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Abstract: Attention-deficit hyperactivity disorder (ADHD) is a common behavioural disorder that affects not only children and adolescents but also adults; however, diagnosis of adult ADHD is difficult because patients seem to have reduced externalized behaviour. ADHD is a multifactorial disorder in which many genes, all with small effects, are thought to cause the disorder in the presence of unfavourable environmental conditions. Therefore, in this pilot study, we explored the expression profile of a list of previously established candidate genes in peripheral blood samples from adult ADHD subjects (n = 108) and compared these results with those of healthy controls (n = 35). We demonstrate that combining the gene expression levels of dopamine transporter (SLC6A3), dopamine D5 receptor, tryptophan hydroxylase-1, and SNAP25 as predictors in a regression model resulted in sensitivity and specificity of over 80 % (ROC: max R(2) = 0.587, AUC = 0.917, P < 0.001, 95 % CI: 0.900-0.985). In conclusion, the combination of these four genes could represent a potential method for estimating risk and could be of diagnostic value for ADHD. Nevertheless, further investigation in a larger independent population including different subtypes of ADHD (inattentive, hyperactive, or combined type) patients is required to obtain more specific sets of biomarkers for each subtype as well as to differentiate between child, adolescent, and adulthood forms.

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Pilot study: potential transcription markers for adult attention-deficit hyperactivity disorder in whole blood

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Abstract

Objectives: Attention-deficit hyperactivity disorder (ADHD) is a common behavioural disorder that affects not only children and adolescents but also adults; however, diagnosis of adult-ADHD is difficult because patients seem to have reduced externalized behaviour. ADHD is a multifactorial disorder in which many genes, all with small effects, are thought to cause the disorder in the presence of unfavourable environmental conditions.

Methods: Therefore, in this pilot study we explored the expression profile of a list of previously established candidate genes in peripheral blood samples from adult-ADHD subjects (n=108) and compared these results to those of healthy controls (n=35).

Results: We demonstrate that combining the gene expression levels of dopamine transporter (*SLC6A3)*, dopamine D5 receptor (*DRD5)*, tryptophan hydroxylase-1 (*TPH1)*, and *SNAP25* as predictors in a regression model resulted in sensitivity and specificity of over 80% (ROC: max R^2=0.587, AUC=0.917, P<0.001, 95% CI: 0.900–0.985).

Conclusion: In conclusion, the combination of these four genes could represent a potential method for estimating risk and could be of diagnostic value for ADHD. Nevertheless, further investigation in a larger independent population including different subtypes of ADHD (inattentive, hyperactive, or combined type) patients is required to obtain more specific sets of biomarkers for each subtype as well as to differentiate between child, adolescent, and adulthood forms.

Key Words: Genetic, Adult ADHD, Peripheral, biomarker, Biological Psychiatry

Introduction

Attention-deficit hyperactivity disorder (ADHD) is one of the most common behavioural disorders in child and adolescent psychiatry (prevalence variance of 5–12%) (Faraone et al. 2003; Polanczyk et al. 2007), while recently it was demonstrated in adults with a prevalence variance of 3–6% (Klassen et al. 2010; Simon et al. 2009; Lara et al. 2009). The disorder is characterized by impaired attention, motor hyperactivity, and increased impulsivity (Haavik et al. 2010; American Psychiatric Association 2000). Adult ADHD is a well characterized condition, although it is generally accepted that the onset of ADHD usually occurs in childhood (Biederman 2005; American Psychiatric Association 2000). The diagnosis and treatment of adult ADHD can be challenging since hyperactive symptoms and externalized behaviours tend to wane with age, making diagnosis difficult (Klassen et al. 2010).

Despite evidence pointing towards a primarily genetic basis for ADHD, no specific genetic variants are yet known (Wood and Neale 2010; Banaschewski et al. 2010; Franke et al. 2009). The candidate genes explored so far confirm only small to moderate effects, with odds ratios (OR) ranging from 1.03–1.83 (Faraone and Mick 2010). The most frequently studied genes affecting the susceptibility to ADHD are the dopamine D4 receptor (*DRD4*), *DRD5*, dopamine transporter (SLC6A3, *DAT1*), 25-kDa synaptosomal-associated protein (*SNAP25*), serotonin transporter (SLC6A4, *SERT*), and tryptophan hydroxylase 2 (*TPH2*) (Faraone and Mick 2010; Gizer et al. 2009), but all seem to have mild effects. Additionally, environmental factors like stress and prenatal alcohol or nicotine consumption could also influence the ADHD phenotype and prevalence, which are part of many components regarded as Gene x Environmental interaction (Nigg et al. 2010). Therefore, there is probably no single gene indicating the presence of ADHD, but rather polygenetic markers that in combination increase the risk for the disorder. Such an approach, using a wide array of parameters, is referred to as biomarkers.

Biomarkers can be used as diagnostic tools to identify or stage disease, as indicators of disease prognosis or predictors, or to monitor clinical response to therapy. As defined by the Biomarkers Definitions Working Group, a biomarker is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention" (Biomarkers Definitions Working Group 2001). Consequently, in this pilot study we explore a group of candidate genes comprising the described risk genes known in the literature (Faraone and Mick 2010; Gizer et al. 2009), and corticotropin-releasing factor binding protein (*CRHBP*) was selected as a response to stress, which is also thought to be a risk factor for ADHD, to assess their expression profile in peripheral blood samples taken from adult ADHD subjects compared with samples from healthy controls. Such concept was previously reported in which transcriptional profiling resulted in identification of several candidate genes in blood and brain, especially for schizophrenic patients (Glatt et al. 2005; Tsuang et al. 2005; Mehler-Wex et al. 2006; Noori-Daloii et al. ; Taurines et al. 2010) and Alzheimer's and Parkinson's disease patients (Grünblatt et al. 2009; Grünblatt et al. 2010; Scherzer et al. 2007).

The concept of peripheral blood cells as a "window" into the central nervous system (CNS) was proposed nearly a decade ago (Gladkevich et al. 2004; Burczynski and Dorner 2006), whereas CNS versus peripheral expression has proven to moderately correlate (Sullivan et al. 2006; Cai et al. 2010). We hypothesised that none of the biomarkers would individually provide high sensitivity and specificity; therefore, we used the best known candidates in a combined gene expression analysis and a comprehensive regression model.

Methods

Subject recruitment

Patients and controls were recruited and phenotypically characterized by a team of experienced psychiatrists at the outpatient units of the Department of Psychiatry, Psychosomatics, and Psychotherapy, University of Würzburg, Germany, according to DSM-

IV criteria (American Psychiatric Association 2000; Jacob et al. 2007). The diagnosis of childhood and youth manifestation of ADHD was retrospectively assessed with the DSM-IV symptom list for ADHD, which was used as a structured clinical interview. Additional information from parents and school report cards was included if available. Adult symptomatology was assessed with the DSM-IV symptom list for ADHD. To ensure diagnostic validity, informative input from partners, relatives, and friends was also collected. Co-morbidity was assessed with the structured clinical interviews of axis I (SCID I) disorders (First et al. 1997). Exclusion criteria were: autistic disorder, psychotic disorder, bipolar affective disorder, history of epilepsy, other neurologic disorder, severe chronic medical condition, IQ < 80 (Jacob et al. 2007). Additionally, detailed information on medication, alcohol abuse, and smoking habits was collected. Control subjects underwent diagnostic procedures similar to those of the patients to exclude any other psychiatric disorders. Controls were recruited through local newspaper ads. The subjects provided informed consent according to the Helsinki Declaration. The study protocol was approved by the local ethics committee of the Universities of Würzburg, Germany. Written informed consent was obtained from all subjects or their primary caregivers.

One hundred and eight patients diagnosed with ADHD (46 males and 62 females) and 35 healthy controls (19 males and 16 females) were included in this study (Table S1, supplemental data). All subjects were with German ancestry that ensured a homogenous sample analysis. The gender distribution was similar in both diagnostic groups (P=0.247, Table S1), whereas the mean age was significantly older in the control group (controls = 39.6±9.49 years, ADHD = 34.7±10.52 years; P=0.009). The most frequent lifetime co-morbidities were substance-related disorders (alcohol 11.1%, cannabis 22.2%), and other co-morbidities were present in 11.1% of patients. In ADHD patients, the combined subtype was diagnosed in 70.4% (n=76), whereas 21.3% (n=23) were primarily inattentive, and 5.5% (n=6) were primarily hyperactive. Whole blood was collected with the *PAXgene™ Blood*

*RNA system (*Becton Dickinson GmbH, Heidelberg, Germany), and EDTA-blood tubes. Blood samples were frozen at -20°C until further processing for total RNA extraction, whereas fresh blood was processed immediately for DNA isolation.

Genomic DNA extraction

DNA was prepared from 2 ml EDTA-blood using proteinase K as described previously (Grünblatt et al. 2005). The DNA was then aliquoted into cryo-Vials (Nunk GmbH, Wiesbaden, Germany) and frozen at -70^{0}C until processing.

Genotyping

Genotyping was conducted as previously described for *SNAP25* +2015 (rs6077690), SNAP25 +80609 (rs363006), *SNAP25* -523 (rs6039769) (Renner et al. 2008), serotonin transporter linked polymorphic region (*HTTLPR*; *SLC6A4* tri allelic) (Lesch et al. 1996; Hu et al. 2005), *TPH2* SNP25 (rs4570625) (Walitza et al. 2005), *SLC6A3* 3'UTR VNTR, and *DRD4* exon 3 VNTR (Walitza et al. 2008).

Total RNA extraction

Total RNA was prepared with the *PAXgene*™ Blood RNA Kit 50 (PreAnalytiX, Qiagen and BD, Germany). RNA isolation reagents were prepared from 0.2 μM filtered, diethyl pyrocarbonate (DEPC)-treated water (Fermentas Inc., Hanover, MD, USA) throughout the isolation procedure. Total RNA samples were spectrophotometrically scanned (Experion, BioRad Co., Hercules, CA, USA) from 220 to 320 nm; the A260/A280 of total RNA was typically >1.9.

Quantitative real-time RT-PCR analysis

Quantitative real-time RT-PCR was used for 7 genes (Table S2, supplemental data) as follows: *SLC6A3*; *DRD4*; *DRD5*; *TPH1* (variant 1 was tested in the peripheral samples since variant 2 [*TPH2*] is not expressed in the peripheral blood but only in the CNS); SLC6A4; SNAP25; CRHBP; additionally, 6 reference genes were used as internal controls. Total RNA (500 ng) from each sample was reverse transcribed with the random hexamer & oligo-dT

primer mix using iScript (BioRad Co., Hercules, CA, USA). Quantitative real-time PCR was performed in the iCycler iQ system (BioRad Co., Hercules, CA, USA) as described previously (Grünblatt et al. 2009). Real-time PCR was subjected to thermocycler amplification as described previously (Grünblatt et al. 2009). All PCR reactions were run in duplicate. The amplified transcripts were quantified using the comparative C_T method analyzed with the BioRad iCycler iQ system program. Gene expression levels were normalized to the 6 reference genes according to GeNorm (Vandesompele et al. 2002). Data was analyzed with Microsoft Excel 2000 to generate raw expression values.

Statistical analysis

Because of non-normal data distribution, parametric procedures including all potential moderating variables could not be employed; therefore, we tried to control for the influence of age on individual mRNA levels by looking at the correlation between age and gene expression. The relationship between genotype, diagnosis, and gender was explored with a Chi^2 test on 2 (diagnosis) x 3 (genotype) cross-tabs both with gender as a layer variable for each gene and for both genders combined. For analysis of our main variable of interest – gene expression differences between diagnostic groups – non-parametric Mann-Whitney-U tests for differences in mean rank between ADHD subjects and controls were performed. For those genes returning significant main effects of diagnosis, we also checked for the influence of genotype on gene expression within each diagnostic group.

Assuming a combination of markers would yield the best prediction, we employed binary logistic regression to first assess the diagnostic utility of the expression of our candidate genes for the ability to differentiate between ADHD patients and healthy controls. To that end, we entered those genes showing differential expression between ADHD and controls as well as the potentially confounding variables age and gender into the model. We applied a filter excluding cases rated as outliers on any one of the respective RNA levels. Outliers were identified using Box plots, with values more than 1.5 and 2 box-lengths away from the box

ends are characterised as such. In total, 19 samples had to be excluded, because they were classified as outliers on the expression level of at least one gene. The predicted probabilities from significant predictors from this whole model were then ultimately used for building a receiver operating characteristic (ROC) curve and assessing sensitivity and specificity. Since odds ratios (OR) were extremely large due to the small unit of the detected gene expression levels (e.g. 6666.13 for TPH1), the original mRNA values were multiplied by a factor of 100. Significances remained unchanged by this procedure. The significance level was set as 0.05 (nominal p-values). All computations were completed using the statistical computing environment, PASW Statistics 18.

Results

All genotypes were in Hardy-Weinberg-Equilibrium. For exact genotype distributions, see supplementary Table S3. From the results of the different genotypes, we detected no association between ADHD and any of the polymorphisms.

Except for lower *TPH1* levels (r=-0.174, *P*=0.039) being weakly associated with older age, no significant correlations between age and any of the genes were observed (all *P*>0.105; for details, see supplementary data, Table S4).

The dopaminergic system showed the most prominent differences between ADHD patients and controls, with patients having elevated *SLC6A3* expression (*P*=0.001), and both *DRD4* and *DRD5* showed higher expression levels in ADHD patients compared with controls (*P*=0.005 and *P*<0.001, respectively; Table 1; supplementary Figure S1). Furthermore, there was a notable increase in *SNAP25* in samples from ADHD subjects compared with controls (*P*=0.01). With regard to the serotonergic genes, no difference was found for *SLC6A4* (*P*=0.696); however, ADHD subjects had higher expression of the rate-limiting enzyme *TPH1* mRNA (*P*<0.001). *CRHBP* mRNA was the only gene expressed at lower levels in ADHD cases (*P*=0.037). Within the diagnostic groups, no influence of genotype on gene expression was observed (Table 1; Supplementary Figure S2).

For our first binary logistic regression model we used a forward stepwise approach, in which all variables were successively entered. In this case, we used the expression levels of all candidate genes (SLC6A3, DRD4, DRD5, TPH1, SNAP25 and CRHBP) along with age and gender as potential predictors. Concerning multicolinearity, all correlations of variables within this model were below 0.510. The whole model returned the following significant regressors: *DRD5* (p=0.011, OR=1.40, 95% CI: 1.08–1.81), *TPH1* (p<0.009, OR=1.10, 95% CI: 1.02–1.17) and *SNAP25* (p=0.021, OR=1.07, 95% CI: 1.01–1.13) and a trend-level contribution for *SLC6A3* (p=0.069, OR=1.06, 95% CI: 1.00–1.13) (Supplementary Table S5). The receiver operating characteristic (ROC) curve was built using these four genes (Figure 1: max R^2=0.587, AUC=0.917, P<0.001, 95% CI: 0.900–0.985). Combining gene expression levels of *SLC6A3*, *DRD5*, *TPH1*, and *SNAP25* as predictors in a regression model, 80% of cases could be correctly classified as ADHD subjects or controls. Any other combination with other genes or with three or two of the above four resulted in a worse prediction of ADHD compared with this four-gene combination. We achieved sensitivity and specificity of over 80% (e.g. sensitivity of 81% and specificity of 82% at a cut-off of 0.69) according to the whole-model ROC analysis. The combined approach was superior to any individual variables predictive ability, where separate logistic models returned the following results: SLC6A3 (AUC=0.694, sensitivity = 0.696, specificity = 0.645), DRD5 (AUC=0.749, sensitivity = 0.752, specificity = 0.625), SNAP25 (AUC=0.689, sensitivity = 0.637, specificity = 0.618) and TPH1 (AUC=0.812, sensitivity = 0.776, specificity = 0.706).

Discussion

We showed that a combination of expression patterns for four ADHD-associated genes in the peripheral blood of adult ADHD patients could be a potential method for estimating risk and have possible diagnostic value for ADHD. Since ADHD is a heterogeneous disorder with a high comorbidity rate, a complex diagnostic tool with more than one marker was expected (Wallis 2010). We show that a combination of expression levels of four genes, *SLC6A3*,

DRD5, *TPH1* and *SNAP25*, provide relatively high sensitivity and specificity, indicating the potential use of this combination of biomarkers to identify the risk of ADHD in adults. Three of the genes studied were unaffected by age, whereas TPH1 showed a very mild decrease in expression with age. Recently, in a study for mRNA alterations in whole blood from child and adolescent ADHD compared to autism spectrum disorder (ASD) and to controls no group differences were found for TPH1 mRNA expression (Taurines et al. 2011) Still, TPH1 expression of the ADHD group (n=51) was slightly higher than the ASD (n=26) or the control (n=39) group (Taurines et al. 2011), which might indicate the beginning of alterations with age for ADHD group. As an additional parameter, we analysed the association of the genotypes for the same genes studied with ADHD. We observed no overall association between genotype and ADHD for any of the genes tested; moreover, we checked for an additional influence of genotype on the mRNA levels of those genes found to be differently expressed in patients and controls; however, this also showed no difference. Since transcript expression seems to be influence rather by multiple factors such as multiple genetic polymorphism as well as environmental factors, the notion that multiple transcript analysis would be necessary to identify ADHD cases is pointed out.

The four candidate genes identified as the best biomarkers in this study were previously described in other ADHD association studies (Stergiakouli and Thapar 2010; Gizer et al. 2009). The most consistent evidence for an association with ADHD exists for the DRD5 gene microsatellite (Squassina et al. 2008) and the SLC6A3 gene variable tandem repeat (VNTR) at the 3' UTR (Stergiakouli and Thapar 2010; Gizer et al. 2009). In contrast, very weak individual associations were reported for TPH1 (Gizer et al. 2009; Johansson et al. 2010) and SNAP25 (Gizer et al. 2009; Forero et al. 2009; Kim et al. 2007), but the 3' UTR of the latter was significant in a recent meta-analysis (Gizer et al. 2009). Each of these genes often showed a very modest genetic effect on the risk of ADHD (meta-analysis: SLC6A3 10 repeats OR=1.1, 95% CI: 1.03–1.17; DRD5 5' Flank OR=1.22, 95% CI: 1.10–1.36; TPH1 intron 6

OR=0.99, 95% CI: 0.89–1.10; SNAP25 3' UTR OR=1.15, 95% CI: 1.01–1.31) (Gizer et al. 2009). Interestingly, SLC6A3 seems to have an opposite effect in persistent adult ADHD compared with childhood ADHD (Franke et al. 2010). Therefore, the need for sensitive biomarkers that in combination achieve a much higher OR than the ones investigated to-date is necessary. In our study, we combined multiple genetic markers with relatively good success, which confirms the potential of this strategy.

A limitation of this study is the small sample size; however, since this was planned as an exploratory study the sample size still pointed to the possible candidate genes that should be than confirmed in the future. Additionally, the different co-morbidities existing in our population might have affected the results, which we could not control for properly because of the limited group size. Since adult ADHD is highly co-morbid with other ICD diagnoses (Wallis 2010), there might be common pathways for the array of these disorders that still provides a good diagnostic tool. It will be necessary to look at subgroups in future studies with a larger population, and to analyze markers for a specific diagnosis. Furthermore, a larger population would enable us to incorporate the different pharmacological treatments to rule out any effects of these on gene expression. The biggest limitation might be the fact that the populations we examined and determined the test's sensitivity and specificity from were extensively evaluated by trained professionals and the subjects included in the study had either a very clear-cut case of ADHD or were completely healthy. However, in real-life patients, presentations are often a lot more ambiguous, making the development of a highly sensitive and specific test a top priority. In that case, our markers might do less well, so results need to be validated with a broader spectrum of ADHD phenotypes and severities and the predictive value needs to be determined.

In conclusion, we demonstrate the potential of peripherally-expressed genes as biomarkers that could be used to diagnose ADHD. Further investigation of additional candidate

transcripts in a larger independent population including different ADHD sub-groups (inattentive, hyperactive, and combined type) and compare it not only to healthy controls but also to other psychiatric disorders is required to obtain more specific sets of biomarkers for each sub-disorder and to differentiate between childhood, adolescent, and adult forms of ADHD.

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Conflict of interest statement

The authors have no conflicts of interest to declare.

References

American Psychiatric Association A (2000) American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders- DSM-IV-TR. 4th edition edn. American Psychiatric Association, Washington, DC

Banaschewski T, Becker K, Scherag S, Franke B, Coghill D (2010) Molecular genetics of attention-deficit/hyperactivity disorder: an overview. Eur Child Adolesc Psychiatry 19 (3):237-257. doi:10.1007/s00787-010-0090-z

Biederman J (2005) Attention-deficit/hyperactivity disorder: a selective overview. Biol Psychiatry 57 (11):1215-1220. doi:S0006-3223(04)01100-X [pii]

10.1016/j.biopsych.2004.10.020

Biomarkers Definitions Working Group (2001) Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 69 (3):89-95. doi:S0009-9236(01)63448-9 [pii]

10.1067/mcp.2001.113989

Burczynski ME, Dorner AJ (2006) Transcriptional profiling of peripheral blood cells in clinical pharmacogenomic studies. Pharmacogenomics 7 (2):187-202

Cai C, Langfelder P, Fuller TF, Oldham MC, Luo R, van den Berg LH, Ophoff RA, Horvath S (2010) Is human blood a good surrogate for brain tissue in transcriptional studies? BMC Genomics 11:589. doi:1471-2164-11-589 [pii]

10.1186/1471-2164-11-589

Faraone SV, Mick E (2010) Molecular genetics of attention deficit hyperactivity disorder. Psychiatr Clin North Am 33 (1):159-180. doi:S0193-953X(09)00106-3 [pii]

10.1016/j.psc.2009.12.004

Faraone SV, Sergeant J, Gillberg C, Biederman J (2003) The worldwide prevalence of ADHD: is it an American condition? World Psychiatry 2 (2):104-113

First MB, Spitzer RL, Gibbon M, Williams JBW (eds) (1997) Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I), Clinician Version. American Psychiatric Publishing Inc., Arlington, VA, USA

Forero DA, Arboleda GH, Vasquez R, Arboleda H (2009) Candidate genes involved in neural plasticity and the risk for attention-deficit hyperactivity disorder: a meta-analysis of 8 common variants. J Psychiatry Neurosci 34 (5):361-366

Franke B, Neale BM, Faraone SV (2009) Genome-wide association studies in ADHD. Hum Genet 126 (1):13-50. doi:10.1007/s00439-009-0663-4

Franke B, Vasquez AA, Johansson S, Hoogman M, Romanos J, Boreatti-Hummer A, Heine M, Jacob CP, Lesch KP, Casas M, Ribases M, Bosch R, Sanchez-Mora C, Gomez-Barros N, Fernandez-Castillo N, Bayes M, Halmoy A, Halleland H, Landaas ET, Fasmer OB, Knappskog PM, Heister AJ, Kiemeney LA, Kooij JJ, Boonstra AM, Kan CC, Asherson P, Faraone SV, Buitelaar JK, Haavik J, Cormand B, Ramos-Quiroga JA, Reif A (2010) Multicenter analysis of the SLC6A3/DAT1 VNTR haplotype in persistent ADHD suggests differential involvement of the gene in childhood and persistent ADHD. Neuropsychopharmacology 35 (3):656-664. doi:npp2009170 [pii]
10.1038/npp.2009.170

Gizer IR, Ficks C, Waldman ID (2009) Candidate gene studies of ADHD: a meta-analytic review. Hum Genet 126 (1):51-90. doi:10.1007/s00439-009-0694-x

Gladkevich A, Kauffman HF, Korf J (2004) Lymphocytes as a neural probe: potential for studying psychiatric disorders. Prog Neuropsychopharmacol Biol Psychiatry 28 (3):559-576

Glatt SJ, Everall IP, Kremen WS, Corbeil J, Sasik R, Khanlou N, Han M, Liew CC, Tsuang MT (2005) Comparative gene expression analysis of blood and brain provides concurrent validation of SELENBP1 up-regulation in schizophrenia. Proc Natl Acad Sci U S A 102 (43):15533-15538. doi:0507666102 [pii]

10.1073/pnas.0507666102

Grünblatt E, Bartl J, Zehetmayer S, Ringel TM, Bauer P, Riederer P, Jacob CP (2009) Gene expression as peripheral biomarkers for sporadic Alzheimer's disease. J Alzheimers Dis 16 (3):627-634. doi:C335V1166L813870 [pii]

10.3233/JAD-2009-0996

Grünblatt E, Schlößer R, Fischer P, Fischer MO, Li J, Koutsilieri E, Wichart I, Sterba N, Rujescu D, Moller HJ, Adamcyk W, Dittrich B, Muller F, Oberegger K, Gatterer G, Jellinger KJ, Mostafaie N, Jungwirth S, Huber K, Tragl KH, Danielczyk W, Riederer P (2005) Oxidative stress related markers in the "VITA" and the centenarian projects. Neurobiol Aging 26 (4):429-438

Grünblatt E, Zehetmayer S, Jacob CP, Muller T, Jost WH, Riederer P (2010) Pilot study: peripheral biomarkers for diagnosing sporadic Parkinson's disease. J Neural Transm. doi:10.1007/s00702-010-0509-1

Haavik J, Halmoy A, Lundervold AJ, Fasmer OB (2010) Clinical assessment and diagnosis of adults with attention-deficit/hyperactivity disorder. Expert Rev Neurother 10 (10):1569-1580. doi:10.1586/ern.10.149

Hu X, Oroszi G, Chun J, Smith TL, Goldman D, Schuckit MA (2005) An expanded evaluation of the relationship of four alleles to the level of response to alcohol and the alcoholism risk. Alcohol Clin Exp Res 29 (1):8-16. doi:00000374-200501000-00002 [pii]

Jacob CP, Romanos J, Dempfle A, Heine M, Windemuth-Kieselbach C, Kruse A, Reif A, Walitza S, Romanos M, Strobel A, Brocke B, Schafer H, Schmidtke A, Boning J, Lesch KP (2007) Co-morbidity of adult attention-deficit/hyperactivity disorder with focus on personality traits and related disorders in a tertiary referral center. Eur Arch Psychiatry Clin Neurosci 257 (6):309-317. doi:10.1007/s00406-007-0722-6

Johansson S, Halmoy A, Mavroconstanti T, Jacobsen KK, Landaas ET, Reif A, Jacob C, Boreatti-Hummer A, Kreiker S, Lesch KP, Kan CC, Kooij JJ, Kiemeney LA, Buitelaar JK, Franke B, Ribases M, Bosch R, Bayes M, Casas M, Ramos-Quiroga JA, Cormand B, Knappskog P, Haavik J (2010) Common variants in the TPH1 and TPH2 regions are not associated with persistent ADHD in a combined sample of 1,636 adult cases and 1,923 controls from four European populations. Am J Med Genet B Neuropsychiatr Genet 153B (5):1008-1015. doi:10.1002/ajmg.b.31067

Kim JW, Biederman J, Arbeitman L, Fagerness J, Doyle AE, Petty C, Perlis RH, Purcell S, Smoller JW, Faraone SV, Sklar P (2007) Investigation of variation in SNAP-25 and ADHD and relationship to co-morbid major depressive disorder. Am J Med Genet B Neuropsychiatr Genet 144B (6):781-790. doi:10.1002/ajmg.b.30522

Klassen LJ, Katzman MA, Chokka P (2010) Adult ADHD and its comorbidities, with a focus on bipolar disorder. J Affect Disord 124 (1-2):1-8. doi:S0165-0327(09)00317-6 [pii]

10.1016/j.jad.2009.06.036

Lara C, Fayyad J, de Graaf R, Kessler RC, Aguilar-Gaxiola S, Angermeyer M, Demytteneare K, de Girolamo G, Haro JM, Jin R, Karam EG, Lepine JP, Mora ME, Ormel J, Posada-Villa J, Sampson N (2009) Childhood predictors of adult attention-deficit/hyperactivity disorder: results from the World Health Organization World Mental Health Survey Initiative. Biol Psychiatry 65 (1):46-54. doi:S0006-3223(08)01203-1 [pii]

10.1016/j.biopsych.2008.10.005

Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Muller CR, Hamer DH, Murphy DL (1996) Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 274 (5292):1527-1531.

Mehler-Wex C, Duvigneau JC, Hartl RT, Ben-Shachar D, Warnke A, Gerlach M (2006) Increased mRNA levels of the mitochondrial complex I 75-kDa subunit. A potential peripheral marker of early onset schizophrenia? Eur Child Adolesc Psychiatry 15 (8):504-507. doi:10.1007/s00787-006-0560-5

Nigg J, Nikolas M, Burt SA (2010) Measured gene-by-environment interaction in relation to attention-deficit/hyperactivity disorder. J Am Acad Child Adolesc Psychiatry 49 (9):863-873. doi:S0890-8567(10)00289-3 [pii]

10.1016/j.jaac.2010.01.025

Noori-Daloii MR, Kheirollahi M, Mahbod P, Mohammadi F, Astaneh AN, Zarindast MR, Azimi C, Mohammadi MR Alpha- and beta-synucleins mRNA expression in lymphocytes of schizophrenia patients. Genet Test Mol Biomarkers 14 (5):725-729. doi:10.1089/gtmb.2010.0050

Polanczyk G, de Lima MS, Horta BL, Biederman J, Rohde LA (2007) The worldwide prevalence of ADHD: a systematic review and metaregression analysis. Am J Psychiatry 164 (6):942-948. doi:164/6/942 [pii]

10.1176/appi.ajp.164.6.942

Renner TJ, Walitza S, Dempfle A, Eckert L, Romanos M, Gerlach M, Schafer H, Warnke A, Lesch KP, Jacob C (2008) Allelic variants of SNAP25 in a family-based sample of ADHD. J Neural Transm 115 (2):317-321. doi:10.1007/s00702-007-0840-3

Scherzer CR, Eklund AC, Morse LJ, Liao Z, Locascio JJ, Fefer D, Schwarzschild MA, Schlossmacher MG, Hauser MA, Vance JM, Sudarsky LR, Standaert DG, Growdon JH, Jensen RV, Gullans SR (2007) Molecular markers of early Parkinson's disease based on gene expression in blood. Proc Natl Acad Sci U S A 104 (3):955-960

Simon V, Czobor P, Balint S, Meszaros A, Bitter I (2009) Prevalence and correlates of adult attention-deficit hyperactivity disorder: meta-analysis. Br J Psychiatry 194 (3):204-211. doi:194/3/204 [pii]

10.1192/bjp.bp.107.048827

Squassina A, Lanktree M, De Luca V, Jain U, Krinsky M, Kennedy JL, Muglia P (2008) Investigation of the dopamine D5 receptor gene (DRD5) in adult attention deficit hyperactivity disorder. Neurosci Lett 432 (1):50-53. doi:S0304-3940(07)01260-8 [pii]

10.1016/j.neulet.2007.12.003

Stergiakouli E, Thapar A (2010) Fitting the pieces together: current research on the genetic basis of attention-deficit/hyperactivity disorder (ADHD). Neuropsychiatr Dis Treat 6:551-560. doi:10.2147/NDT.S11322

Sullivan PF, Fan C, Perou CM (2006) Evaluating the comparability of gene expression in blood and brain. Am J Med Genet B Neuropsychiatr Genet 141 (3):261-268

Taurines R, Grünblatt E, Schecklmann M, Schwenck C, Albantakis L, Reefschlager L, Walitza S, Renner T, Gerlach M, Thome J, Romanos M (2011) Altered mRNA expression of monoaminergic candidate genes in the blood of children with attention deficit hyperactivity disorder and autism spectrum disorder. World J Biol Psychiatry 12 Suppl 1:104-108. doi:10.3109/15622975.2011.600297

Taurines R, Thome J, Duvigneau JC, Forbes-Robertson S, Yang L, Klampfl K, Romanos J, Muller S, Gerlach M, Mehler-Wex C (2010) Expression analyses of the mitochondrial complex I 75-kDa subunit in early onset schizophrenia and autism spectrum disorder: increased levels as a potential biomarker for early onset schizophrenia. Eur Child Adolesc Psychiatry 19 (5):441-448. doi:10.1007/s00787-009-0074-z

Tsuang MT, Nossova N, Yager T, Tsuang MM, Guo SC, Shyu KG, Glatt SJ, Liew CC (2005) Assessing the validity of blood-based gene expression profiles for the classification of schizophrenia and bipolar disorder: a preliminary report. Am J Med Genet B Neuropsychiatr Genet 133B (1):1-5. doi:10.1002/ajmg.b.30161

Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F (2002) Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol 3 (7):RESEARCH0034

Walitza S, Renner TJ, Dempfle A, Konrad K, Wewetzer C, Halbach A, Herpertz-Dahlmann B, Remschmidt H, Smidt J, Linder M, Flierl L, Knolker U, Friedel S, Schafer H, Gross C, Hebebrand J, Warnke A, Lesch KP (2005) Transmission disequilibrium of polymorphic variants in the tryptophan hydroxylase-2 gene in attention-deficit/hyperactivity disorder. Mol Psychiatry 10 (12):1126-1132. doi:4001734 [pii]

10.1038/sj.mp.4001734

Walitza S, Scherag A, Renner TJ, Hinney A, Remschmidt H, Herpertz-Dahlmann B, Schulz E, Schafer H, Lange KW, Wewetzer C, Gerlach M (2008) Transmission disequilibrium studies in early onset of obsessive-compulsive disorder for polymorphisms in genes of the dopaminergic system. J Neural Transm 115 (7):1071-1078. doi:10.1007/s00702-008-0051-6

Wallis D (2010) The search for biomarkers for attention deficit/hyperactivity disorder. Drug News Perspect 23 (7):438-449. doi:1472296 [pii]

10.1358/dnp.2010.23.7.1472296

Wood AC, Neale MC (2010) Twin studies and their implications for molecular genetic studies: endophenotypes integrate quantitative and molecular genetics in ADHD research. J Am Acad Child Adolesc Psychiatry 49 (9):874-883. doi:S0890-8567(10)00481-8 [pii]

10.1016/j.jaac.2010.06.006

Figure legend:

Figure 1: Receiver operating characteristic (ROC) curve for the candidate markers, dopamine transporter (*SLC6A3*), dopamine D5 receptor (*DRD5*), tryptophan hydroxylase 1 (*TPH1*), and synaptosomal-associated protein of 25 kDa (*SNAP25*) mRNA.

Supplementary figures:

Figure S1: Gene expression levels in peripheral blood samples of attention-deficit hyperactivity disorder (ADHD) patients compared with controls. Statistical significance **P*<0.05, ***P*<0.0001. Annotation: *SLC6A3*, dopamine transporter (DAT); *DRD4*, dopamine D4 receptor; *DRD5*, dopamine D6 receptor; *CRHBP*, corticotropin-releasing factor binding protein; *SNAP25*, synaptosomal-associated protein of 25 kDa; *TPH1*, tryptophan hydroxylase 1; *SLC6A4*, serotonin transporter.

Figure S2: Gene expression levels in peripheral blood samples from attention-deficit hyperactivity disorder (ADHD) patients compared with controls showing the genotype influence of expression. The genotypes are: *SNAP25* +2015 (rs6077690), +80609 (rs363006), -523 (rs6039769), *TPH2* (rs4570625), and *SLC6A3* (3'UTR VNTR). Statistical significance **P*<0.05, ***P*<0.001. Annotation: *SLC6A3*, dopamine transporter (DAT); *SNAP25*, synaptosomal-associated protein of 25 kDa; *TPH1*, tryptophan hydroxylase 1; *TPH2*, tryptophane hydroxylase 2.

Table 1: Gene expression patterns in attention-deficit hyperactivity disorder (ADHD) patients versus control subjects and genotype effects.

	ADHD subjects (mean ± SD)		**Controls subjects** (mean ± SD)	***P***	**Genotype Effects (*P*-values)**	
					ADHD	***Controls***
SLC6A3	.37 ± .19	>	.25 ± .09	0.001	.537	.783
DRD4	.36 ± .27	>	.24 ± .14	0.005	.684	.206
DRD5	.13 ± .09	>	.07 ± .04	<0.001	N/A	N/A
CRHBP	.17 ± .11	<	.21 ± .12	0.037		
SNAP25	.41 ± .29	>	.24 ± .08	0.001	.311	.126
TPH1	.44 ± .30	>	.19 ± .06	<0.001	.800	.598
SLC6A4	.16 ± .09		.17 ± .09	N/S	N/D	N/D

Annotation: N/A, data not available since no genotyping was conducted; N/S, not significant; N/D, not done (since the main result was not significant); *SLC6A3*, dopamine transporter (DAT); *DRD4*, dopamine D4 receptor; *DRD5*, dopamine D6 receptor; *CRHBP*, corticotropin-releasing factor binding protein; *SNAP25*, synaptosomal-associated protein of 25 kDa; TPH1, tryptophan hydroxylase; *SLC6A4*, serotonin transporter.

Table 2: Individual logistic regression models for each biomarker

	***P*-value**	**OR (95% CI)**	**AUC**
Age	0.018	0.95 (0.92–0.99)	0.646
Gender	0.229	0.63 (0.29–1.35)	0.558
SLC6A3	0.003	1.06 (1.02–1.10)	0.694
DRD4	0.015	1.04 (1.01–1.06)	0.656
DRD5	0.001	1.21 (1.08–1.35)	0.749
CRHBP	0.074	0.97 (0.94–1.00)	0.381
SNAP25	0.002	1.05 (1.02–1.08)	0.689
TPH1	< 0.001	1.09 (1.05–1.14)	0.812
SLC6A4	0.705	0.99 (0.95–1.04)	0.478

OR: higher value refers to attention-deficit hyperactivity disorder (ADHD) cases, P <0.05 (uncorrected).

Annotation: AUC = area under the curve (for gender 1= male, 2 = female); OR, odds ratio; *SLC6A3*, dopamine transporter (DAT), *DRD4*, dopamine D4 receptor; *DRD5*, dopamine D6 receptor; *CRHBP*, corticotropin-releasing factor binding protein; *SNAP25*, synaptosomal-associated protein of 25 kDa; TPH1, tryptophan hydroxylase; *SLC6A4*, serotonin transporter.

line figure 1
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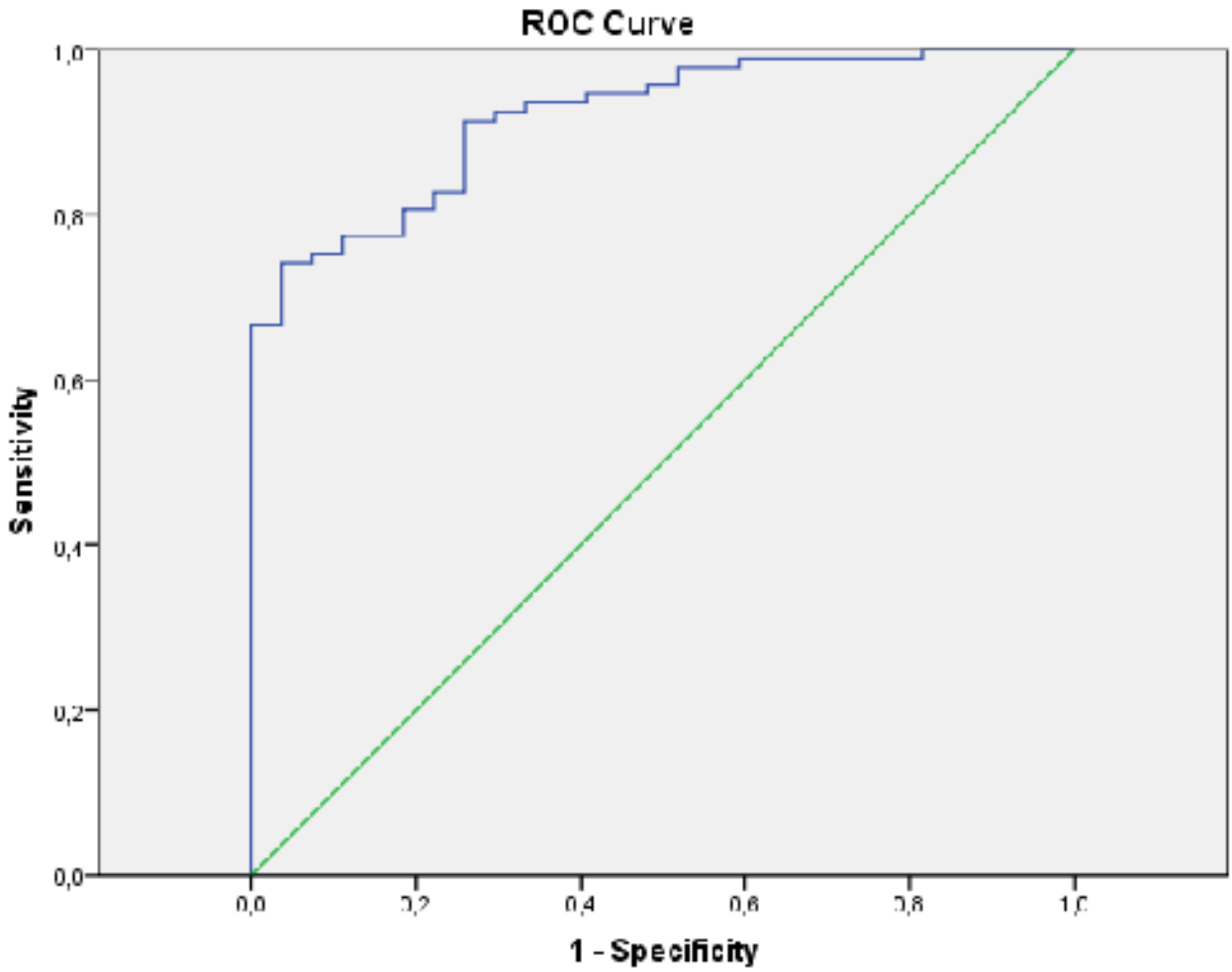

Supplementary Table S1: Characteristics of the attention deficit disorder hyperactivity (ADHD) patients and controls.

	ADHD (N = 108)		**Controls (N = 35)**		
	Mean	SE	Mean	SE	p
Age (years)	34.70	1.61	39.63	1.02	.009
	N	%	N	%	p
Gender (male)	46	54.3	19	42.6	.247
Medication[a] Yes	32	29.6			
No	55	50.9	0	0	n.a.
Unknown	21	19.4			
Comorbidities lifetime					
• Alcohol	12	11.1			.034
• Cannabis	24	22.2	-	-	.002
• Other	12	11.1			.034
Comorbidities current					
• Alcohol	7	6.5			n.s. (.122)
• Cannabis	3	2.8	-	-	n.s. (.073)
• Other[b]	1	0.9			n.s. (.097)
ADHD					
	N_{child}	N_{child} %	N_{adult}	N_{adult} %	
Subtypes[c]					
• inattentive	35	32.4	23	21.3	
• hyperactive	9	8.3	6	5.5	
• combined	58	53.7	76	70.4	

[a] Medication- methylphenidate.

[b] