( n = 22 )</td>
<td>(.44 )</td>
</tr>
<tr>
<td>Pacific Islander</td>
<td>0.68%, ( n = 1 )</td>
<td>0.0%, ( n = 0 )</td>
<td>(.49 )</td>
</tr>
<tr>
<td>Middle Eastern</td>
<td>0%, ( n = 0 )</td>
<td>0.47%, ( n = 1 )</td>
<td>(.51 )</td>
</tr>
<tr>
<td>Total household income (U.S. $)</td>
<td>87,300 (45,800)</td>
<td>68,000 (40,100)</td>
<td>(&lt; .001 )</td>
</tr>
<tr>
<td>Global Assessment Functioning score</td>
<td>81.2 (9.4)</td>
<td>67.5 (9.1)</td>
<td>(&lt; .001 )</td>
</tr>
<tr>
<td>Estimated Full Scale IQ</td>
<td>111.5 (12.6)</td>
<td>103.8 (14.6)</td>
<td>(&lt; .001 )</td>
</tr>
<tr>
<td>Inattention symptoms</td>
<td>1.7 (2.0)</td>
<td>8.5 (1.4)</td>
<td>(&lt; .001 )</td>
</tr>
<tr>
<td>Hyperactivity-impulsivity symptoms</td>
<td>1.0 (1.5)</td>
<td>5.9 (3.1)</td>
<td>(&lt; .001 )</td>
</tr>
<tr>
<td>Oppositional deficit disorder (%)</td>
<td>2.7</td>
<td>26.5</td>
<td>(&lt; .001 )</td>
</tr>
<tr>
<td>Conduct disorder (%)</td>
<td>0.0</td>
<td>4.7</td>
<td>(.008 )</td>
</tr>
<tr>
<td>Blood lead level (µg/dl)</td>
<td>0.74 (0.35)</td>
<td>0.94 (0.52)</td>
<td>(&lt; .001 )</td>
</tr>
<tr>
<td>Iron hemoglobin level (g/dl)</td>
<td>14.1 (1.2)</td>
<td>14.0 (1.2)</td>
<td>(.23 )</td>
</tr>
<tr>
<td>( \text{HFE} ) C282Y genotype (%)</td>
<td>11.0</td>
<td>11.4</td>
<td>(.89 )</td>
</tr>
<tr>
<td>( \text{HFE} ) H63D genotype (%)</td>
<td>24.0</td>
<td>31.0</td>
<td>(.15 )</td>
</tr>
<tr>
<td>Conners’ Rating Scale–Parent Inattention (cognitive) ( T ) score</td>
<td>46.8 (6.7)</td>
<td>71.1 (10.3)</td>
<td>(&lt; .001 )</td>
</tr>
<tr>
<td>Hyperactivity ( T ) score</td>
<td>48.2 (6.9)</td>
<td>66.5 (15.1)</td>
<td>(&lt; .001 )</td>
</tr>
<tr>
<td>Conners’ Rating Scale–Teacher Inattention (cognitive) ( T ) score</td>
<td>49.3 (9.0)</td>
<td>57.7 (13.0)</td>
<td>(&lt; .001 )</td>
</tr>
<tr>
<td>Hyperactivity ( T ) score</td>
<td>48.0 (7.1)</td>
<td>59.1 (10.2)</td>
<td>(&lt; .001 )</td>
</tr>
</tbody>
</table>

Note: Values in parentheses are standard deviations. Twenty-four children with a diagnosis of ADHD not otherwise specified were excluded from these group comparisons. Parental and teacher ratings were collected using the Conners’ Rating Scale–Revised (Conners, 1997), and Full Scale IQ was estimated with the Wechsler Intelligence Scale for Children–4th edition (Wechsler, 2003). \( \text{HFE} \) = hemochromatosis gene.
$p$ values for the tests of interaction reliability, using an $\alpha$ level of .0125 for both parental and teacher data.

**Data-analytic strategy**

Unless otherwise noted, analyses were conducted in Mplus Version 7.0 (Muthén & Muthén, 2015). The CLUSTER option was used to account for nonindependence issues stemming from sibling data. Missing data were handled using full-information maximum-likelihood procedures in Mplus; missing data were minimal except for income (7% missing). Linear regression was the primary method of examining Gene $\times$ Environment effects. All models included the main effects of the respective $HFE$ genotypes (examined separately), blood lead level, and their interaction. All continuous variables were standardized so they would be centered on zero. Effect coding was used to designate genotype groups, centered on 0 (wild type $= -1$, one or two mutation copies $= 1$). Standardized parameter estimates were computed and are presented along with their 95% CIs.

