Journal of the American Academy of Child and Adolescent Psychiatry

Impact of a common genetic variation associated with putamen volume on neural mechanisms of ADHD

--Manuscript Draft--

Manuscript Number:	JAACAP-D-16-00498R1
Full Title:	Impact of a common genetic variation associated with putamen volume on neural mechanisms of ADHD
Short Title:	effect of rs945270 on putamen and ADHD
Article Type:	Research Article
Keywords:	rs945270, putamen, ADHD, reward anticipation, response control.
Corresponding Author:	Sylvane Desrivières, Ph.D King's College London london, London UNITED KINGDOM
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	King's College London
Corresponding Author's Secondary Institution:	
First Author:	Bing Xu
First Author Secondary Information:	
Order of Authors:	Bing Xu
	Tianye Jia
	Christine Macare
	Tobias Banaschewski
	Arun L.W. Bokde
	Uli Bromberg
	Christian Büchel
	Anna Cattrell
	Patricia J. Conrod
	Herta Flor
	Vincent Frouin
	Jürgen Gallinat
	Hugh Garavan
	Penny Gowland
	Andreas Heinz
	Bernd Ittermann
	Jean-Luc Martinot
	Marie-Laure Paillère Martinot
	Frauke Nees
	Dimitri Papadopoulos Orfanos
	Tomáš Paus

	Luise Poustka
	Michael N. Smolka
	Henrik Walter
	Robert Whelan
	Gunter Schumann
	Sylvane Desrivières
Order of Authors Secondary Information:	
Manuscript Region of Origin:	UNITED KINGDOM
Abstract:	Objective: In a recent genome-wide association study of subcortical brain volumes,1 we have identified common genetic variation at rs945270 as having the strongest effect on putamen volume, a brain measure linked to familial risk for attention-deficit/hyperactivity disorder (ADHD). To determine whether rs945270 is a genetic determinant of ADHD, we now explored it impacts on ADHD-related symptoms and on neural mechanisms of ADHD, such as response inhibition and reward sensitivity. Method: We used a large population sample of 1,834 14-years old adolescents to test the effects of rs945270 on (i) ADHD symptoms accessed through the Strengths and Difficulties Questionnaire (SDQ) and (ii) Region-of-interest (ROI) analyses of putamen activation by functional magnetic resonance imaging (fMRI) using the Stop Signal (SST) and monetary incentive delay (MID) tasks, assessing response inhibition and rewards sensitivity, respectively. Results: We found a significant link between rs945270 and ADHD symptoms scores, the C-allele being associated with lower symptoms scores, most notably hyperactivity. We also observed sex-specific effects of this variant on the brain. In boys, the C-allele associated with lower putamen activity during successful response inhibition, a brain response that was not associated with ADHD symptoms. In girls, putamen activation during reward anticipation increased with the number of C-alleles, most significantly in the right putamen. Remarkably, right putamen activation during reward anticipation tended to negatively correlate with ADHD symptoms. Conclusions: Our results indicate that rs945270 may contribute to the genetic risk of ADHD partly through its effects on hyperactivity and reward processing in girls.

Impact of a Common Genetic Variation Associated With Putamen Volume on Neural Mechanisms of Attention-Deficit/Hyperactivity Disorder

RH: Effect of rs945270 on Putamen and ADHD

Bing Xu, PhD, Tianye Jia, PhD, Christine Macare, PhD, Tobias Banaschewski, MD, PhD, Arun L.W. Bokde, PhD, Uli Bromberg, Dipl-Psych, Christian Büchel, MD, Anna Cattrell, PhD, Patricia J. Conrod, PhD, Herta Flor, PhD, Vincent Frouin, PhD, Jürgen Gallinat, MD, Hugh Garavan, PhD, Penny Gowland, PhD, Andreas Heinz, MD, PhD, Bernd Ittermann, PhD, Jean-Luc Martinot, MD, PhD, Marie-Laure Paillère Martinot, MD, PhD, Frauke Nees, PhD, Dimitri Papadopoulos Orfanos, PhD, Tomáš Paus, MD, PhD, Luise Poustka, PhD, Michael N. Smolka, MD, Henrik Walter, MD, PhD, Robert Whelan, PhD, Gunter Schumann, MD, Sylvane Desrivières, PhD, and the IMAGEN Consortium (https://imagen-europe.com)

Supplemental material cited in this article is available online.

Accepted March 1, 2017

Drs. Xu, Jia, Macare, Cattrell, Schumann, and Desrivières are with Institute of Psychiatry, Psychology and Neuroscience, King's College London, UK. Drs. Banaschewski and Poustka are with Clinical Faculty Mannheim, Central Institute of Mental Health, University of Heidelberg, Germany. Dr. Bokde is with Discipline of Psychiatry, School of Medicine and Trinity College Institute of Neurosciences, Trinity College Dublin, Ireland. Drs. Bromberg and Büchel are with University Medical Centre Hamburg-Eppendorf, Germany. Dr. Conrod is with Université de Montreal, Centre Hospitalier Universitaire Sainte-Justine, Montreal, Canada. Drs. Flor and Nees are with Central Institute of Mental Health, Medical Faculty Mannheim, Germany. Drs. Frouin and Orfanos are with Neurospin, Commissariat à l'Energie Atomique, CEA-Saclay Center, Paris, France. Dr. Gallinat is with University Medical Center Hamburg-Eppendorf (UKE), Hamburg, Germany. Dr. Garavan is with University of Vermont, Burlington, USA. Dr. Gowland is with Sir Peter Mansfield Imaging Centre School of Physics

and Astronomy, University of Nottingham, United Kingdom. Drs. Heinz and Walter are with Campus Charité Mitte, Charité, Universitätsmedizin Berlin, Germany. Dr. Ittermann is with Physikalisch-Technische Bundesanstalt, Braunschweig and Berlin, Germany. Drs. Martinot and Paillère-Martinot are with Institut National de la Santé et de la Recherche Médicale, INSERM Unit 1000 Neuroimaging and Psychiatry, University Paris Sud, University Paris Descartes - Sorbonne Paris Cité; and Maison de Solenn, Paris. Dr. Paillere-Martinot is also with Maison de Solenn, Cochin Hospital, Paris. Dr. Paus is with Rotman Research Institute, Baycrest and University of Toronto, Canada. Dr. Smolka is with Technische Universität Dresden, Germany. Dr. Whelan is with University College Dublin, Ireland.

This work received support from the following sources: the European Union-funded FP6 Integrated Project IMAGEN (Reinforcement-related behaviour in normal brain function and psychopathology) (LSHM-CT- 2007-037286), the FP7 projects IMAGEMEND (602450; IMAging GEnetics for MENtal Disorders), AGGRESSOTYPE (602805) and MATRICS (603016), the Innovative Medicine Initiative Project EU-AIMS (115300-2), the Medical Research Council Grants ‘‘Developmental pathways into adolescent substance abuse’’ (93558) and Consortium on Vulnerability to Externalizing Disorders and Addictions [c-VEDA] (MR/N000390/1), the Swedish funding agencies VR, FORTE and FORMAS, the Medical Research Council and the Wellcome Trust (Behavioural and Clinical Neuroscience Institute, University of Cambridge), the National Institute for Health Research (NIHR) Biomedical Research Centre at South London and Maudsley NHS Foundation Trust and King’s College London, the Bundesministeriumfür Bildung und Forschung (BMBF grants 01GS08152; 01EV0711; eMED SysAlc01ZX1311A; Forschungsnetz AERIAL), the Deutsche Forschungsgemeinschaft (DFG grants SM 80/7-1, SM 80/7-2, SFB 940/1), the National Institutes of Health, USA (Axon, Testosterone and Mental Health during Adolescence; RO1 MH085772-01A1), and by the NIH Consortium grant U54 EB020403,

supported by a cross-NIH alliance that funds Big Data to Knowledge Centres of Excellence.

This study was presented as a poster presentation at the Organization for Human Brain Mapping's 22nd Annual Meeting, Geneva, Switzerland, June 26-30, 2016.

Dr. Jia served as the statistical expert for this research.

The authors wish to thank all members of the IMAGEN Consortium: Tobias Banaschewski, MD, PhD, Heidelberg University; Gareth Barker, PhD, King's College London; Arun L.W. Bokde, PhD, Trinity College Dublin; Uli Bromberg, Dipl-Psych, University Medical Centre Hamburg-Eppendorf; Christian Büchel, MD, University Medical Centre Hamburg-Eppendorf; Erin Burke Quinlan, PhD, King's College London; Sylvane Desrivières, PhD, King's College London; Herta Flor, PhD, Heidelberg University and University of Mannheim; Vincent Frouin, PhD, Commissariat à l'Energie Atomique; Hugh Garavan, PhD, University of Vermont; Penny Gowland, PhD, University of Nottingham; Andreas Heinz, MD, PhD, Charité, Universitätsmedizin Berlin; Bernd Ittermann, PhD, Physikalisch-Technische Bundesanstalt (PTB), Braunschweig and Berlin; Jean-Luc Martinot, MD, PhD, University Paris Sud, University Paris Descartes and Maison de Solenn; Marie-Laure Paillère Martinot, MD, PhD, University Paris Sud, University Paris Descartes and Maison de Solenn; Eric Artiges, MD, PhD, University Paris Sud, University Paris Descartes and Orsay Hospital; Herve Lemaitre, PhD, University Paris Sud and University Paris Descartes; Frauke Nees, PhD, Heidelberg University; Dimitri Papadopoulos Orfanos, PhD, Commissariat à l'Energie Atomique; Tomáš Paus, MD, PhD, University of Toronto; Luise Poustka, MD, Heidelberg University & Medical University of Vienna; Michael N. Smolka, MD, Technische Universität Dresden; Nora C. Vetter, PhD, Technische Universität Dresden; Sarah Jurk, Dipl-Psych, Technische Universität Dresden; Eva Mennigen, MD, Technische Universität Dresden; Henrik Walter, MD, PhD, Charité, Universitätsmedizin Berlin; Robert Whelan, PhD, University College Dublin; Gunter Schumann, MD, King's College London.

