HHS Public Access

Author manuscript

Addict Biol. Author manuscript; available in PMC 2017 July 01.

Published in final edited form as:

Addict Biol. 2016 July ; 21(4): 915–923. doi:10.1111/adb.12230.

Dopamine and serotonin genetic risk scores predicting substance and nicotine use in Attention-Deficit/Hyperactivity Disorder

Annabeth P. Groenman[1,2], **Corina U. Greven**[2,3,4], **Marjolein M.J. van Donkelaar**[5], **Arnt Schellekens**[6,7], **Kimm J.E. van Hulzen**[5], **Nanda Rommelse**[6,4], **Catharina A. Hartman**[8], **Pieter J. Hoekstra**[8], **Marjolein Luman**[1], **Barbara Franke**[5,6], **Stephen V. Faraone**[9,10], **Jaap Oosterlaan**[1], and **Jan K. Buitelaar**[2,4]

[1]VU University Amsterdam, Department of Clinical Neuropsychology, Amsterdam, The Netherlands [2]Radboud University Medical Centre Nijmegen, Donders Institute for Brain, Cognition and Behavior, Centre for Neuroscience, Department of Cognitive Neuroscience, Nijmegen, The Netherlands [3]King's College London, MRC Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, U.K. [4]Karakter, Child and Adolescent Psychiatry University Centre, Nijmegen, The Netherlands [5]Radboud University Medical Centre Nijmegen, Donders Institute for Brain, Cognition and Behaviour, Department of Human Genetics, Nijmegen, The Netherlands [6]Radboud University Medical Centre Nijmegen, Donders Institute for Brain, Cognition and Behavior, Centre for Neuroscience, Department of Psychiatry, Nijmegen, The Netherlands [7]Nijmegen Institute for Science Practitioners in Addiction (NISPA) [8]Department of Psychiatry, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands [9]Departments of Psychiatry and of Neuroscience and Physiology, SUNY Upstate Medical University, Syracuse, USA [10]K.G. Jebsen Centre for Research on Neuropsychiatric Disorders, University of Bergen, Bergen, Norway

Abstract

Corresponding Author: Stephen Faraone, Department of Psychiatry and Behavioral Sciences, SUNY Upstate Medical University, 750 East Adams St., Syracuse, NY 13210, USA., sfaraone@childpsychresearch.org.

Author contributions:

AG, AS, and JB were responsible for the study concept and design. AG contributed to the data collection of phenotypical data, MvD was responsible for data acquisition of genetic data and drafted part of the manuscript. NR oversaw data collection of the phenotypic data. AG was responsible for data analyses, and drafted the manuscript. KvH advised in data analyses. CH, PJ, BF, SV, JO and JB obtained funding for the study. CUG, NR, CH, PJ, BF, ML, MvD, KvH, AS, SV, JO and JB provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved final version for publication.

Conflicted interest:

JO has received an unrestricted grant from Shire. PJH has received honoraria for advice from Eli Lilly and Shire. JAS is a member of advisory board of Shire en Eli Lilly, has received a research grant from Lilly and speaker fees from Shire, Lilly, Janssen-Cillag, Novartis. In the past 3 years, JKB has been a consultant to / member of advisory board of / and/or speaker for Janssen Cilag BV, Eli Lilly, Bristol-Myer Squibb, Shering Plough, UCB, Shire, Novartis and Servier. He is not an employee of any of these companies, and not a stock shareholder of any of these companies. He has no other financial or material support, including expert testimony, patents or royalties. In the past year, SVF received consulting income and/or research support from Shire, Otsuka and Alcobra and research support from the National Institutes of Health (NIH). In previous years, he received consulting fees or was on Advisory Boards or participated in continuing medical education programs sponsored by: Shire, McNeil, Janssen, Novartis, Pfizer and Eli Lilly. SVF receives royalties from books published by Guilford Press: *Straight Talk about Your Child's Mental Health* and Oxford University Press: *Schizophrenia: The Facts*. APG, AS, MvD, KvH, NNJR, BF, CAH and CUG have no conflicts of interest to declare.

Individuals with attention-deficit/hyperactivity disorder (ADHD) are at increased risk of developing substance use disorders (SUDs) and nicotine dependence. The co-occurrence of ADHD and SUDs/nicotine dependence may in part be mediated by shared genetic liability. Several neurobiological pathways have been implicated in both ADHD and SUDs, including dopamine and serotonin pathways. We hypothesized that variations in dopamine and serotonin neurotransmission genes were involved in the genetic liability to develop SUDs/nicotine dependence in ADHD. The current study included participants with ADHD (n=280) who were originally part of the Dutch International Multicenter ADHD Genetics study. Participants were aged 5–15 years and attending outpatient clinics at enrollment in the study. Diagnoses of ADHD, SUDs, nicotine dependence, age of first nicotine and substance use, and alcohol use severity were based on semi-structured interviews and questionnaires. Genetic risk scores were created for both serotonergic and dopaminergic risk genes previously shown to be associated with ADHD and SUDs and/or nicotine dependence. The serotonin genetic risk score significantly predicted alcohol use severity. No significant serotonin*dopamine risk score or effect of stimulant medication was found. The current study adds to literature by providing insight into genetic underpinnings of the comorbidity of ADHD and SUDs. While the focus of the literature so far has been mostly on dopamine, our study suggests that serotonin may also play a role in the relationship between these disorders.