**Results**

**Descriptive statistics and bivariate correlations**

Table 1 presents demographic and clinical data and the percentage of children in our sample who were homozygous and heterozygous for the mutation. As we reported previously for an almost identical sample, blood lead level was higher in the ADHD group than in the non-ADHD group, $d = 0.45$, 95% CI $= [0.24, 0.66]$. Iron hemoglobin levels did not differ reliably by group. In a random population sample of more than 3,500 White individuals in Michigan, Barry et al. (2005) found an $HFE$ C282Y allele frequency of 5.7% (compared with 5.6% for our overall sample). This means that 11.4% of the Barry et al. sample were either homozygous or heterozygous for the C282Y mutation, compared with 10.9% of our sample (10.8% in the non-ADHD group and 11.4% in the ADHD group). In their study, by extension 28.0% were either homozygous or heterozygous for the $HFE$ H63D mutation, compared with our 28.5% (24.4% non-ADHD group, 31.1% ADHD group).

The average blood lead level in our sample was almost identical to the national average reported by the U.S. Centers for Disease Control and Prevention at the time of collection (for discussion, see Nigg et al., 2008; Nigg, Nikolas, Mark Knottnerus, Cavanagh, & Friderici, 2010). Thus, as intended, our sample provided a reasonable representation of allele frequency and lead exposure and allowed us to take advantage of a case-control design. Table 2 shows the bivariate correlations among blood lead and hemoglobin levels, $HFE$ genotype, teacher and parental ratings of ADHD symptoms, and selected demographic indicators. Sex was associated with ADHD symptoms (i.e., boys were overrepresented in the ADHD group) and was also associated with lead level, such that males had higher mean levels of both, further justifying our decision to covary sex and to secondarily examine interactions with sex.

As reported previously (Nigg et al., 2008), blood lead level correlated with scores calculated from both parental and teacher reports of the inattention and hyperactivity-impulsivity factor, even at these low, population-typical blood lead levels. Contrary to expectations, blood lead level was only weakly positively correlated with iron hemoglobin level. However, as expected, higher blood lead level was associated with lower income, lower full-scale IQ, and the $HFE$ C282Y variant genotype, whereas the association between blood lead level and the H63D variant genotype was not statistically reliable.

**Primary tests of Gene $\times$ Environment effects**

**Parental reports.** The interaction between $HFE$ C282Y genotype and blood lead level was a statistically reliable predictor of parental reports of hyperactivity-impulsivity, $\beta = 0.22$, 95% CI $= [0.11, 0.40]$, $\Delta R^2 = .02$, total $R^2 = .28$, $p < .05$. **p < .01. **p < .001.

<table>
<thead>
<tr>
<th>Table 2. Correlations Among Blood Lead Level, Genotype, and Demographic Indicators</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
</tr>
<tr>
<td>----------------------------------------</td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Household income</td>
</tr>
<tr>
<td>Estimated Full Scale IQ</td>
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<tr>
<td>Global assessment functioning</td>
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<tr>
<td>Parental ratings (composite)</td>
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<tr>
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<tr>
<td>Hyperactivity-impulsivity</td>
</tr>
<tr>
<td>Teacher ratings (composite)</td>
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<tr>
<td>Hyperactivity-impulsivity</td>
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<tr>
<td>Blood lead level</td>
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<tr>
<td>$HFE$ C282Y genotype</td>
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<tr>
<td>$HFE$ H63D genotype</td>
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<td>Iron hemoglobin</td>
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Note: Parental and teacher inattention and hyperactivity-impulsivity scores are factor scores based on the two-factor latent-variable model constructed to capture variance across the parents’ and teachers’ reports on the ADHD measures, as described in the Method section. $N = 386$, including all children with a diagnosis of attention-deficit/hyperactivity disorder (ADHD) not otherwise specified. $HFE = hemochromatosis$ gene.
but not parental reports of inattention, $\beta = 0.16$, 95% CI = [-0.04, 0.32], $\Delta R^2 = .005$, total $R^2 = .14$. The association between blood lead level and hyperactivity was significantly stronger for youths with the $HFE$ C282Y mutation, $\beta = 0.74$, 95% CI = [0.52, 0.96], than for those with the wild-type genotype, $\beta = 0.28$, 95% CI = [0.15, 0.41]. Figure 2 illustrates this effect, which passed our modified Bonferroni threshold. The interactions between the $HFE$ H63D genotype and blood lead level as a predictor of parental and teacher reports of ADHD symptoms were very small and not statistically reliable, $\Delta R^2$s < .01, $ps > .3$.