Disclosure: Dr. Banaschewski has served as an advisor or consultant to Bristol-Myers Squibb, Desitin Arzneimittel, Eli Lilly and Co., Medice, Novartis, Pfizer, Shire, UCB, and Vifor Pharma. He has received conference attendance support, conference support, or speaking fees from Eli Lilly and Co., Janssen McNeil, Medice, Novartis, Shire, and UCB. He is involved in clinical trials conducted by Eli Lilly and Co., Novartis, and Shire; the present work is unrelated to these relationships. Dr. Gallinat has received research funding from the German Federal Ministry of Education and Research, AstraZeneca, Eli Lilly and Co., Janssen-Cilag, and Bristol-Myers Squibb. He has received speaking fees from AstraZeneca, Janssen-Cilag, and Bristol-Myers Squibb. Dr. Paillère-Martinot has received compensation from Janssen-Cilag for CME activities. Prof. Poustka has received speaking fees from Shire, Eli Lilly and Co., and Medice. Drs. Xu, Jia, Macare, Bokde, Bromberg, Büchel, Cattrell, Conrod, Flor, Frouin, Garavan, Gowland, Heinz, Ittermann, Martinot, Nees, Orfanos, Paus, Smolka, Walter, Whelan, Schumann, and Desrivières report no biomedical financial interests or potential conflicts of interest.

Correspondence to Sylvane Desrivières, PhD, MRC - Social, Genetic and Developmental Psychiatry Centre, Institute of Psychiatry, Psychology and Neuroscience, King's College London, 16 De Crespigny Park, SE5 8AF, London, UK; email: sylvane.desrivieres@kcl.ac.uk

ABSTRACT

Objective: In a recent genome-wide association study of subcortical brain volumes, we identified common genetic variation at rs945270 as having the strongest effect on putamen volume, a brain measure linked to familial risk for attention-deficit/hyperactivity disorder (ADHD). To determine whether rs945270 is a genetic determinant of ADHD, we now explore its impacts on ADHD-related symptoms and neural mechanisms of ADHD, such as response inhibition and reward sensitivity.

Method: We used a large population sample of 1,834 14-year-old adolescents to test the effects of rs945270 on (*i*) ADHD symptoms accessed through the Strengths and Difficulties Questionnaire (SDQ) and (*ii*) region-of-interest (ROI) analyses of putamen activation by functional magnetic resonance imaging (fMRI) using the stop signal (SST) and monetary incentive delay (MID) tasks, assessing response inhibition and reward sensitivity, respectively.

Results: We found a significant link between rs945270 and ADHD symptom scores, with the C-allele associated with lower symptom scores, most notably hyperactivity. We also observed sex-specific effects of this variant on the brain. In boys, the C-allele was associated with lower putamen activity during successful response inhibition, a brain response that was not associated with ADHD symptoms. In girls, putamen activation during reward anticipation increased with the number of C-alleles, most significantly in the right putamen. Remarkably, right putamen activation during reward anticipation tended to negatively correlate with ADHD symptoms.

Conclusion: Our results indicate that rs945270 may contribute to the genetic risk of ADHD partly through its effects on hyperactivity and reward processing in girls.

Key words: rs945270, putamen, ADHD, reward anticipation, response control.

INTRODUCTION

Abnormalities in subcortical brain structures, notably in the putamen,[1,2] have been linked to attention-deficit/hyperactivity disorder (ADHD), a highly heritable and multifactorial neurodevelopmental disorder characterized by symptoms of inattention and hyperactivity/impulsivity.[3] The age-dependent reductions in putamen volume that normally occur during childhood throughout early adulthood are not observed in patients with ADHD and their non-affected siblings,[4,5] suggesting that heritable differences in developmental putamen trajectories contribute to the risk for ADHD. Thus, the examination of individual differences in ADHD-related measures including the putamen in a nonclinical sample of youth may provide valuable insight into the disease.

That abnormalities within the putamen contribute to the pathology of ADHD is further supported by findings showing that lesions of the putamen tend to be associated with ADHD symptomatology.[6,7] Functional neuroimaging approaches have been helpful in identifying the neural mechanisms underlying ADHD symptoms. Task-based functional magnetic resonance imaging (fMRI) studies have shown that ADHD associates with poor response inhibition (i.e., a deficit in the ability to stop an already planned or initiated action), due to abnormalities in frontal-striatal and frontal-parietal networks in adolescents and young adults with ADHD,[8-10] which may underlie some of the deficits in impulse control observed in ADHD.[11] On the other

hand, several fMRI studies have observed decreased activation of the ventral striatum, an area including the ventromedial putamen,[12] during anticipation of reward in both adolescents and adults with ADHD, which led to the hypothesis that ADHD is part of a reward deficiency syndrome reflecting the impact of dysregulation of dopamine on the reward system.[13,14] Despite evidence implicating the putamen in the pathology of ADHD, its distinctive contributions in the neurobiology of ADHD remains understudied, possibly because small subcortical brain regions like the putamen are relatively difficult to observe in whole-brain fMRI.

Progress to identify genetic factors contributing to putamen development was made through our recent genome-wide meta-analysis that identified several single nucleotide polymorphisms (SNPs) associating with subcortical regions, with the strongest effects observed for rs94527 on the volume of the putamen. Genotypes at this SNP appear to have functional consequences, as the C-allele at this locus, which associates with increased putamen volume, also associates with increased expression of the nearby *KTN1* gene in the brain and in blood.[15] This gene is known to play a role in intracellular organelle motility, and it is interesting to note that its expression was found to be decreased in patients with Parkinson's disease[16] and in women with depression undergoing antidepressant treatment.[17] Results of these analyses also indicated that while rs945270 associated with putamen volume across the lifespan, the effects of this SNP on putamen volume tended to decrease with the mean age of each participating cohort.[15] This, and the age-dependent reductions of putamen volume from childhood through early adulthood in controls but not in individuals with ADHD or their unaffected siblings,[5] suggested that

rs945270's effect on the putamen might predispose to ADHD.

To test this, we analyzed the effects of rs945270 on putamen function and its relation to ADHD-related phenotypes in a large healthy cohort of 14-year-old adolescents, using the stop-signal (SST) and the monetary incentive delay (MID) fMRI tasks that assess motor control and reward processing, respectively.

METHOD

Participants

Participants of the study were tested at eight assessment centers (London, Nottingham, Dublin, Mannheim, Berlin, Hamburg, Paris, and Dresden). Local ethics research committees at each site approved the study. On the day of assessment, written consent was obtained from the parent or guardian, and verbal assent was obtained from the adolescent. A detailed description of the recruitment and assessment procedures and inclusion/exclusion criteria have been published elsewhere.[18] After quality control, complete and reliable datasets were available from 1,129 participants during the reward anticipation phase of the monetary incentive delay task (MID), and from 1,227 participants for the successful inhibition in the stop signal task (SST). Demographics information is displayed in Table 1.

ADHD Symptoms (Strength and Difficulties Questionnaire)

ADHD symptoms were assessed using parental reports of the Strength and Difficulties Questionnaire (SDQ), a 25-item behavioral screening tool probing for ADHD-type problems (hyperactivity, inattention, and impulsivity), emotional symptoms, conduct problems, peer problems, and prosocial behavior.[19] For this study, we used the SDQ ADHD symptoms scale,

which provides a sum score (ranging from 0 to 10) for both hyperactivity/impulsivity (with a 3-item subscale, with scores ranging from 0 to 6) and inattention (with a 2-item subscale, scores ranging from 0 to 4). The Development and Well-Being Assessment (DAWBA)[20] was also used to administer a structured interview to parents and children, which was used by experienced clinical raters to assign *DSM-IV* diagnoses blind to the SDQ scores.

Genotyping and Quality Control

DNA purification and genotyping was performed by the Centre National de Génotypage in Paris. DNA was extracted from whole blood samples preserved in ethylene-diamine-tetra-acetic acid (EDTA) vacutainer tubes (BD, Becton, Dickinson and Company, Oxford, UK) using the Gentra Puregene Blood Kit (QIAGEN, Valencia, CA) according to the manufacturer's instructions. Genotype information was collected at 582,982 markers using the Illumina HumanHap610 Genotyping BeadChip (Illumina, San Diego, CA).[18] A total of 705 and 1,382 individuals were genotyped with the Illumina (Little Chesterford, UK) Human610-Quad Beadchip and Illumina Human660-Quad Beadchip, respectively. SNPs with call rates <98%, minor allele frequency <1%, or deviation from the Hardy–Weinberg equilibrium ($P < 1 \times 10^{-4}$) were excluded from analyses. Individuals with an ambiguous sex code, excessive missing genotypes (failure rate >2%), and outlying heterozygosity (heterozygosity rate of 3 SDs from the mean) were also excluded. Identity by state similarity was used to estimate cryptic relatedness for each pair of individuals using the PLINK software (pngu.mgh.harvard.edu/~purcell/plink/). Closely related individuals with identity by descent >0.1875 were eliminated from the subsequent analysis. Population

stratification for the genome-wide association study (GWAS) was examined using multidimensional scaling (MDS) analysis with HapMap populations as reference groups. Individuals with divergent ancestry (from Utah residents with ancestry from northern and western Europe) were excluded through visual inspection of the first two components. Figure S1 (available online) shows that our sample is genetically homogeneous (overlapping with the Utah residents with northern and western ancestry [CEU] population). The imputation protocol used MaCH software for haplotype phasing and minimac for imputation.[21]

MRI Data Acquisition and Preprocessing

MRI data were acquired with 3T MRI scanners of different manufacturers (Siemens, Munich, Germany; Philips, Best, The Netherlands; General Electric, Chalfont St Giles, UK; Bruker, Ettlingen, Germany) using the same scanning protocol at all sites (see supplemental material, available online). High-resolution T1-weighted 3D structural images were acquired for anatomical localization and coregistration with the functional time series. Forty slices, tilted to the anterior-posterior commissure line, were acquired in descending order (2.4 mm thickness, 1 mm gap) using a gradient-echo T2*-weighted echo-planar imaging (EPI) sequence, and the following parameters: TR = 2200 ms; TE = 30 ms; matrix size 64 × 64 over a 21.8-cm field of view, giving an in-plane resolution voxel size of 3.4 × 3.4 mm. For anatomical reference, a 3D gradient echo-based sequence of the whole brain was obtained, using protocol based on the Alzheimer's Disease Neuroimaging Initiative (ADNI) protocol (http://www.loni.ucla.edu/ADNI/Cores/index.shtml).

fMRI data were analyzed with SPM8 (Statistical Parametric Mapping,

http://www.fil.ion.ucl.ac.uk/spm). Spatial preprocessing included: slice time correction to adjust for time differences due to multi-slice imaging acquisition, realignment to the first volume in line, non-linearly warping to the Montreal Neurological Institute (MNI) space (based on a custom EPI template [53x63x46 voxels] created out of an average of the mean images of 200 adolescents), resampling at a resolution of 3x3x3mm^3 and smoothing with an isotropic Gaussian kernel of 5 mm full width at half maximum. At the first level of analysis, changes in the blood oxygen level-dependent (BOLD) response for each participant were assessed by linear combinations at the individual participant level; for each experimental condition, each trial (e.g., anticipation of high gain in the MID task) was convolved with the hemodynamic response function to form regressors that account for potential noise variance, e.g. head movement. Estimated movement parameters were added to the design matrix in the form of 18 additional columns (3 translations, 3 rotations, 3 quadratic and 3 cubic translations, and each 3 translations with a shift of ±1 TR).