Keywords

ADHD; Adolescence; Genetic risk scores; Nicotine use; Substance use

Introduction

Numerous studies have shown that individuals with attention-deficit/hyperactivity disorder (ADHD) are at increased risk of developing substance use disorders (SUDs) and nicotine dependence (Charach et al., 2011). In addition, the prevalence of ADHD is much higher in substance-dependence populations compared to the general population (van de Glind et al., 2014). The heritability of ADHD is high, ranging from .71 to .73 (Nikolas and Burt, 2010). SUDs also cluster within families, with heritability estimates of SUDs and nicotine dependence lying between .40 and .55 (Kendler et al., 2012). Family-genetic studies of probands with ADHD have reported higher rates of SUDs/nicotine dependence in the relatives of probands with ADHD compared to the relatives of controls (Monuteaux et al., 2008). On a symptom level ADHD and SUDs are both characterized by impulsive behavior (American Psychiatric Association, 2000). The overlap between ADHD and SUDs/nicotine dependence might be mediated by shared genetic liability (Carpentier et al., 2013). It is, though, still unclear, which genetic pathways are involved in such a shared liability. Several neurobiological pathways have been implicated in both ADHD and SUDs/nicotine dependence, including dopamine and serotonin neurotransmission pathways.

Alterations in brain dopamine function are considered to play a crucial role in SUDs and nicotine dependence. The use of substances of abuse leads to an increase in extracellular dopamine, mostly in the ventral striatum (Volkow et al., 2009). Chronic use of substances leads to neuroadaptations within the dopamine reward pathway (e.g. reduced D2 receptor

availability), which are thought to be a hallmark of addiction (Bobzean et al., 2014; Volkow et al., 2009). In addition, reduced functioning of the dopamine inhibitory control pathway (mainly mediated by the prefrontal cortex) has also been implicated in the pathogenesis of SUDs/nicotine dependence (Bobzean et al., 2014). Compelling evidence also points to deficits in the dopaminergic reward and inhibitory control pathways in ADHD (Cortese et al., 2012). Indeed, the first line pharmacological treatment of ADHD involves treatment with stimulants, which are hypothesized to normalize dopamine functioning in fronto-striato-cerebellar brain circuits (del Campo et al., 2011). Taken together, these studies suggest that alterations in dopamine functioning, e.g. genes involved in dopaminergic transmission might mediate the risk for SUDs/nicotine dependence in ADHD.

Besides dopamine, serotonin has also been implicated in ADHD, and SUDs/nicotine dependence. The serotonin raphe nucleus projects to the striatum, modulating the dopamine reward system (Johnson, 2004). Alcohol and nicotine intake have been associated with increased serotonergic functioning (LeMarquand et al., 1994; Olausson et al., 2002). Indeed, serotonergic antagonists have been shown to be effective in the treatment of alcoholism (Johnson, 2004). Although serotonergic drugs do not reduce behavioral symptoms of ADHD (Verbeeck et al., 2009), serotonin dysfunction has also been implicated in the pathogenesis of ADHD (Oades, 2010). Thus, genes that influence the serotonergic system may also be involved in the clinical overlap/co-morbidity between SUDs/nicotine dependence and ADHD.

Genetic liability for complex disorders such as ADHD and SUDs/nicotine dependence is thought to be multifactorial, with contributions of multiple risk variants, each with a small effect size (Manolio et al., 2009). In comparison to analyzing single polymorphisms, analysis of genetic risk scores may capture a greater proportion of the genetic contribution to complex disorders (Plomin et al., 2009). Previously, genetic risk scores have been successfully applied to, for example, disruptive behavior disorders in ADHD (Kotte et al., 2013) and food addiction (Davis et al., 2013). Here we created genetic risk scores for both serotonergic and dopaminergic neurotransmission risk genes previously found associated with ADHD, and/or SUDs/nicotine dependence. In this study, we tested the hypothesis that variations in such dopamine and serotonin genetic risk scores are involved in the genetic liability to develop SUDs and nicotine dependence in ADHD.

Methods

Participants

Participants were selected from the Dutch part of the International Multicenter ADHD Genetics (IMAGE) study (Brookes et al., 2006). Data collection for IMAGE took place between 2003 and 2006. ADHD families were recruited from outpatient clinics and included at least one child aged 5 to 17 years with combined type ADHD and at least one biological sibling regardless of ADHD diagnosis. Exclusion criteria applying to all participants included autism, epilepsy, IQ < 70, brain disorders and any single gene disorder associated with externalizing behaviors that might mimic ADHD (e.g. fragile-x).

In 2008 and 2009 participants were re-invited to participate in the current follow-up study. Ethical approval for the study was obtained from the National Institute of Health registered ethical review boards; we obtained written informed consent from all participants and/or their parents. A total of 280 of 358 participants with ADHD had data available for the current study of which 250 participants had complete data available for the selected dopamine risk alleles, and 278 for the selected serotonin risk alleles. Of all 280 subjects complete information was available for the substance use and nicotine related outcomes (the full dataset is described here Groenman et al., 2013). We found no differences between those subjects with and without complete data concerning number of SUDs, conduct disorder (CD), IQ, age, or ADHD symptoms (all $p > .1$); however, those with missing data in their genetic risk score were less likely to smoke ($p = .032$)

Measures

Diagnostic assessment

Assessment of ADHD, oppositional defiant disorder and conduct disorder: At study entry, ADHD families, probands and siblings, were screened for ADHD, using standard procedures of the IMAGE project described fully elsewhere (Müller et al., 2011). Only siblings that met all DSM-IV criteria for ADHD were included. The Long Version of the Conners Parent (CPRS-R:L) and Teacher Rating Scale (CTRS-R:L) were used to identify and quantify ADHD symptoms. T-scores ≥ 63 on the Conners ADHD subscales (L, M, and N) were considered clinically significant. The Parental Account of Childhood Symptoms (PACS) interview was administered if clinically significant scores were obtained on any of the Conners ADHD rating scales. A trained interviewer administered the PACS to the parents, who were asked for detailed descriptions of the child's typical behavior in a range of specified situations. The PACS covers the Diagnostic and Statistical Manual of mental disorders (DSM-IV; American Psychiatric Association, 2000) symptoms of ADHD, oppositional defiant disorder (ODD) and CD (also see Müller et al., 2011).