**Teacher reports.** $HFE$ C282Y genotype and blood lead level also interacted in predicting teacher reports of hyperactivity-impulsivity, although the 95% CI did include zero, $\beta = 0.19$, 95% CI = [-0.002, 0.41], $\Delta R^2 = .015$, total $R^2 = .22$. The association between blood lead and hyperactivity was qualitatively stronger for youths with the $HFE$ C282Y mutation, $\beta = 0.47$, 95% CI = [0.22, 0.72], than for those with the wild-type genotype, $\beta = 0.29$, 95% CI = [0.18, 0.40]. The interaction of $HFE$ C282Y genotype and blood lead level was not a statistically reliable predictor of teacher-reported inattention, $\beta = 0.06$, 95% CI = [-0.04, 0.12], $\Delta R^2 = .002$, total $R^2 = .11$, just as it was not for parent-reported inattention. As with parental reports, the interaction effects of $HFE$ H63D genotype and blood lead level were small and not reliably different from zero. For secondary analyses, we focused on the C282Y genotype.

**Secondary analyses**

**Sex.** Males and females differed significantly in mean level of ADHD symptoms and blood lead level, and there is a substantial theoretical literature on biological sex as a moderator of ADHD vulnerability (Martel, 2013). Therefore, we conducted secondary analyses to determine whether sex moderated the impact of lead exposure on ADHD symptoms. Interaction terms were not reliably different from zero for analyses of either parental or teacher reports of inattention symptoms. The Sex x Blood Lead interaction term was reliably different from zero in the model predicting teacher reports of hyperactivity-impulsivity, $\beta = 0.26$, 95% CI = [0.11, 0.41], but not the model predicting reports of hyperactivity-impulsivity, $\beta = 0.10$, 95% CI = [-0.04, 0.24]. The direction of the effect for teacher reports indicated that the effect was larger in boys than in girls. We then examined effects separately within sex. For completeness, we report results for both teacher and parental reports.

In parental reports for boys, the Blood Lead x C282Y Genotype interaction was associated with hyperactivity-impulsivity, $\beta = 0.31$, 95% CI = [0.14, 0.48], $\Delta R^2 = .033$, total $R^2 = .24$. The association between blood lead level and hyperactivity-impulsivity was stronger for boys with the $HFE$ C282Y mutation, $\beta = 0.50$, 95% CI = [0.10, 0.90], than for boys with the wild-type genotype, $\beta = 0.21$, 95% CI = [0.06, 0.27]. In the smaller sample of females, the interaction between $HFE$ C282Y mutation and blood lead level was not reliable, $\beta = 0.09$, 95% CI = [-0.16, 0.34], and the association between blood lead level and hyperactivity did not differ much between girls with the $HFE$ C282Y mutation, $\beta = 0.22$, 95% CI = [-0.02, 0.46], and those with the wild-type genotype, $\beta = 0.17$, 95% CI = [-0.10, 0.54].

In teacher reports for boys, the interaction between $HFE$ C282Y genotype and blood lead level was a reliable predictor of reports of hyperactivity-impulsivity, $\beta = 0.19$, 95% CI = [0.07, 0.31], $\Delta R^2 = .016$, total $R^2 = .15$. Once again, the association between blood lead level and ADHD symptoms was stronger for boys with the $HFE$ C282Y mutation, $\beta = 0.46$, 95% CI = [0.23, 0.69], than for boys with the wild-type genotype, $\beta = 0.15$, 95% CI = [0.02, 0.28]. In the smaller sample of girls, again, the interaction was not reliable, $\beta = 0.11$, 95% CI = [-0.03, 0.25]. The associations between blood lead level and hyperactivity-impulsivity were similar for girls with the $HFE$ C282Y mutation, $\beta = 0.20$, 95% CI = [0.04, 0.36], and those with the wild-type genotype, $\beta = 0.16$, 95% CI = [0.07, 0.25].

**Race.** Following the method of Keller (2014), we also created a model that included an interaction term for $HFE$ C282Y genotype and each of the seven racial groups shown in Table 1 to determine whether these interactions accounted for the interactive effect of C282Y genotype and blood lead level on ADHD symptoms. This was repeated for H63D. The results for this model were not discernibly different from the results already reported.