We extracted the putamen ROI using the Marsbar toolbox (http://marsbar.sourceforge.net), based on the MNI Automated Anatomical Labeling. Averaged beta values based on all voxels in the left and right putamen were used for ROI analyses. The current study used the contrast successful stop vs. successful go trials to access brain activity associated with inhibition of a motor response in the SST. For the MID task, we used the contrast anticipation of high win vs. anticipation of no win based on prior research suggesting reliable associations between ADHD symptoms and fMRI BOLD responses measured during reward anticipation.[22]

Monetary Incentive Delay Task

To examine neural responses to reward anticipation and outcome, participants performed an age-adapted version of the monetary incentive delay (MID) task[23] in which sweets instead of money were used as reward. It is a reaction time task that assesses how quickly the participant presses a button with left or right index finger to hit a target (white square) that only appears for a short time on the left or right side of the screen.

Stop Signal Task

The stop signal task (SST) was used to study neural responses to successful and unsuccessful inhibitory control.[24] Participants were instructed to make a single, button-press response to either of two regularly presented, visual-go stimuli (arrows pointing left or right shown centrally for 1000 ms), unless followed by an unpredictable stop signal (arrow pointing upwards shown for 100–300 ms). Twenty percent of the go stimuli were followed by a stop signal. The dependent variable of the task is the stop signal reaction time (SSRT), which was calculated by subtracting the mean stop signal delay (the average time between go and stop signal, where the participant managed to inhibit 50% of trials) from the mean reaction time to go trials.[25] Because of problems in the tracking algorithm, SSRT data was only available for half of participants (see supplemental material, available online). Participants had first completed a practice session outside the scanner (for ~5 minutes).

Statistical Analyses

The general linear model was used to calculate associations between ADHD symptom scores, BOLD responses, behavioral measures and genotype, controlling for covariates of sex (depending on the analyses), scanning sites (7 dummy variables), and handedness. All analyses

were two-sided. Since those hypotheses for brain activity involved the two hemispheres and two contrasts, the significance threshold was set to 0.0125 using Bonferroni correction.

To investigate the stability of our main findings, we have carried out two internal replications. First, we tested the same association in eight research sites separately. The regression coefficients (β) and their corresponding standard errors (SEs) were then used to conduct a meta-analysis. The R package rmeta (https://cran.r-project.org/web/packages/rmeta/ rmeta.pdf) was then used to generate the forest plot. Second, a resampling of the original data with replacement (a bootstrapping process) was conducted to generate a new sample with the same sample size as the original data. The process was replicated 10,000 times, and all of the t statistics were used to calculate a bootstrapping p-value. By these two replications, we demonstrated both the stability of the results and the fact that they are unlikely to reflect sample-specific variance.

RESULTS

Relationship of rs945270 With ADHD Symptoms

In our population cohort of 14-year-old adolescents, each copy of the C allele was associated with a 77.4 mm^3 increase in the averaged volume of the bilateral putamen (β = 77.4, 1.1% variance explained, $p = 1.3 \times 10^{-5}$). While we noted a sex difference for putamen volume (smaller volume in girls than in boys: β= -554.5, $p < 10^{-10}$), there was no interaction between sex and genotype at rs945270 on putamen volume (β = -16.1, p = .65). Investigating the relationship between rs945270 and ADHD symptoms, we found that rs945270 significantly associated with ADHD symptoms in the full sample. The C-allele at rs945270 associated with

lower scores for ADHD symptoms compared to the G-allele, such that each C-allele is expected to reduce symptoms scores by 0.067 SD (β = -0.21, β_{stand} = 0.067, p = .0053; bootstrapping: p = .0070; meta-analysis: β = -0.21, p = .0063; Figure 1 and Table 2). Testing for the specificity of rs945270 on ADHD symptoms, we found that variation at rs945270 was mostly associated with hyperactivity symptoms (β = -0.126, β_{stand} = -0.065, p = .0054 and β = -0.093, β_{stand} = -0.051, p = .028 for the hyperactivity and inattention subscales, respectively). Testing for possible sex differences in ADHD symptoms, we found that girls scored lower for ADHD symptoms than boys (β = -0.77, β_{stand} = -0.169, p = 1.12 x 10^{-12}), differing relatively more in inattentive (β = -0.406, β_{stand} = -0.162, p = 6.77 x 10^{-12}) than in hyperactive (β = -0.361, β_{stand} = -0.134, p = 1.37 x 10^{-8}) symptoms. There was no sex-by- rs945270 genotype interaction on ADHD symptoms (β = 0.049, p = .71). ADHD symptom scores were not associated with putamen volume in this sample (β = -0.00012, p = .28); the association between rs945270 genotypes and ADHD symptoms remained significant after controlling for the whole putamen volume.

Testing for the specificity of rs945270 effects, we analyzed its association with the other four symptom domains measured by the SDQ. We found nominal association of rs945270 with the emotional symptoms score (β = -0.15, p = .021), not with the conduct (β = -0.048, p = .37), peer problem (β = -0.074, p = .17), and prosocial (β = 0.075, p = .21) scores.

As about 2.5% of our sample had received a diagnosis of ADHD (11 girls and 35 boys), based on the DAWBA,[20] we tested how well the SDQ symptom scores correlated with the ADHD diagnosis. There was a moderate positive correlation (β = 0.304, p = 4.23 x10^{-40})

between the two variables, confirming the utility of the SDQ as a screening tool for ADHD in the community.[26]

Response Inhibition

We next tested the effects of rs945270 on inhibitory control, assessing BOLD responses in the putamen during successful inhibition of motor response in the SST fMRI task. The C-allele of rs945270 associated with decreased putamen activity (left putamen: β = -0.088, p = .0030; bootstrapping p = .0060; meta-analysis, β = -0.080, p = .0039; right putamen: β = -0.089, p = .0018, bootstrapping p = .0020; meta-analysis, β = -0.094, p = .0016) in the full sample (Table 3). Girls had lower BOLD responses than boys, most significantly in the left putamen (left: β = -0.15, p = .002; right: β = -0.084, p = .035), and we observed a significant sex-by-genotype interaction in the left (β = 0.16, p = .0080; bootstrapping p = .0080; meta-analysis β = 0.17, p = .013), but not in the right putamen (β = 0.087, p = .12) (Table 3). Sex-specific analyses indicated that the associations of rs945270 with putamen activity observed in the full sample were mainly driven by boys; the C-allele was associated with decreased putamen activation in this group (left putamen: β = -0.16, p = .0005; right putamen: β = -0.13, p = .004), but not in girls (left putamen: β = -0.010, p = .78; right putamen: β = -0.047, p = .20) (Figure 2A, B). To test if these effects of rs945270 on putamen activation in boys were localized rather that distributed throughout the whole putamen, we performed post hoc, voxel-wise association within the putamen. These revealed significant clusters of activity only in the right putamen, in caudal regions (Figure S2, Table S1, available online). This suggests that rs945270 influences response inhibition in boys throughout global rather than

localized effects on the left putamen. Although we observed significant negative association between putamen volume and left putamen activation in the full sample (left: β = -0.000093, p = .018, right: β = -0.000063, p = .12), the association between genotype and putamen activation remained significant after controlling for putamen volume. However, while rs945270 variation associated with putamen activation during successful inhibition in boys, we found no significant association between putamen activation and ADHD symptoms, neither in the full sample (Table 3) nor when analyzing boys only (left: β = 0.16, p = .19, right: β = 0.10, p = .43). This indicates differences in putamen activation during successful inhibition are unlikely to account for the association between rs945270 and ADHD symptoms noted above.

To test if these differences in putamen activation were reflected at the behavioral level, we analyzed a subsample of n = 671 individuals with information on duration of the stop process (SSRT), a known proxy for ADHD symptoms that also correlated with ADHD symptom scores in our sample (β = 1.44, p = .014). Putamen activation during successful stopping negatively correlated with SSRT in the full sample (left: β = -12.28, $p = 2.50 \times 10^{-8}$, right: β = -11.83, $p = 1.0 \times 10^{-6}$), so that individuals who stopped their responses more quickly activated this region more strongly. While there were no sex differences for SSRT (β = -1.532, p = .68), an interaction between sex and left putamen activation on SSRT (p = .009) was observed. Left putamen activation during response inhibition negatively correlated with SSRT in boys, not in girls (boys: β = -16.97, , β_{stand} = -0.309, $p = 10^{-10}$; girls: β = -5.52, β_{stand} = -0.112, p = .090).