A standardized algorithm was applied to combine PACS and CTRS-R:L to derive the 18 symptoms of ADHD (American Psychiatric Association, 2000). Situational pervasiveness of ADHD was defined as at least two symptoms being present in two or more different situations as assessed with PACS interview, as well as the presence of one or more items scored 2 or more from the ADHD scale of the CTRS-R:L. ODD and CD were defined according to DSM-IV criteria (American Psychiatric Association, 2000) based on information from the PACS (algorithm fully described in Rommelse et al., 2007).

Substance use: A full description of follow-up measures can be found elsewhere (Groenman et al., 2013). Briefly, a parental report of SUDs was obtained using the substance use disorder module of the Diagnostic Interview Schedule for Children (DISC-IV-P). Participants above 12 years completed the Alcohol Use Disorders Identification Test (AUDIT), Drug Abuse Screening Test–20 (DAST), and Fagerström Test for nicotine dependence (FTND), about their substance and nicotine use in the past 12 months. Diagnoses of SUDs were present if *either* self- or parent-report measures were positive. We created summary diagnostic groups by aggregating diagnostic information across instruments and informants. For Alcohol Use Disorder (AUD), the AUDIT (self-report) and

alcohol module of the DISC-IV-P (parental-report) were used. For Drug Use Disorder (DUD), DAST (self-report) and the marihuana and other drugs module of the DISC-IV-P (parental-report) were used. AUD and DUD were collapsed into one category to form an overall measure of SUDs and used as dependent measure in the analyses. Daily smoking below the age of 18, as assessed with the FTND (self-report) or the tobacco module of the DISC-IV-P (parental-report), were used to establish nicotine dependence. Furthermore, age of first substance use (DISC-IV-P), age of first nicotine use (FTND), and severity of alcohol use (total score of the AUDIT) served as dependent variables.

Pharmacy and self-report data on stimulant medication use were obtained. These were combined to form two groups, an untreated (n=36) and a pharmacologically treated group (n=212; data on 248 participants).

Genotyping

An overview of genotyping procedures in the IMAGE sample and the Dutch follow-up study NeuroIMAGE can be found elsewhere (von Rhein et al., 2014). For the IMAGE sample, DNA was extracted from blood samples or lymphocyte cell lines at Rutgers University Cell and DNA Repository, New Jersey, USA (Brookes et al., 2006). DNA isolation for additional samples from the NeuroIMAGE study was performed from blood or saliva at the department of Human Genetics of the Radboud university medical center. DNA was isolated from saliva using Oragene containers (DNA Genotek, Ottawa, Ontario, Canada) according to the protocol supplied by the manufacturer, DNA from blood was isolated using standard procedures. Genome-wide genotyping for IMAGE was performed as part of the GAIN study using the Perlegen 600K genotyping platform, which comprises approximately 600,000 tagging Single Nucleotide Polymorphisms (SNPs; (Neale et al., 2008). For NeuroIMAGE samples not genotyped during IMAGE, genotyping was performed on the Human CytoSNP-12 version 2 genotyping BeadChip (Illumina Inc., San Diego, California, USA) at the Lifelines facility (Groningen, The Netherlands). Quality control steps were performed for the genotype data from both platforms separately. SNPs were excluded if the call rate per SNP was less than 95%, the minor allele frequency was less than 1%, or the SNPs failed the Hardy-Weinberg equilibrium test at a threshold of p≤10–6 (genome-wide). Participants were excluded if the call rate per individual was lower than 95%. In order to increase genomic coverage and to harmonize genotyping, imputation was performed separately for both data sets using IMPUTE2 (Howie et al., 2009) and the 1000 Genomes Phase 1. V3 reference data downloaded from http://mathgen.stats.ox.ac.uk/impute/Data_download_1000G_phase1_integrated.html (The 1000 Genomes Project Consortium, 2010). SNPs with low imputation quality (INFO < 0.8) and minor allele frequency < 0.01 were not included in the analysis.

Genotyping of the *DRD4* exon 3, *DAT1/SLC6A3* 3′ untranslated region, and 5-*HTT/SLC6A4 HTTLPR* variable number tandem repeat (VNTR) polymorphisms had been performed previously by the IMAGE consortium (Brookes et al., 2006). Standard PCR protocols were used for all VNTR markers, and amplified products were visualized on 2% agarose under UV light. For the additional NeuroIMAGE samples, VNTRs were genotyped using standard PCR protocols. After the PCR, fragment length analysis was performed on

the ABI Prism 3730 Genetic Analyser (Applied Biosystems, Nieuwekerk a/d Ijssel, The Netherlands), and results were analyzed with GeneMapper® Software, version 4.0 (Applied Biosystems). No deviations from Hardy-Weinberg Equilibrium were found for the VNTR polymorphisms at $p < .05$.

For this study, genetic variants were selected that have been implicated in the dopamine (see Table 1) and serotonin neurotransmission systems (see Table 2) in prior genetic studies of ADHD or SUD/nicotine dependence. To be included, we required that the SNP was in a gene directly associated with the dopamine or serotonin system and had been associated in at least one study with ADHD *and* in at least one study with SUDs and/or nicotine dependence. For example, rs4680 in the Catechol-*O*-methyltransferase (*COMT)* gene was included because it has been associated with SUD/nicotine dependence in multiple studies and was found to be associated with ADHD (and CD) in one study (also see Table S1 and Table S2 for a complete overview of association studies showing all selected polymorphisms).

Genetic Risk Score

Across all SNPs the high-risk genotypes were assigned a score of 1, low risk genotypes a score of 0, and if applicable an intermediate risk genotypes with a score of .5. The literature was checked to assess which allele imparted risk; regression models were built for individual polymorphisms and the two main outcome measures (SUDs and nicotine dependence) to check this direction. For each subject, risk scores were calculated by multiplying the scores by genotype dosages for each SNP included in the analysis. The risk scores for each SNP were added to create a risk score for the dopamine and the serotonin system separately. For example, a subject carrying none of the high-risk dopamine genotypes would have a risk score of 0. In Tables 1 and 2 we describe the selected polymorphisms, and the assigned risk-scores for each genotype.