**Other interactions.** Also following the method of Keller (2014), we created models with blood lead level and terms for the interaction between $HFE$ genotype and all other covariates (i.e., age, ethnicity, income, iron hemoglobin). Again, the results for this model were essentially the same as those reported earlier.

**Comorbidity.** When ODD-CD score was included as a covariate, results were essentially the same. The interaction between C282Y genotype and blood lead level was still a significant predictor of parental reports of hyperactivity on the K-SADS-E, $\beta = 0.20$, 95% CI = [0.04, 0.36], $\Delta R^2 = .020$, total $R^2 = .28$; the interaction between C282Y genotype and blood lead level was not a reliable predictor of parental reports of inattention, $\beta = 0.15$, 95% CI = [-0.003, 0.31], $\Delta R^2 = .005$, total $R^2 = .14$. When teacher-reported ODD-CD sum score was included as a covariate, the interaction between C282Y genotype and blood lead level was not reliably different from zero for either hyperactivity, $\beta = 0.17$, 95% CI = [-0.003, 0.43], $\Delta R^2 = .018$,
total $R^2 = .22$, or for inattention, $\beta = 0.07$, 95% CI $=[-0.05$, $0.19]$, $\Delta R^2 = .003$, total $R^2 = .11$. Conversely, in a model predicting ODD-CD score (controlling for ADHD symptoms), the interaction of C282Y genotype and blood lead level was small and not reliably different from zero, $\beta = 0.08$, 95% CI $=[-0.07$, $0.23]$, $\Delta R^2 = .006$, total $R^2 = .17$.

**Specificity.** As a cross-check on specificity of effects, we conducted analyses examining $HFE$ C282Y genotype as a moderator of the impact of parental monitoring, an environment not expected to be related to ADHD or to interact with $HFE$ genotype (Ellis & Nigg, 2009). Parental monitoring was evaluated by parental self-report using the Alabama Parenting Questionnaire (Shelton et al., 1996). As expected, there was no hint that the interaction between parental monitoring and genotype was a reliable predictor of parental or teacher reports of hyperactivity or inattention, all $R$’s $< .01$, all $p$s $> .50$.

**Discussion**

We used Mendelian randomization to evaluate effects of blood lead level in childhood ADHD. Consistent with the proposition that lead plays a causal role in the development of some cases of ADHD, the $HFE$ C282Y mutation heightened the association between lead and ADHD, principally for hyperactivity-impulsivity symptoms. The reliability of effects for hyperactivity but not for inattention echoes the literature, which suggests that the neurobiology and etiology of the two ADHD symptom domains is partially distinct (Sonuga-Barke, 2005; Willcutt et al., 2012). Lead may preferentially act in DA-related reward circuits, for example, and thus preferentially affect the hyperactivity-impulsivity symptom domain.

In this school-age sample, the association between the $HFE$ C282Y mutation and lead level itself was rather weak. Although similar associations were seen in prior studies of U.S. adults (Wright et al., 2004), the direction of association was reversed in a study of Mexican preschoolers (Hopkins et al., 2008). Aside from markedly greater lead exposure and different allele frequencies in the Mexican preschoolers compared with the U.S. adults, the functional effect of $HFE$ mutations on lead level may vary across development in relation to developmental changes in iron demand in the body (Delatycki, Powell, & Allen, 2004; Gundacker et al., 2010). Park et al. (2009) proposed that if the C282Y effect primarily alters lead’s influence on secondary metabolic activity (rather than lead uptake per se), then C282Y could alter lead’s effect on hyperactivity without markedly altering blood lead readings. Although their specific hypothesis remains unproven, it would be consistent with the effects seen here.

The same may hold for iron level. In this sample, $HFE$ variants were not associated with increased iron hemoglobin level. Perhaps a different measure, such as serum iron, would have given different results (Zimmermann, 2008). More likely, this result is related to greater iron demand during development, which is known to attenuate the $HFE$ association with absolute iron stores in the body. Nonetheless, it is clear that confirmation of the
hypothesis proposed here will require a better understanding of how absolute iron and lead levels contribute to altered metabolism or cell damage in the presence of particular genotypes, particularly in children. We reported previously that lead’s association with ADHD was mediated by effects on cognitive control (Nigg et al., 2008; Nigg et al., 2010). To better evaluate this theory and its pathophysiological meaning, future researchers could consider intermediate phenotypes, such as cognitive control and, in particular, reward processing related to hyperactivity-impulsivity.