Reward Anticipation

In the fMRI reward anticipation task, we observed lesser activation in the putamen bilaterally in girls compared to boys (left: β = -0.10, p = 5 x 10^{-6}; right: β = -0.090, p = 3.9 x 10^{-5}). No significant main effect of rs945270 genotype on putamen activation was observed (β = 0.016, p = .66 and β = 0.022, p = .38, for the left and right putamen, respectively). However, there was a significant sex-by-genotype interaction for activation in the right putamen (β = 0.084, p = .0065; bootstrapping p = .011; meta-analysis, β = 0.065, p = .0044; Left putamen: β = 0.054, p = .086; Figure 3A, B). Sex-specific analyses indicated that putamen activation increased with the number of C-alleles at rs945270 in girls, most significantly in the right putamen (left: β = 0.045, p = .035; right: β = 0.066, p = .002). We performed post hoc voxel-wise association analysis within the putamen to test if these effects of rs945270 on right putamen activation in girls were due to localized effects of rs945270 in the putamen. These identified significant clusters in the right anterior ventral putamen associating with rs945270 (Figure S3, Table S2, available online). This indicates that rs945270 influences reward sensitivity in girls through localized effects on the right putamen. Analyses in boys did not reveal genotype differences (left: β= -0.009, p = .71, right: β = -0.019, p = .4). There were significant associations between putamen volume and putamen activation in this task (left: β = 0.000085, p = .000041, right: β = 0.000078, p = .00023), which were driven by girls (left: β = 0.00012, p = .000016, right: β = 0.00011, p = .00007), not by boys. Besides, the associations between genotype and putamen activation remained significant after controlling for putamen volume.

Given the reported decrease in ventral striatal activation during anticipation of reward in

both adolescents and adults with ADHD,[27-30] we investigated this link further in our sample. We found significant negative association between right putamen activation during reward anticipation and ADHD symptom scores in the full sample (left: β = -0.009, p = .089, right: β = -0.012, p = .014). We found a negative trend for association between right putamen activation during reward anticipation and ADHD symptoms in the full sample (left: β = -0.009, p = .089, right: β = -0.012, p = .014), reflecting an association with hyperactivity rather than inattention (Table 3). This suggests that the G-allele at rs945270 may partly contribute to the risk of ADHD through its negative effects on reward processing and positive effect on hyperactivity, particularly in girls.

DISCUSSION

Our study provides the first genetic link between putamen structure and function and risk for ADHD. We demonstrate that the rs945270 variant, identified in a previous genome-wide meta-analysis of putamen volume, associates with ADHD symptom scores at age 14 years, notably with hyperactivity. We also found it may contribute to the genetic risk of ADHD by influencing neural mechanisms in a sex-specific manner. Specifically, the G-allele at this locus may partly contribute to the risk of ADHD in girls through its negative effects on reward processing in the right anterior ventral putamen and positive effect on hyperactivity. We also found that rs945270 genotypes affected putamen activation during successful response inhibition in boys, an effect that did not correlate with ADHD symptoms.

Our findings that a genetic polymorphism influences neural mechanisms related to ADHD (i.e., reward sensitivity) confirms previous evidence suggesting that a common set of genes

may contribute to the distinct symptoms of ADHD.[31] They also considerably extend these conclusions by providing a genetic mechanism that clarifies sexually dimorphic phenomena associated with ADHD.[32] Sexual dimorphisms were evident during both response inhibition and reward anticipation, the putamen being activated to a lesser extent in girls compared to boys. The G-allele at rs945270 not only associated with higher scores of ADHD symptoms; it also accentuated sexually dimorphic responses by increasing putamen activity during response inhibition in boys and decreasing that in girls during reward anticipation. That girl carriers of the risk G-allele at rs945270 exhibit significantly lowered putamen activation compared to carriers of the C-allele when anticipating a reward suggests that this locus influences reward sensitivity in girls. The negative correlation between BOLD responses in the right putamen during reward anticipation and ADHD symptoms is indeed consistent with a blunted reward system,[29] as evidenced by hypo-responsiveness of the ventral striatum (VS) in patients with ADHD.[27, 28, 33, 34]

Our voxel-wise analyses showing specific effects of rs945270 on the anterior and the posterior putamen in the reward- and motor response-related tasks respectively provide further support for earlier reports of putamen segmentation[35-38] indicating gradual functional specialization within the putamen. Rostral subregions are important for goal-direct behaviors, motivation, and emotional processing, and higher-order cognitive aspects of motor function, such as learning new movements, through connections to the anterior cingulate cortex, presupplementary motor area, and prefrontal cortex.[39] In contrast, more caudal regions are implicated in planning and execution of well-learned, skilled movements, through connections

to primary and supplementary motor cortices.[39,40] A major implication of our study is that genes may impact these putamen-related functions differently in men and women.

Our use of parental reports for the SDQ has a number of limitations. The SDQ has demonstrated reasonable efficiency in screening for ADHD in the community,[26] and it is notable that its hyperactivity/inattention scale performs similarly to the "any disorder" scale despite its brevity. However, this screening efficiency depends on the informant, with the combination of parent and teacher reports being a better predictor of ADHD. Thus, our use of single informant limited our ability to detect ADHD symptoms. It has also limited our ability to properly measure the impact of emotional disorders, which are better captured by self-reports. These limitations are further exacerbated by the poor ability of the SDQ to identify other disorders such as specific phobias, panic disorder/agoraphobia, eating disorders, and separation anxiety. Thus, comorbidity and other unmeasured covariates, such as socioeconomic status, parental psychopathology, and life events could have contributed to our findings.

Nonetheless, our findings illustrate the sex-dependant impact of a genetic variation on human brain function and potential implications for ADHD. Further studies of patients and their controls, taking the above limitations into account, are needed to determine if rs945270 can help the detection and classification of ADHD and its subtypes and disease outcome.

References

1. Sobel LJ, Bansal R, Maia TV, et al. Basal ganglia surface morphology and the effects of stimulant medications in youth with attention deficit hyperactivity disorder. *Am J Psychiatry.* 2010;167:977-986.

2. Ellison-Wright I, Ellison-Wright Z, Bullmore E. Structural brain change in attention deficit hyperactivity disorder identified by meta-analysis. *BMC Psychiatry.* 2008;8:51.

3. Thapar A, Cooper M. Attention deficit hyperactivity disorder. *Lancet.* 2016;387:1240-50.

4. Castellanos FX, Giedd JN, Marsh WL, et al. Quantitative brain magnetic resonance imaging in attention-deficit hyperactivity disorder. *Arch Gen Psychiatry.* 1996;53:607-16.

5. Greven CU, Bralten J, Mennes M, et al. Developmentally stable whole-brain volume reductions and developmentally sensitive caudate and putamen volume alterations in those with attention-deficit/hyperactivity disorder and their unaffected siblings. *JAMA Psychiatry.* 2015;72:490-499.

6. Mous SE, Hammerschlag AR, Polderman TJ, et al. A population-based imaging genetics study of inattention/hyperactivity: basal ganglia and genetic pathways. *J Am Acad Child Adolesc Psychiatry.* 2015;54:745-752.

7. Max JE, Fox PT, Lancaster JL, et al. Putamen lesions and the development of attention-deficit/hyperactivity symptomatology. *J Am Acad Child Adolesc Psychiatry.* 2002;41:563-571.

8. van Rooij D, Hoekstra PJ, Mennes M, et al. Distinguishing adolescents with ADHD from their unaffected siblings and healthy comparison subjects by neural activation

patterns during response inhibition. *Am J Psychiatry.* 2015;172:674-683.

9. Rubia K, Smith AB, Brammer MJ, Toone B, Taylor E. Abnormal brain activation during inhibition and error detection in medication-naive adolescents with ADHD. *Am J Psychiatry.* 2005;162:1067-1075.

10. Dickstein SG, Bannon K, Xavier Castellanos F, Milham MP. The neural correlates of attention deficit hyperactivity disorder: An ALE meta- analysis. *J Child Psychol Psychiatry.* 2006;47:1051-1062.

11. Urcelay GP, Dalley JW. Linking ADHD, impulsivity, and drug abuse: a neuropsychological perspective. In Stanford C, Tannock R, eds. *Behavioral Neuroscience of Attention Deficit Hyperactivity Disorder and Its Treatment*. Heidelberg: Springer; 2011:173-197.

12. Heimer L, De Olmos J, Alheid G, et al. Chapter II The human basal forebrain. Part II. *The primate nervous system. Part III. Handbook of chemical neuroanatomy.* 1999;15:57-226.

13. Blum K, Chen A, Braverman ER, et al. Attention-deficit-hyperactivity disorder and reward deficiency syndrome. *Neuropsychiatr Dis Treat.* 2008;4:893-918.

14. Castellanos FX, Tannock R. Neuroscience of attention-deficit/hyperactivity disorder: the search for endophenotypes. *Nat Rev Neurosci.* 2002;3:617-628.

15. Hibar DP, Stein JL, Renteria ME, et al. Common genetic variants influence human subcortical brain structures. *Nature*. 2015;520:224-229.

16. van Dijk KD, Berendse HW, Drukarch B, et al. The proteome of the locus ceruleus in

Parkinson's disease: relevance to pathogenesis. *Brain pathology.* 2012;22:485-498.

17. Olivier JD, Akerud H, Skalkidou A, Kaihola H, Sundstrom-Poromaa I. The effects of antenatal depression and antidepressant treatment on placental gene expression. *Frontiers in cellular neuroscience.* 2014;8:465.

18. Schumann G, Loth E, Banaschewski T, et al. The IMAGEN study: reinforcement -related behaviour in normal brain function and psychopathology. *Mol Psychiatry.* 2010;15:1128-39.

19. Goodman R. The Strengths and Difficulties Questionnaire: a research note. *J Child Psychol Psychiatry.* 1997;38:581-586.

20. Goodman R, Ford T, Richards H, Gatward R, Meltzer H. The Development and Well-Being Assessment: description and initial validation of an integrated assessment of child and adolescent psychopathology. *J Child Psychol Psychiatry.* 2000;41:645-55.

21. Howie B, Fuchsberger C, Stephens M, Marchini J, Abecasis GR. Fast and accurate genotype imputation in genome-wide association studies through pre-phasing. *Nature genetics.* 2012;44:955-959.