Data analyses

First, differences in gender, age, ADHD-severity, and ODD/CD comorbidity were compared between those followed-up and those lost to follow-up using t-test and chi-square tests. For each risk score, we excluded subjects with any missing data. This led to 250 subjects with a dopamine risk score, and 278 with a serotonin risk score. All analyses were done using SPSS (IBM SPSS Statistics version 20).

First, regression models (either logistic for binary outcomes [i.e., SUDs, nicotine dependence] or linear for continuous outcomes [i.e., age of substance use, alcohol use severity, and age of first nicotine use]) using generalized estimated equations (GEE; Norton et al., 1996) were built for all outcome measures to account for age, gender, and intra-familial correlation (i.e., multiple siblings within one family). Family number was used as repeated measure, and robust estimators and exchangeable structure were used for working correlation matrices. From these models, residuals were saved and used in further analyses.

Second, we estimated linear regression models predicting the residuals (obtained in the first step of our analyses from the GEE models) from the genetic risk score. Separate models were built for the serotonin and dopamine risk scores. Since genome-wide genotyping was

performed on two different platforms that can not be combined in a mega-analysis, models were run separately for the two different platforms (i.e. Perlegen 600K, CytoSNP-12), and combined using the weighted Stouffer overall Z method (Stouffer et al., 1949). This meta-analytic method takes sample size into account.

To correct for multiple testing, we calculated the effective number of independent tests (Meff) (Li and Ji, 2005). This method uses the correlation matrix of the five outcome measures to calculate the eigenvalues for each of the outcomes assessed using an online-algorithm (http://gump.qimr.edu.au/general/daleN/matSpD/). This led to a Meff of 4, with an associated *p*-value of .013. Because we looked at two genetic risk scores, we divided this value by two, and used $p = .0065$ as significance level for all analyses.

In exploratory analyses, we assessed the interaction between the genetic risk scores for dopamine and serotonin. Also, analyses were re-run excluding participants who took no medication (n=36) to assess whether possible effects were dependent on those.

Results

Dopamine neurotransmission genes

After correction for multiple comparisons, the dopamine risk score did not predict age of first substance use, age of first smoke, or SUDs in participants with ADHD. Nominal significant results were found for the dopamine risk score to predict alcohol use severity ($p = .013$) and nicotine dependence ($p=0.014$) (see upper panel Table 4).

Serotonin neurotransmission genes

After correction for multiple comparisons the serotonin risk score significantly predicted alcohol use severity ($p < .001$) in participants with ADHD. Nominal significant results were found for the serotonergic risk score to predict SUDs. The serotonin risk score did not predict age of first substance use, age of first smoke, or nicotine dependence (see lower panel Table 4).

Exploratory analyses

No significant interactions were found between the dopamine and serotonin genetic risk scores (Table 6). Results hardly changed when analyses were re-run in the stimulant treatment group only (Table 5).

Discussion

In this study we tested the hypothesis that variation in dopamine or serotonin neurotransmission genes is involved in the genetic liability for the development of SUDs and nicotine dependence in adolescents or young adults with ADHD. Instead of studying individual genetic variants, we employed genetic risk scores to capture more of the genetic variance. Although we hypothesized that both dopaminergic and serotonergic genetic risk scores would predict SUDs/nicotine dependence-related outcomes, only the serotonin genetic risk score significantly predicted alcohol use severity. The serotonin risk score produced marginally significant results for SUDs. The dopamine genetic risk score yielded

only marginally significant results in predicting nicotine dependence and alcohol use severity. We found no significant interactions between the dopaminergic and serotonergic risk scores.

In adolescents with ADHD, the serotonin genetic risk score was a significant predictor of alcohol use severity (5.0% of variance explained). Our findings add to the literature implicating serotonin in the development of SUDs (Johnson, 2004), by demonstrating the role of serotonin in early stages of the development of SUDs in ADHD (i.e. alcohol use severity in adolescence). Previous studies have shown a high heritability for age of first nicotine and substance use (Vink et al., 2006). However, neither the dopamine nor the serotonin genetic risk score predicted age of first nicotine use, indicating that other genetic variants may underlie this trait.

Serotonin and dopamine play a role in impulsivity (Dalley and Roiser, 2012), and impulsivity is a diagnostic feature of ADHD, that has also been crucially related to SUDs/nicotine dependence (Moeller et al., 2001). However, impulsivity is not a unitary concept. One can distinguish multiple aspects of impulsivity, such as impulsive choices (e.g. motor inhibition) and impulsive actions (e.g. delay aversion). It has been shown that dopamine and serotonin are differentially associated with these aspects of impulsivity (Dalley and Roiser, 2012). Serotonergic neurotransmission appears to be more related to impulsive action than impulsive choice (Bari and Robbins, 2013). Animal studies have shown that serotonin depletion does not affect stop signal reaction time (SSRT) measuring impulsive choice, while it did affect impulsive action. Although serotonin depletion did not affect SSRT, treatment with d-amphetamines did (Eagle et al., 2009), suggesting that the dopaminergic system is involved in impulsive choice. In previous work, we found that SSRT could not differentiate between ADHD with and without SUDs (Groenman et al., 2014). Although subjects with ADHD show performance on tasks of impulsive choice, and impulsive action (Pauli-Pott et al., 2013), only serotonin-related impulsivity (i.e. impulsive action) seems to have a mediating role in the development of SUDs in ADHD.

Dopamine is implicated in the pathogenesis of SUDs and nicotine dependence (Bobzean et al., 2014; Volkow et al., 2009), and compelling evidence points to dopaminergic reward deficits in ADHD (Cortese et al., 2012). However, we did not find that dopamine genes could differentiate between those subjects with ADHD only and ADHD and SUDs/nicotine dependence. There are indications that increased risk for substance and nicotine use, found for certain dopaminergic polymorphisms, is under the influence of environmental factors. For example, carriers of the *DRD4* 7-repeat allele were found to be more frequent cannabis users, but only under the influence of low parental monitoring (Otten et al., 2012). Similarly, the *COMT Val158Met* genotype interacted with childhood trauma in predicting alcohol dependence (Schellekens et al., 2013) and alcohol use was higher in carriers of the *DRD2* A1-allele when parental rule setting was low (van der Zwaluw et al., 2010). Also, life-course effects have been reported for dopaminergic genes on alcohol consumption, showing only association with alcohol consumption in young adulthood, but not in adolescence (Guo et al., 2007). Longitudinal follow-up studies are necessary to examine the effect of dopaminergic neurotransmission genes on SUDs/nicotine dependence throughout the lifespan.