Effects were reliable for C282Y but not for H63D. Although some past work has indicated moderation of lead’s clinical effects by H63D, effects have been more consistent for C282Y. Evidence suggests that C282Y has a more powerful effect on iron-related metabolism than that of H63D. The $HFE$ C282Y mutation causes a greater loss of $HFE$ protein function than does the H63D mutation and confers a greater risk for development of hemochromatosis and iron overload in adulthood (Hanson et al., 2001).

Findings were most robust for boys. Boys are more vulnerable than girls are to early neurodevelopmental insult, and ADHD is more common in boys, possibly because of hormone-mediated effects (Martel, 2013). It may be that additional biochemical pathways interact with lead and iron handling in boys. The boys in this sample also had higher lead levels than the girls, so it is also possible that C282Y’s moderating effects are stronger at slightly higher lead levels.

The timing of the exposure to lead in this cross-sectional sample is unknown; obviously, if lead exposure is causal, it should precede development of ADHD. In keeping with this idea, results from broader population studies have shown that peak lead exposure in children occurs in the toddler years, whereas ADHD is typically not observed or diagnosed until later in preschool or school age. Even so, it is unclear whether early exposure or sustained exposure drove the effects we observed.

The size of the Blood Lead Level × $HFE$ interaction effect on ADHD was modest. Although this effect passed multiple-testing correction and tests of specificity, and therefore is reliably different from zero and indicates a causal association, it is also likely that $HFE$ genotype is only one of the elements moderating the strength of association between blood lead level and ADHD.

Lead is associated with conduct problems and delinquency as well as ADHD. In the current study, effects were specific to ADHD. Many youths with ODD will not develop CD or delinquency, and few youths with CD were included. In addition, many of these youths were not old enough to display conduct problems. ADHD is a typical precursor to CD, particularly for boys. Thus, as these youths mature, an effect with CD might emerge.

This study highlights lead as an exemplar environmental exposure for understanding the interplay among environmental, genetic, and (potentially) epigenetic inputs to the development of psychopathology in the case of ADHD. The results enable speculation on the larger question of Gene × Environment etiology of ADHD, although this larger Gene × Environment question was not our focus. Twin studies demonstrate substantial heritability for ADHD (Nikolas & Burt, 2010). Genetic factors have been shown to influence the uptake of toxic elements, including lead (Whitfield et al., 2007). Widespread and common environmental exposures (such as that to lead) likely function as shared environments (Nigg, 2006), which means that lead exposure is correlated among siblings within the same family and serves to increase their similarity. However, given that shared environmental effects may influence ADHD within the context of genetic risk, gene-by-environment interactions have been proposed (Nigg, 2006; Nigg et al., 2010). Shared environment is of specific interest in the current study, because the Gene × Shared Environment interaction is subsumed within the additive genetic variance in twin studies, inflating the typical heritability estimate (Purcell, 2002). Thus, Nigg (2006) proposed that studies of environment and ADHD should emphasize common environmental exposures, among which low-level lead exposure is salient.

Our results add to the growing picture of how ADHD and related disorders involve dynamic, genetically modified responses to common environments, and they should stimulate development of appropriate theoretical conceptualizations. At the same time, this work should encourage further research on other neurotoxics and neurodevelopmental disorders. For example, even low-level lead exposure may result in epigenetic changes during development (e.g., histone modification), and such changes can mediate increased hyperactive behavior (Luo et al., 2014). Thus, future work could specifically examine changes in expression in ADHD-relevant genes related to lead, as well as other toxicants.

Studies of specific genes in specific environments move the field toward biology-based nosology and personalized treatments for developmental psychopathology. Although achievement of those goals remains some distance away, the present findings suggest that further investigation of environmental toxicants may provide clues regarding causal pathways for neurodevelopmental disorders.

**Author Contributions**

J. T. Nigg and K. H. Friderici developed the study concept and design. Data collection, including genotyping of samples analyzing blood lead levels, was overseen by J. T. Nigg, K. H. Friderici, and M. A. Nikolas. M. A. Nikolas, A. L. Elmore, and N. Natarajan performed the data analysis with supervision and
guidance by J. T. Nigg, J. T. Nigg, A. L. Elmore, and M. A. Nikolos. Drafted the manuscript, and all authors provided critical revisions. All authors approved the final manuscript before submission.

Declaration of Conflicting Interests
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