22. Nymberg C, Jia T, Lubbe S, et al. Neural mechanisms of attention-deficit/hyperactivity disorder symptoms are stratified by MAOA genotype. *Biol Psychiatry.* 2013;74:607-14.

23. Knutson B, Westdorp A, Kaiser E, Hommer D. FMRI visualization of brain activity during a monetary incentive delay task. *NeuroImage.* 2000;12:20-27.

24. Rubia K, Smith AB, Taylor E, Brammer M. Linear age-correlated functional development of right inferior fronto-striato-cerebellar networks during response inhibition

and anterior cingulate during error-related processes. *Hum brain map.* 2007;28:1163-77.

25. Logan GD, Schachar RJ, Tannock R. Impulsivity and inhibitory control. *Psychol Sci.* 1997;8:60-4.

26. Goodman R, Ford T, Simmons H, Gatward R, Meltzer H. Using the Strengths and Difficulties Questionnaire (SDQ) to screen for child psychiatric disorders in a community sample. *Br J Psychiatry.* 2000;177:534-539.

27. Boecker R, Holz NE, Buchmann AF, et al. Impact of early life adversity on reward processing in young adults: EEG-fMRI results from a prospective study over 25 years. *PloS one.* 2014;9:e104185.

28. Scheres A, Milham MP, Knutson B, Castellanos FX. Ventral striatal hyporesponsiveness during reward anticipation in attention-deficit/hyperactivity disorder. *Biol Psychiatry.* 2007;61:720-724.

29. Strohle A, Stoy M, Wrase J, et al. Reward anticipation and outcomes in adult males with attention-deficit/hyperactivity disorder. *NeuroImage.* 2008;39:966-972.

30. Plichta MM, Vasic N, Wolf RC, et al. Neural hyporesponsiveness and hyperresponsiveness during immediate and delayed reward processing in adult attention-deficit/hyperactivity disorder. *Biol Psychiatry.* 2009;65:7-14.

31. Greven CU, Rijsdijk FV, Plomin R. A twin study of ADHD symptoms in early adolescence: hyperactivity-impulsivity and inattentiveness show substantial genetic overlap but also genetic specificity. *J Abnorm Child Psychol.* 2011;39:265-275.

32. Trent S, Davies W. The influence of sex-linked genetic mechanisms on attention and

impulsivity. *Biol Psychol.* 2012;89:1-13.

33. Nixon DC, Prust MJ, Sambataro F, et al. Interactive effects of DAOA (G72) and catechol-O-methyltransferase on neurophysiology in prefrontal cortex. *Biol Psychiatry.* 2011;69:1006-1008.

34. Hoogman M, Aarts E, Zwiers M, et al. 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.

35. Postuma RB, Dagher A. Basal ganglia functional connectivity based on a meta-analysis of 126 positron emission tomography and functional magnetic resonance imaging publications. *Cereb Cortex.* 2006;16:1508-1521.

36. Seger CA, Spiering BJ. A critical review of habit learning and the Basal Ganglia. *Frontiers in systems neuroscience.* 2011;5:66.

37. Di Martino A, Scheres A, Margulies DS, et al. Functional connectivity of human striatum: a resting state FMRI study. *Cereb Cortex.* 2008;18:2735-2747.

38. Draganski B, Kherif F, Klöppel S, et al. Evidence for segregated and integrative connectivity patterns in the human basal ganglia. *J Neurosci.* 2008;28:7143-7152.

39. Helmich RC, Derikx LC, Bakker M, Scheeringa R, Bloem BR, Toni I. Spatial remapping of cortico-striatal connectivity in Parkinson's disease. *Cereb Cortex.* 2010;20:1175-86.

40. Tricomi E, Balleine BW, O'Doherty JP. A specific role for posterior dorsolateral striatum in human habit learning. *Eur J Neurosci.* 2009;29:2225-32.

Table 1: Demographics Split by Tasks (Monetary Incentive Delay and Stop Signal) and Sex

	Reward Anticipation		**Successful Inhibition**	
	Boys (n = 543)	**Girls (n = 586)**	**Boys (n = 577)**	**Girls (n = 650)**
Age (in years)	14.4 ± 0.4	14.4 ± 0.4	14.4 ± 0.4	14.4 ± 0.4
Handedness	84 L/A 459 R	62 L/A 524 R	78 L/A 499 R	62 L/A 588 R
rs945270 genotypes	GG: 103 GC: 229 CC: 211	GG: 97 GC: 302 CC: 187	GG: 107 GC: 267 CC: 203	GG: 106 GC: 336 CC: 208
ADHD symptoms scores	3.2 ± 2.2	2.4 ± 2.1	3.1 ± 2.3	2.4 ± 2.1

Note: Means, standard deviations are presented (Mean ± SD). ADHD = attention-deficit/hyperactivity disorder; L/A = left-handed or ambidextrous; R = right-handed.

Table 2: Associations of rs945270 and Sex With Attention-Deficit/Hyperactivity Disorder (ADHD) Symptoms

	rs945270 (effect C-allele)	Sex	Sex * rs945270
ADHD symptoms	β = -0.21, ***p* = .005**	β = -0.77, ***p* = 1.12 x 10^{-12}**	β = 0.049, *p* = .71
Hyperactivity	*β = -0.126* ***p* = .005**	β = -0.361 ***p* = 1.37 x 10^{-8}**	na
Inattention	*β = -0.093* *p = .028**	β = -0.406 ***p* = 6.77 x 10^{-12}**	na

Note: Associations significant affect Bonferroni correction for multiple testing are in bold. Na = not assessed.

* nominally significant association

Table 3: Associations of rs945270, Sex on Attention-Deficit/Hyperactivity Disorder (ADHD) Symptoms on Putamen Activation Split by Tasks (Monetary Incentive Delay [MID] and Stop Signal [SST])

	SST		MID	
	Left putamen	Right putamen	Left putamen	Right putamen
rs945270 (effect C-allele)	β = -0.088, ***p* = .0030**	β = -0.089 ***p* = .0018**	β = 0.016, *p* = .66	β = 0.022, *p* = .38
Sex	β = -0.15, ***p* = .002**	β = -0.084, *p* = .035	β = -0.10, ***p* = 5 x 10^{-6}**	β = -0.090, ***p* = 3.9 x 10^{-5}**
Sex * rs945270	β = 0.16, ***p* = .0080**	β = 0.087, *p* = .12	β = 0.054, *p* = .086	β = 0.084, ***p* = .0065**
ADHD symptoms	β = 0.002 *p* = .80	β =0.003 *p* = .76	β = -0.009, *p* = .089	β = -0.012, *p* = .014*
Hyperactivity	β =0.010 *p* = .52	β =0.008 *p* = .60	β = -0.015, *p* = .072	β = -0.021, *p* = .013*
Inattention	β =-0.004 *p* = .83	β =-6.4*10^{-5} *p* = .99	β = -0.011, *p* = .25	β = -0.016, *p* = .076
Sex * ADHD	β = -0.036, *p* = .06	β = -0.02, *p* = .27	β = 0.008, *p* = .45	β = -0.006, *p* = .55
Sex * Hyperactivity	β =-0.033, *p* = .30	β = -0.014, *p* = .64	β =0.006, *p* = .73	β =0.008, *p* = .64
Sex * Inattention	β = -0.075, *p* = .028*	β = -0.47, *p* = .15	β = 0.018, *p* = .34	β = -0.01, *p* = .59

Note: Associations significant affect Bonferroni correction for multiple testing are in bold.
* nominally significant associations.

Figures

Figure 1: A) Relationship between attention-deficit/hyperactivity disorder (ADHD) symptom scores and rs945270 in 14-year-old adolescents (error bars denote standard error of the mean [SEM]). B) Forest plots of associations between ADHD symptom scores and rs945270 in the 8 research sites and meta-analysis. Note: The β values obtained at each research site are shown as black squares, and the ranges between upper and lower CI95s are shown as the black lines. The integrated β as well as its upper and lower CI95s were calculated from the meta-analysis by using the inversion of squared standard errors (SEs) as weight, and the result is illustrated as the black diamond, where the mean is indicated vertically and the range between upper and lower CI95s is indicated horizontally.

Figure 2: Relationship between rs945270 genotypes and blood oxygen level-dependent (BOLD) responses within the left (A) and right (B) putamen during successful inhibition of motor response in the stop signal (SST) functional magnetic resonance imaging (fMRI) task (blue and red represent boys and girls, respectively; error bars denote standard error of the mean [SEM]).

Figure 3: Blood oxygen level-dependent (BOLD) responses within the left (A) and right (B) putamen during reward anticipation in the monetary incentive delay (MID) functional magnetic resonance imaging (fMRI) task stratified by rs945270 genotypes (blue and red represent boys and girls, respectively, error bars denote standard error of the mean [SEM]).

Supplement 1

Monetary Incentive Delay Task

The task consisted of 66 10-second trials. In each trial, participants were presented with one of three cue shapes (cue, 250 ms) denoting whether a target (white square) would subsequently appear on the left or right side of the screen and whether 0, 2, or 10 points could be won in that trial. After a variable delay (4,000-4,500 ms) of fixation on a white crosshair, participants were instructed to respond with left/right button-press as soon as the target appeared. Feedback on whether and how many points were won during the trial was presented for 1,450 ms after the response. Using a tracking algorithm, task difficulty (i.e. target duration varied between 100 and 300 ms) was individually adjusted such that each participant successfully responded on ~66% of trials. Participants had first completed a practice session outside the scanner (~5 minutes), during which they were instructed that for each 5 points won they would receive one food snack in the form of small chocolate candies. Only successfully "hit" trials were included here.

Stop Signal Task

The task was composed of Go trials and Stop trials. During Go trials (83%; 480 trials), participants were presented with arrows pointing either to the left or to the right. During these trials, participants were instructed to make a button response with their left or right index finger corresponding to the direction of the arrow. In the unpredictable Stop trials (17%; 80 trials), the arrows pointing left or right were followed (on average 300 ms later) by arrows pointing upwards; participants were instructed to inhibit their motor responses during these

trials. A tracking algorithm changes the time interval between Go signal and Stop signal onsets according to each participant's performance on previous trials (average percentage of inhibition over previous Stop trials, recalculated after each Stop trial), resulting in 50% successful and 50% unsuccessful inhibition trials. The inter-trial interval was 1800ms.

Stop Signal Reaction Times (SSRT)

The SST works according to a horse race model, where the stop-signal and go-signal develop independently of each other. If the stop-process can be implemented before the go-process reaches an ultimate threshold, the response will be successfully inhibited. Stop signal reaction times were collected during the SST. However, a problem with the tracking algorithm during functional magnetic resonance imaging (fMRI) acquisition of the SST resulted in loss of behavioral SSRT data. The problem was that if the participant responded prior to a stop stimulus appearing on a stop trial, then that trial was repeated once (a maximum of seven such trials; stop-too-early [STE] trials were repeated). This may have affected the SSRT as some participants had more STE trials than others. Therefore, for participants with more than eight STEs, we calculated the SSRT up to the point of the eighth STE. However, for some participants, this occurred early in their run. Therefore, we restricted the SSRT analysis only to participants who did not reach their 8th STE before their 300th trial. The SSRT can be computed by various methods. In the present study, the SSRT was computed by taking the Go RT at the percentile corresponding to the proportion of unsuccessfully inhibited Stop trials and subtracting the mean stop signal delay.

In order to determine that the SSRT-data sample (n=671) is representative of the entire sample (n=1,129), we performed analyses in terms of sex,

attention-deficit/hyperactivity disorder (ADHD) symptoms, IQ, and socioeconomic status. There were 54% girls in the full sample and 55% girls in the SSRT sample; mean ADHD symptoms were 2.72 (std.: 2.20) in the full sample and 2.55 (std: 2.11) in the SSRT sample. Mean verbal IQ was 112 (std: 14.6) in the full sample and 113 (std: 14.2) in the SSRT sample. We also collected information regarding family stresses in our sample through the Development and Wellbeing Assessment (DAWBA) questionnaire, which was completed by the parent. This questionnaire collects information regarding unemployment, work situation, financial difficulties, housing situation, and physical and mental health of the parent to create a total score ranging from 1 to 26. In the full sample, this value was 2.69 (std. 2.47) and 2.65 (std. 2.36) in the SSRT sample. Based on these results, we can only conclude that the SSRT sample is representative of the entire tested population.

Standardization of MRI Protocols Across Sites

Protocols were developed iteratively, with input from experts from sites with scanners from each of the manufacturers involved (GE, Siemens, Philips and Bruker). For structural imaging, the well-validated Alzheimer's Disease Neuroimaging Initiative (ADNI) protocols were used with only minimal modifications; for other types of scan, a set of desired values for parameters such as voxel size were determined by consensus, and a set of dependent parameters (e.g. TE, TR) that was possible for all scanners was determined. Phantom scans and then in vivo scans were then used to assess signal to noise and contrast to noise ratios at each site. More details are given in[1]. As even minor difference between sites in image quality (or other image features that cannot be standardized, such as subtle differences in geometric distortion) could still be statistically significant, a variable coding for "site" was included as a variable of no interest in all analyses.

Supplemental Results

For the voxel-wise analyses within the whole brain and the putamen, we performed both voxel-wise and cluster-wise inference, using random fields method for family wise error correction in each contrast, especially setting the uncorrected p-value threshold for cluster formation to .001. Only voxels that are significant at both voxel-level and cluster-level are illustrated.

Response Inhibition

We did not observe significant results from whole-brain voxel-wise association analysis with rs945270 in the whole sample, nor in boys or girls when analyzed separately. Voxel-wise association analysis within the putamen in boys revealed significant clusters of activity in caudal regions of the right putamen that associated with rs945270 genotypes during inhibition of motor responses (Table S1).

Reward Anticipation

We did not observe significant results from whole-brain voxel-wise association analysis with rs945270 in the whole sample, or in boys or girls when analyzed separately. But voxel-wise association analysis within the putamen in girls identified significant clusters in the right anterior ventral putamen associating with rs945270 (Table S2).

Table S1: Voxel-Wise Association Analysis With rs945270 in Response Inhibition

	Peak T value's MNI coordinates	**Peak T value**	**Peak *p*-value**	**Cluster size**	**Cluster *p*-value**
Boys	27, -13, 13	4.36	.003	11	.025

Note: MNI = Montreal Neurological Institute.

Table S2: Voxel-Wise Association Analysis With rs945270 in Reward Anticipation

	Peak T value's MNI coordinates	**Peak T value**	**Peak *p*-value**	**Cluster size**	**Cluster *p*-value**
Girls	24, 20, -8	3.76	.035	11	.019

Note: MNI = Montreal Neurological Institute.

Figure S1: Multidimensional scaling (MDS) analysis with HapMap populations as reference groups, showing that our sample is genetically homogeneous (overlapping with the CEU population). Note: ASW = African ancestry in southwest USA; CEU = Utah residents with northern and western European ancestry from the Centre d'Etude du Polymorphisme Humain (CEPH) collection; CHD = Chinese in metropolitan Denver, Colorado; GIH = Gujarati Indians in Houston, Texas; LWK = Luhya in Webuye, Kenya; MEX = Mexican ancestry in Los Angeles, California; MKK = Maasai in Kinyawa, Kenya; TSI = Tuscans in Italy; YRI = Yoruba in Ibadan, Nigeria.

Figure S2: Voxel-wise association between rs945270 and blood oxygen level-dependent (BOLD) responses within the putamen in boys during successful response inhibition, showing clusters of activity in the right posterior putamen (peak voxel: 27, -13, 13).

Figure S3: Voxel-wise association between rs945270 and blood oxygen level-dependent (BOLD) responses within the putamen in girls during reward anticipation, showing clusters of activity in the right anterior putamen (peak voxel: 24, 20, -8).

Supplemental References

1. Schumann G, Loth E, Banaschewski T, et al. The IMAGEN study: reinforcement-related behaviour in normal brain function and psychopathology. *Mol Psychiatry.* 2010;15:1128-1139.

Impact of a Common Genetic Variation Associated With Putamen Volume on Neural Mechanisms of ADHD

Bing Xu, PhD, Tianye Jia, PhD, Christine Macare, PhD, Tobias Banaschewski, MD, PhD, Arun L.W. Bokde, PhD, Uli Bromberg, Dipl-Psych, Christian Büchel, MD, Anna Cattrell, PhD, Patricia J. Conrod, PhD, Herta Flor, PhD, Vincent Frouin, PhD, Jürgen Gallinat, MD, Hugh Garavan, PhD, Penny Gowland, PhD, Andreas Heinz, MD, PhD, Bernd Ittermann, PhD, Jean-Luc Martinot, MD, PhD, Marie-Laure Paillère Martinot, MD, PhD, Frauke Nees, PhD, Dimitri Papadopoulos Orfanos, PhD, Tomáš Paus, MD, PhD, Luise Poustka, PhD, Michael N. Smolka, MD, Henrik Walter, MD, PhD, Robert Whelan, PhD, Gunter Schumann, MD, Sylvane Desrivières, PhD, and the IMAGEN Consortium (https://imagen-europe.com)

Funding

This work received support from the following sources: the European Union-funded FP6 Integrated Project IMAGEN (Reinforcement-related behaviour in normal brain function and psychopathology) (LSHM-CT- 2007-037286), the FP7 projects IMAGEMEND (602450; IMAging GEnetics for MENtal Disorders), AGGRESSOTYPE (602805) and MATRICS (603016), the Innovative Medicine Initiative Project EU-AIMS (115300-2), the Medical Research Council Grants ‘‘Developmental pathways into adolescent substance abuse’’ (93558) and Consortium on Vulnerability to Externalizing Disorders and Addictions [c-VEDA] (MR/N000390/1), the Swedish funding agencies VR, FORTE and FORMAS, the Medical Research Council and the Wellcome Trust (Behavioural and Clinical Neuroscience Institute, University of Cambridge), the National Institute for Health Research (NIHR) Biomedical Research Centre at South London and Maudsley NHS Foundation Trust and King’s College London, the Bundesministeriumfür Bildung und Forschung (BMBF grants 01GS08152; 01EV0711; eMED SysAlc01ZX1311A; Forschungsnetz AERIAL), the Deutsche Forschungsgemeinschaft (DFG grants SM 80/7-1, SM 80/7-2, SFB 940/1), the National Institutes of Health, USA (Axon, Testosterone and Mental Health during Adolescence; RO1 MH085772-01A1), and by the NIH Consortium grant U54 EB020403, supported by a cross-NIH alliance that funds Big Data to Knowledge Centres of Excellence.

Presentation Information

This study was presented as a poster presentation at the Organization for Human Brain Mapping's 22nd Annual Meeting, Geneva, Switzerland, June 26-30, 2016.

Acknowledgements

The authors wish to thank all members of the IMAGEN Consortium: Tobias Banaschewski, MD, PhD, Heidelberg University; Gareth Barker, PhD, King's College London; Arun L.W. Bokde, PhD, Trinity College Dublin; Uli Bromberg, Dipl-Psych, University Medical Centre Hamburg-Eppendorf; Christian Büchel, MD, University Medical Centre Hamburg-Eppendorf; Erin Burke Quinlan, PhD, King's College London; Sylvane Desrivières, PhD, King's College London; Herta Flor, PhD, Heidelberg University & University of Mannheim; Vincent Frouin, PhD, Commissariat à l'Energie Atomique; Hugh Garavan, PhD, University of Vermont; Penny Gowland, PhD, University of Nottingham; Andreas Heinz, MD, PhD, Charité, Universitätsmedizin Berlin; Bernd Ittermann, PhD, Physikalisch-Technische Bundesanstalt (PTB), Braunschweig and Berlin; Jean-Luc Martinot, MD, PhD, University Paris Sud, University Paris Descartes & Maison de Solenn; Marie-Laure Paillère Martinot, MD, PhD, University Paris Sud, University Paris Descartes & Maison de Solenn; Eric Artiges, MD, PhD, University Paris Sud, University Paris Descartes & Orsay Hospital; Herve Lemaitre, PhD, University Paris Sud & University Paris Descartes; Frauke Nees, PhD, Heidelberg University; Dimitri Papadopoulos Orfanos, PhD, Commissariat à l'Energie Atomique; Tomáš Paus, MD, PhD, University of Toronto; Luise Poustka, MD, Heidelberg University & Medical University of Vienna; Michael N. Smolka, MD, Technische Universität Dresden; Nora C. Vetter, PhD, Technische Universität Dresden; Sarah Jurk, Dipl-Psych, Technische Universität Dresden; Eva Mennigen, MD, Technische Universität Dresden; Henrik Walter, MD, PhD, Charité, Universitätsmedizin Berlin; Robert Whelan, PhD, University College Dublin; Gunter Schumann, MD, King's College London

Disclosures

Dr. Banaschewski has served as an advisor or consultant to Bristol-Myers Squibb, Desitin Arzneimittel, Eli Lilly and Co., Medice, Novartis, Pfizer, Shire, UCB, and Vifor Pharma. He has received conference attendance support, conference support, or speaking fees from Eli Lilly and Co., Janssen McNeil, Medice, Novartis, Shire, and UCB. He is involved in clinical trials conducted by Eli Lilly and Co., Novartis, and Shire; the present work is unrelated to these relationships.

Dr. Gallinat has received research funding from the German Federal Ministry of Education and Research, AstraZeneca, Eli Lilly and Co., Janssen-Cilag, and Bristol-Myers Squibb. He has received speaking fees from AstraZeneca, Janssen-Cilag, and Bristol-Myers Squibb.

Dr. Paillère-Martinot has received compensation from Janssen-Cilag for CME activities.

Prof. Poustka has received speaking fees from Shire, Eli Lilly and Co., and Medice.

Drs. Xu, Jia, Macare, Bokde, Bromberg, Büchel, Cattrell, Conrod, Flor, Frouin, Garavan, Gowland, Heinz, Ittermann, Martinot, Nees, Orfanos, Paus, Smolka, Walter, Whelan, Schumann, and Desrivières report no biomedical financial interests or potential conflicts of interest.

Figure 1

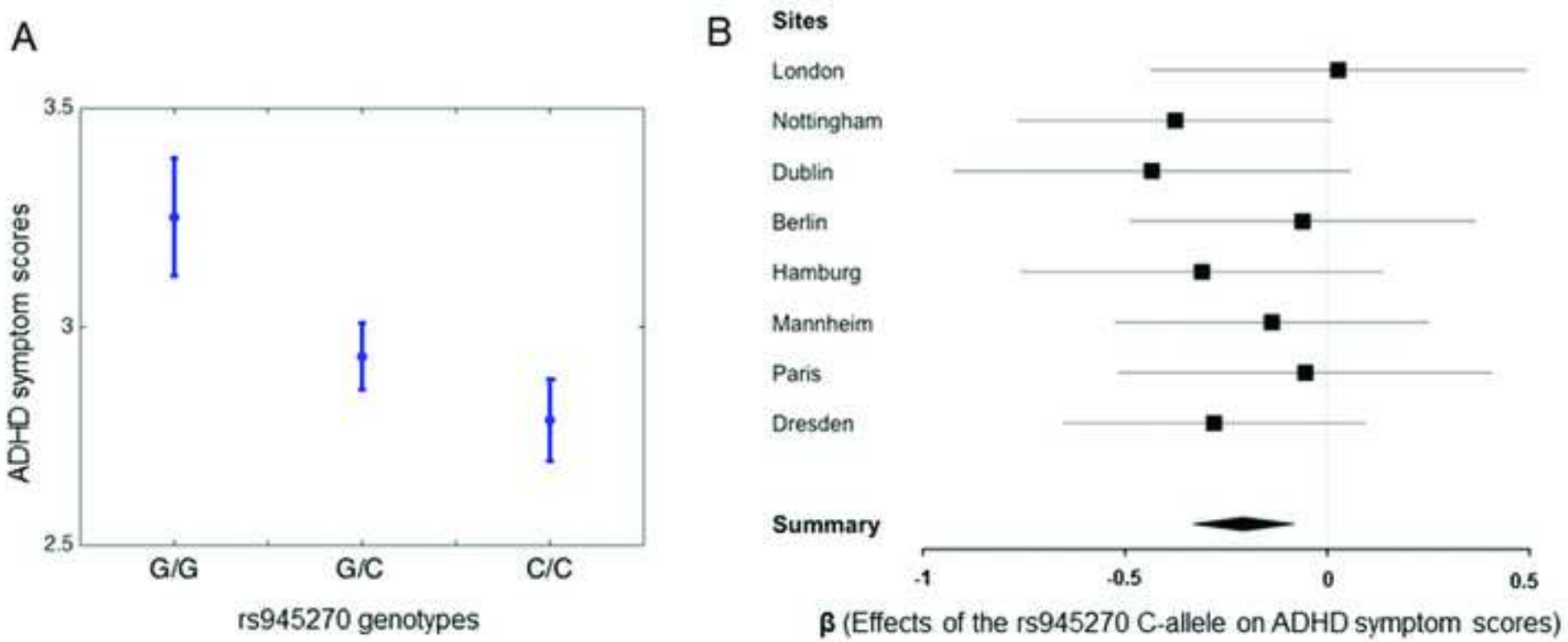

A
3.5
3
2.5
ADHD symptom scores
G/G
G/C
C/C
rs945270 genotypes
B
Sites
London
Nottingham
Dublin
Berlin
Hamburg
Mannheim
Paris
Dresden
Summary
-1
-0.5
0
0.5
β (Effects of the rs945270 C-allele on ADHD symptom scores)

Figure 2

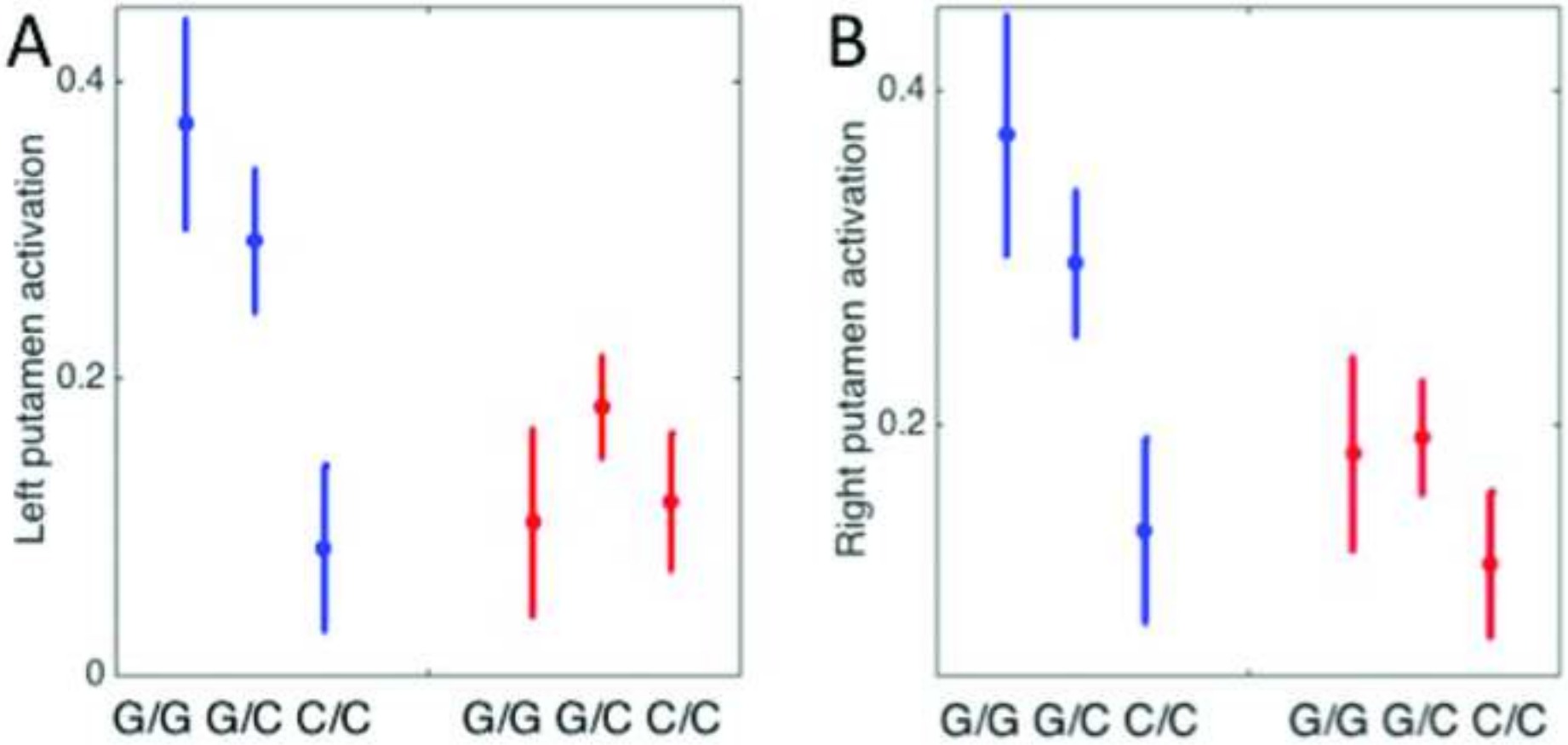
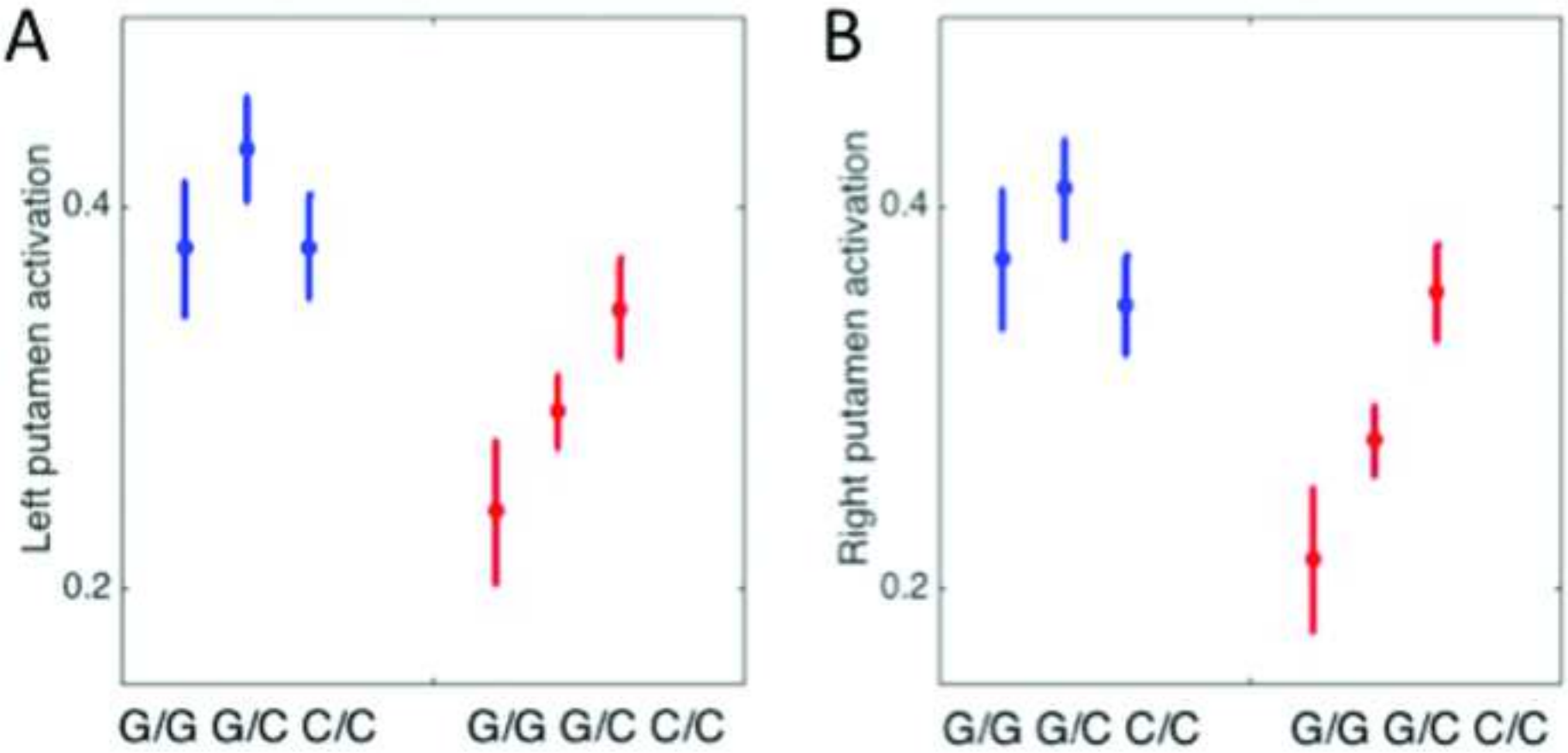
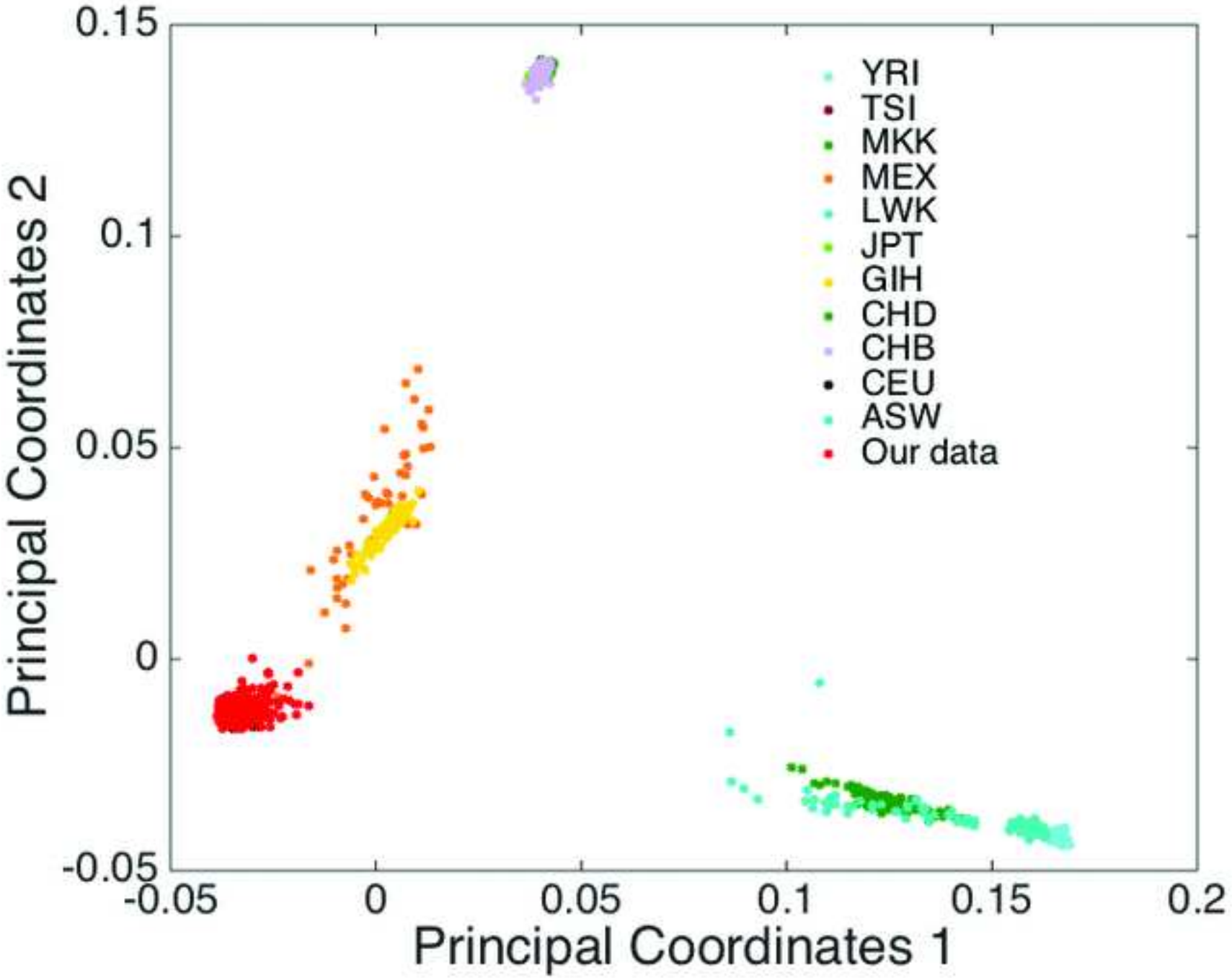

A
Left putamen activation
0.4
0.2
0
G/G G/C C/C
G/G G/C C/C
B
Right putamen activation
0.4
0.2
G/G G/C C/C
G/G G/C C/C

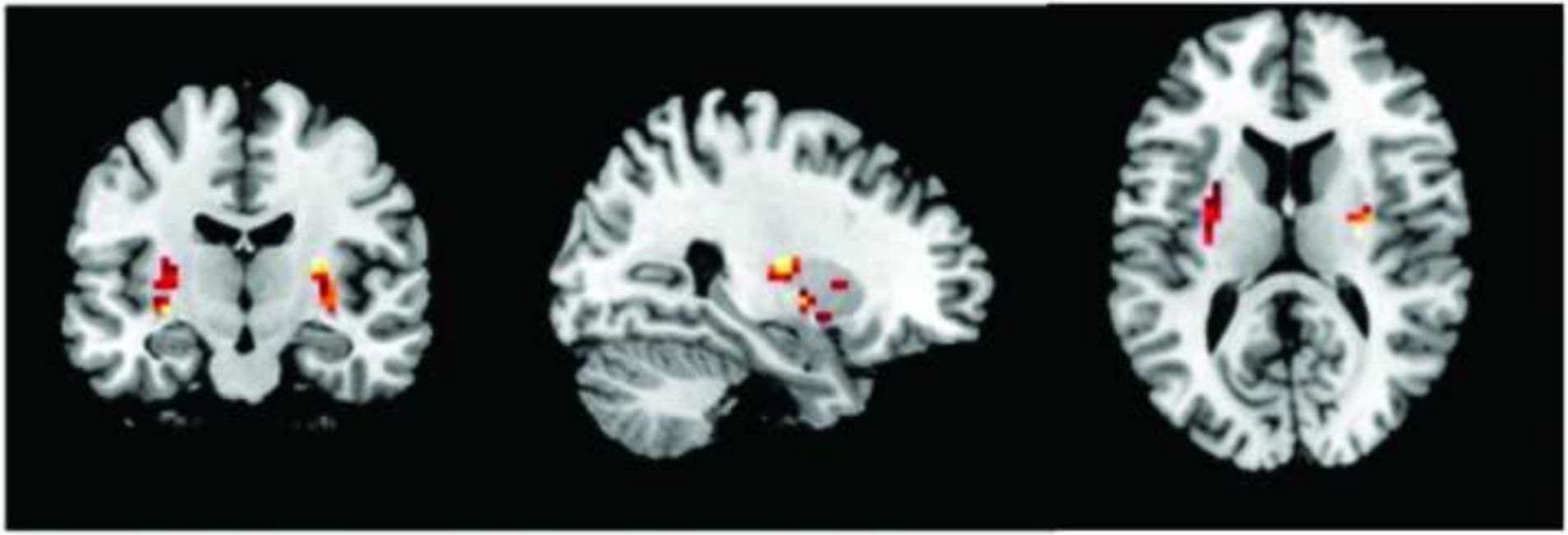
Figure 3


A
Left putamen activation
0.4
0.2
G/G G/C C/C
G/G G/C C/C
B
Right putamen activation
0.4
0.2
G/G G/C C/C
G/G G/C C/C

Supplemental Fig1



Supplemental Fig2



Supplemental Fig3