The current study focused on dopaminergic and serotonergic genes, but there are other candidate genes and neurotransmitter systems, which could mediate the shared risk between ADHD and SUDs/nicotine-related outcomes. For example, the *CDH13* gene encodes cadherin-13, a member of a family of cell–cell adhesion proteins, and regulates neural cell growth. Cadherin-13 expression has been detected, among others, in dopamine neurons in ventral tegmental area, substantia nigra, and locus coeruleus, and as such had been implicated in ADHD and comorbid disorders including SUDs (Rivero et al., 2013). Indeed, the *CDH13* gene has been implicated in several GWAS studies of ADHD (Franke et al., 2009), and SUDs (Treutlein et al., 2009; Uhl et al., 2008). Another possible candidate is the *NOS1* gene. *NOS1* is suggested to modulate ventral striatal activity and impulsivity in adults with ADHD (Hoogman et al., 2011). The ventral striatum is an area implicated in the development of SUDs/nicotine dependence (Volkow et al., 2009), with studies showing a relationship between the *NOS1* gene and for example nicotine dependence (Di Matteo et al., 2010). Other neurotransmitter systems could also be important in both ADHD and SUDs/nicotine dependence, for example the norepinephrine system, which has been implicated in ADHD (Scassellati et al., 2012). Atomoxetine, a norepinephrine reuptake inhibitor, is efficacious in reducing symptoms of ADHD (Garnock-Jones and Keating, 2010). The norepinephrine system plays a crucial part in the rewarding effects of amphetamines, cocaine, and alcohol (Sofuoglu and Sewell, 2009; Weinshenker and Schroeder, 2007). Future studies including risk scores from a broader range of genes or biological processes may be able to account for a larger portion of variance in SUD/nicotine dependence.

Our findings should be viewed in light of some strengths and limitations. Our study has shown the benefits of using genetic risk scores. This approach captures more variation than a single SNP analysis. The use of genetic risk scores in predicting certain outcomes requires smaller samples than studies using single SNP analyses, while it still provides information about the possible etiology of a condition. Our sample consisted of participants of European Caucasian descent, limiting the generalizability of the findings to other populations. While our results hardly changed when rerun in the stimulant treated group, we can not make strong inferences about stimulant naïve individuals with ADHD. Participants were relatively young and may not have fully passed the risk period for the onset of SUDs/nicotine dependence. Because of the relatively young age at the time of assessment (mean age: 16.5 years) AUD and DUD were combined into SUD to increase statistical power. This approach prevented conclusions about the effects on AUD and DUD in isolation. Several nominal significant results were found, and although the use of genetic risk scores increases power, it should be kept in mind that power was possibly too low to detect these effects. Possibly, our sample is an imperfect representation of the clinical ADHD population, as those with missing data in their genetic risk score were less likely to smoke. We did not have the opportunity to assess our genetic risk score in control populations, and therefore cannot make inferences about the effect on ADHD-status of the genetic risk scores assessed in the current paper. Possibly, the genes identified by looking at the overlap between ADHD and SUDs/nicotine dependence could prove indicative for SUDs and nicotine dependence in controls.

To summarize, the current study adds to the literature showing that a risk score derived from serotonergic neurotransmission genes significantly predicts alcohol use severity in

adolescents with ADHD. Thereby, our work provides insight into shared genetic underpinnings of these highly comorbid disorders. The focus of the literature on ADHD and SUDs/nicotine dependence comorbidity has been mostly on the dopaminergic system. Our study suggests that the serotonergic system also plays a role in the relationship between these disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by an unrestricted grant from Shire Pharmaceuticals (to Stephen V. Faraone) and by a grant from The Netherlands Organization for Health Research and Development (ZonMw) (60-60600-97-193 to Jan Buitelaar)

References

American Psychiatric Association. Diagnostic and statistical manual of mental disorders [DSM-IV-TR]. American Psychiatric Association; Washington, DC: 2000.

Bari A, Robbins TW. Inhibition and impulsivity: behavioral and neural basis of response control. Progress in neurobiology. 2013; 108:44–79. [PubMed: 23856628]

Bobzean SA, Denobrega AK, Perrotti LI. Sex differences in the neurobiology of drug addiction. Experimental neurology. 2014

Brookes K, Xu X, Chen W, Zhou K, Neale B, Lowe N, Aneey R, Franke B, Gill M, Ebstein R. The analysis of 51 genes in DSM-IV combined type attention deficit hyperactivity disorder: association signals in DRD4, DAT1 and 16 other genes. Molecular psychiatry. 2006; 11:934–953. [PubMed: 16894395]

Carpentier PJ, Arias Vasquez A, Hoogman M, Onnink M, Kan CC, Kooij JJ, Makkinje R, Iskandar S, Kiemeney LA, de Jong CA, Franke B, Buitelaar JK. Shared and unique genetic contributions to attention deficit/hyperactivity disorder and substance use disorders: a pilot study of six candidate genes. Eur Neuropsychopharmacol. 2013; 23:448–457. [PubMed: 22841130]

Charach A, Yeung E, Climans T, Lillie E. Childhood attention-deficit/hyperactivity disorder and future substance use disorders: comparative meta-analyses. J Am Acad Child Adolesc Psychiatry. 2011; 50:9–21. [PubMed: 21156266]

Cortese S, Kelly C, Chabernaud C, Proal E, Di Martino A, Milham MP, Castellanos FX. Toward systems neuroscience of ADHD: a meta-analysis of 55 fMRI studies. Am J Psychiatry. 2012; 169:1038–1055. [PubMed: 22983386]

Dalley JW, Roiser JP. Dopamine, serotonin and impulsivity. Neuroscience. 2012; 215:42–58. [PubMed: 22542672]

Davis C, Loxton NJ, Levitan RD, Kaplan AS, Carter JC, Kennedy JL. 'Food addiction'and its association with a dopaminergic multilocus genetic profile. Physiology & behavior. 2013; 118:63–69. [PubMed: 23680433]

del Campo N, Chamberlain SR, Sahakian BJ, Robbins TW. The roles of dopamine and noradrenaline in the pathophysiology and treatment of attention-deficit/hyperactivity disorder. Biological psychiatry. 2011; 69:e145–e157. [PubMed: 21550021]

Di Matteo V, Pierucci M, Benigno A, Esposito E, Crescimanno G, Di Giovanni G. Critical role of nitric oxide on nicotine-induced hyperactivation of dopaminergic nigrostriatal system: Electrophysiological and neurochemical evidence in rats. CNS neuroscience & therapeutics. 2010; 16:127–136. [PubMed: 20345972]

Eagle DM, Lehmann O, Theobald DE, Pena Y, Zakaria R, Ghosh R, Dalley JW, Robbins TW. Serotonin depletion impairs waiting but not stop-signal reaction time in rats: implications for

theories of the role of 5-HT in behavioral inhibition. Neuropsychopharmacology. 2009; 34:1311–1321. [PubMed: 19005464]

Franke B, Neale BM, Faraone SV. Genome-wide association studies in ADHD. Hum Genet. 2009; 126:13–50. [PubMed: 19384554]

Garnock-Jones KP, Keating GM. Spotlight on atomoxetine in attention-deficit hyperactivity disorder in children and adolescents. CNS drugs. 2010; 24:85–88. [PubMed: 20030421]

Groenman AP, Oosterlaan J, Greven CU, Vuijk PJ, Rommelse N, Franke B, Hartman CA, Hoekstra PJ, Sergeant J, Faraone SV, Buitelaar J. Neurocognitive predictors of substance use disorders and nicotine dependence in ADHD probands, their unaffected siblings, and controls: a 4-year prospective follow-up. J Child Psychol Psychiatry. 2014

Groenman AP, Oosterlaan J, Rommelse N, Franke B, Roeyers H, Oades RD, Sergeant JA, Buitelaar JK, Faraone SV. Substance use disorders in adolescents with attention deficit hyperactivity disorder: a 4-year follow-up study. Addiction. 2013; 108:1503–1511. [PubMed: 23506232]

Guo G, Wilhelmsen K, Hamilton N. Gene-lifecourse interaction for alcohol consumption in adolescence and young adulthood: five monoamine genes. Am J Med Genet B Neuropsychiatr Genet. 2007; 144b:417–423. [PubMed: 17440951]

Hoogman M, Aarts E, Zwiers M, Slaats-Willemse D, Naber M, Onnink M, Cools R, Kan C, Buitelaar J, Franke B. Nitric oxide synthase genotype modulation of impulsivity and ventral striatal activity in adult ADHD patients and healthy comparison subjects. Am J Psychiatry. 2011; 168:1099–1106. [PubMed: 21724667]

Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. PLoS genetics. 2009; 5:e1000529. [PubMed: 19543373]

Johnson BA. Role of the Serotonergic System in the Neurobiology of Alcoholism. CNS drugs. 2004; 18:1105–1118. [PubMed: 15581381]

Kendler KS, Chen X, Dick D, Maes H, Gillespie N, Neale MC, Riley B. Recent advances in the genetic epidemiology and molecular genetics of substance use disorders. Nat Neurosci. 2012; 15:181–189. [PubMed: 22281715]

Kotte A, Faraone SV, Biederman J. Association of genetic risk severity with ADHD clinical characteristics. Am J Med Genet B Neuropsychiatr Genet. 2013; 162B:718–733. [PubMed: 24132904]

LeMarquand D, Pihl RO, Benkelfat C. Serotonin and alcohol intake, abuse, and dependence: findings of animal studies. Biol Psychiatry. 1994; 36:395–421. [PubMed: 7803601]

Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, McCarthy MI, Ramos EM, Cardon LR, Chakravarti A, Cho JH, Guttmacher AE, Kong A, Kruglyak L, Mardis E, Rotimi CN, Slatkin M, Valle D, Whittemore AS, Boehnke M, Clark AG, Eichler EE, Gibson G, Haines JL, Mackay TF, McCarroll SA, Visscher PM. Finding the missing heritability of complex diseases. Nature. 2009; 461:747–753. [PubMed: 19812666]

Moeller FG, Barratt ES, Dougherty DM, Schmitz JM, Swann AC. Psychiatric aspects of impulsivity. Am J Psychiatry. 2001; 158:1783–1793. [PubMed: 11691682]

Monuteaux MC, Faraone SV, Hammerness P, Wilens TE, Fraire M, Biederman J. The familial association between cigarette smoking and ADHD: a study of clinically referred girls with and without ADHD, and their families. Nicotine & tobacco research: official journal of the Society for Research on Nicotine and Tobacco. 2008; 10:1549–1558. [PubMed: 18946774]

Müller U, Asherson P, Banaschewski T, Buitelaar J, Ebstein R, Eisenberg J, Gill M, Manor I, Miranda A, Oades R. The impact of study design and diagnostic approach in a large multi-centre ADHD study. Part 1: ADHD symptom patterns. BMC Psychiatry. 2011; 11:54. [PubMed: 21473745]

Neale BM, Lasky-Su J, Anney R, Franke B, Zhou K, Maller JB, Vasquez AA, Asherson P, Chen W, Banaschewski T. Genome-wide association scan of attention deficit hyperactivity disorder. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics. 2008; 147:1337–1344.

Nikolas MA, Burt SA. Genetic and environmental influences on ADHD symptom dimensions of inattention and hyperactivity: A meta-analysis. Journal of Abnormal Psychology. 2010; 119:1–17. [PubMed: 20141238]

Oades RD. 5-The Role of Serotonin in Attention-Deficit Hyperactivity Disorder (ADHD). Handbook of Behavioral Neuroscience. 2010; 21:565–584.

Olausson P, Engel JA, Soderpalm B. Involvement of serotonin in nicotine dependence: processes relevant to positive and negative regulation of drug intake. Pharmacol Biochem Behav. 2002; 71:757–771. [PubMed: 11888567]

Otten R, Barker ED, Huizink AC, Engels RCME. The interplay between parental monitoring and the dopamine D4 receptor gene in adolescent cannabis use. PloS one. 2012; 7:e49432. [PubMed: 23209577]

Pauli-Pott U, Dalir S, Mingebach T, Roller A, Becker K. Do different ADHD-related etiological risks involve specific neuropsychological pathways? An analysis of mediation processes by inhibitory control and delay aversion. J Child Psychol Psychiatry. 2013; 54:800–809. [PubMed: 23452329]

Plomin R, Haworth CM, Davis OS. Common disorders are quantitative traits. Nature reviews Genetics. 2009; 10:872–878.

Rivero O, Sich S, Popp S, Schmitt A, Franke B, Lesch KP. Impact of the ADHD-susceptibility gene CDH13 on development and function of brain networks. Eur Neuropsychopharmacol. 2013; 23:492–507. [PubMed: 22795700]

Rommelse NNJ, Oosterlaan J, Buitelaar J, Faraone SV, Sergeant JA. Time reproduction in children with ADHD and their nonaffected siblings. J Am Acad Child Adolesc Psychiatry. 2007; 46:582–590. [PubMed: 17450049]

Scassellati C, Bonvicini C, Faraone SV, Gennarelli M. Biomarkers and Attention-Deficit/Hyperactivity Disorder: A Systematic Review and Meta-Analyses. Journal of the American Academy of Child & Adolescent Psychiatry. 2012; 51:1003–1019.e1020. [PubMed: 23021477]

Schellekens AF, Franke B, Ellenbroek B, Cools A, de Jong CA, Buitelaar JK, Verkes RJ. COMT Val158Met modulates the effect of childhood adverse experiences on the risk of alcohol dependence. Addiction biology. 2013; 18:344–356. [PubMed: 22509987]

Sofuoglu M, Sewell RA. Norepinephrine and stimulant addiction. Addiction biology. 2009; 14:119–129. [PubMed: 18811678]

Treutlein J, Cichon S, Ridinger M, Wodarz N, Soyka M, Zill P, Maier W, Moessner R, Gaebel W, Dahmen N, Fehr C, Scherbaum N, Steffens M, Ludwig KU, Frank J, Wichmann HE, Schreiber S, Dragano N, Sommer WH, Leonardi-Essmann F, Lourdusamy A, Gebicke-Haerter P, Wienker TF, Sullivan PF, Nothen MM, Kiefer F, Spanagel R, Mann K, Rietschel M. Genome-wide association study of alcohol dependence. Arch Gen Psychiatry. 2009; 66:773–784. [PubMed: 19581569]

Uhl GR, Drgon T, Liu QR, Johnson C, Walther D, Komiyama T, Harano M, Sekine Y, Inada T, Ozaki N, Iyo M, Iwata N, Yamada M, Sora I, Chen CK, Liu HC, Ujike H, Lin SK. Genome-wide association for methamphetamine dependence: convergent results from 2 samples. Arch Gen Psychiatry. 2008; 65:345–355. [PubMed: 18316681]

van de Glind G, Konstenius M, Koeter MW, van Emmerik-van Oortmerssen K, Carpentier PJ, Kaye S, Degenhardt L, Skutle A, Franck J, Bu ET, Moggi F, Dom G, Verspreet S, Demetrovics Z, Kapitany-Foveny M, Fatseas M, Auriacombe M, Schillinger A, Moller M, Johnson B, Faraone SV, Ramos-Quiroga JA, Casas M, Allsop S, Carruthers S, Schoevers RA, Wallhed S, Barta C, Alleman P, Levin FR, van den Brink W. Variability in the prevalence of adult ADHD in treatment seeking substance use disorder patients: results from an international multi-center study exploring DSM-IV and DSM-5 criteria. Drug and alcohol dependence. 2014; 134:158–166. [PubMed: 24156882]

van der Zwaluw CS, Engels RC, Vermulst AA, Franke B, Buitelaar J, Verkes RJ, Scholte RH. Interaction between dopamine D2 receptor genotype and parental rule-setting in adolescent alcohol use: evidence for a gene-parenting interaction. Mol Psychiatry. 2010; 15:727–735. [PubMed: 19238152]

Verbeeck W, Tuinier S, Bekkering GE. Antidepressants in the treatment of adult attention-deficit hyperactivity disorder: a systematic review. Adv Ther. 2009; 26:170–184. [PubMed: 19238340]

Vink JM, Posthuma D, Neale MC, Slagboom PE, Boomsma DI. Genome-wide linkage scan to identify loci for age at first cigarette in Dutch sibling pairs. Behavior genetics. 2006; 36:100–111. [PubMed: 16374522]

Volkow ND, Fowler JS, Wang GJ, Baler R, Telang F. Imaging dopamine's role in drug abuse and addiction. Neuropharmacology. 2009; 56(Suppl 1):3–8. [PubMed: 18617195]

von Rhein D, Mennes M, van Ewijk H, Groenman AP, Zwiers MP, Oosterlaan J, Heslenfeld D, Franke B, Hoekstra PJ, Faraone SV, Hartman C, Buitelaar J. The NeuroIMAGE study: a prospective phenotypic, cognitive, genetic and MRI study in children with attention-deficit/hyperactivity disorder. Design and descriptives. Eur Child Adolesc Psychiatry. 2014

Weinshenker D, Schroeder JP. There and back again: a tale of norepinephrine and drug addiction. Neuropsychopharmacology. 2007; 32:1433–1451. [PubMed: 17164822]

Table 1

Genetic risk score for dopamine-based neurotransmission

Gene	Polymorphism	Genotype	Risk-score
DRD2/ANKK1	rs1800497	AA	1
		AG	.5
		GG	0
DRD2	rs1076560	A-allele carriers	1
		GG	0
DRD2	rs4648317	A-allele carriers	1
		GG	*0*
DRD3	rs6280	CC	1
		T-allele carriers	0
DRD4	VNTR	7-repeat carriers	1
		Not 7-repeat carriers	0
COMT	rs4680	GG	1
		A-allele carriers	0
DAT1/SLC6A3	VNTR	10/10	1
		9-repeat carriers	0
DBH	rs2519152	C-allele carriers	1
		TT	0
DBH	rs1611115	C-allele carriers	1
		TT	0
DBH	rs1108580	GG	1
		A-allele carriers	0

Note. 1 indicates high risk, .5 intermediate risk, 0 low risk.

Table 2

Genetic risk score for serotonin-based neurotransmission

Gene	Polymorphism	Genotype	Risk-score
HTR1A	rs7445832	T-allele carriers	1
		AA	0
HTR1B	rs11568817	AA	1
		CC	0
HTR1B	rs6296	G-allele carriers	1
		CC	0
HTR1B	rs130058	T-allele carriers	1
		AA	0
HTR1B	rs13212041	G-allele carriers	1
		AA	0
HTR2A	rs6313	GG	1
		A-allele carriers	0
HTR2A	rs6311	CC	1
		T-allele carriers	0
HTR3B	rs3758987	T-allele carriers	1
		CC	0
TPH1	rs1799913	CC	1
		T-allele carriers	0
TPH2	rs1843809		
5-HTT	VNTR	Short/Short	1
		L-allele carriers	0

Note. 1 indicates high risk, .5 intermediate risk, 0 low risk.

Table 3

Subject characteristics

	Dopamine risk score (n=250)	**Serotonin risk score (n=278)**
Age (mean, SD)	16.53 (2.40)	16.57 (2.41)
Gender (n male, %)	202 (80.8)	222 (79.1)
Risk score (mean, SD)	4.06 (2.16)	5.07 (1.36)
CD (n, %)	42(18.3)	49 (19.2)
IQ	100.39 (13.98)	100.04 (13.97)
Chip (n perlegen600, %)	161 (64.4)	186 (66.9)
Stimulant treated [a]	207 (86.3)	211 (85.4)
SUD (n, %)	59 (23.4)	64 (23.0)
- AUD (n, %)	52 (20.8)	57 (20.5)
- DUD (n, %)	23 (9.3)	24 (8.7)
Nicotine dependence (n,%)	65 (26.0)	72 (25.9)

Note. CD = conduct disorder, SUD = substance use disorder, AUD = alcohol use disorder, DUD = drug use disorder.

[a]Data available on 247 subjects

Table 4

Influences of genetic risk scores on substance and nicotine use-related outcomes

Dopamine							
	Chip 1		**Chip 2**		**Combined**		
	n	***p*-value**	**n**	***p*-value**	**n**	***p*-value**	**ES (r)**
Alcohol use severity	156	.156	87	.024	243	.013	.160
Age first substance	98	.125	66	.655	164	.141	.115
Age first smoke	65	.604	49	.454	114	.378	.083
Nicotine dependence	161	.016	89	.374	250	.014	.156
Substance use disorder	161	.489	89	.788	250	.694	.025

Serotonin							
	Chip 1		**Chip 2**		**Combined**		
	n	***p*-value**	**n**	***p*-value**	**n**	***p*-value**	**ES (r)**
Alcohol use severity	178	.002	90	.049	268	<.001	.223
Age first substance use	115	.815	70	..896	185	.789	.020
Age first smoke	77	.129	51	.126	128	.035	.187
Nicotine dependence	186	.046	92	.496	278	.037	.125
Substance use disorder	186	.038	92	.208	278	.016	.145

Note. chip 1 = Perlegen 600K, chip 2= HumanCytoSNP-12

Table 5

Effect of genetic risk score within stimulant-medicated participants

Dopamine							
	Chip 1		**Chip 2**		**Combined**		
	n	***p*-value**	**n**	***p*-value**	**n**	***p*-value**	**ES (r)**
Alcohol use severity	141	.340	61	.114	202	.133	.106
Age first substance	87	.256	49	.776	136	.259	.097
Age first smoke	56	.514	37	.586	96	.400	.086
Nicotine dependence	144	.014	63	.545	207	.013	.173
Substance use disorder	144	.706	63	.731	207	.630	.034

Serotonin							
	Chip 1		**Chip 2**		**Combined**		
	n	***p*-value**	**n**	***p*-value**	**n**	***p*-value**	**ES (r)**
Alcohol use severity	142	.002	64	.122	206	<.001	.228
Age first substance	87	.619	52	.302	139	.339	.092
Age first smoke	59	.208	38	.593	97	.177	.137
Nicotine dependence	145	.161	66	.373	211	.106	.111
Substance use disorder	145	.031	66	.800	211	.039	.142

Note. chip 1 = Perlegen 600K, chip 2= HumanCytoSNP-12

Table 6

Interaction between dopamine and serotonin genetic risk score

	Chip 1		Chip 2		Combined		
	n	*p*-value	n	*p*-value	n	*p*-value	ES (r)
Alcohol use severity	155	.168	86	.331	241	.094	.108
Age first substance	97	.128	66	.274	163	.061	.147
Age first smoke	65	.228	49	.747	114	.247	.108
Nicotine dependence	186	.338	92	.166	248	.132	.096
Substance use disorder	160	.638	88	.387	248	.407	.053

Note. chip 1 = Perlegen 600K, chip 2= HumanCytoSNP-12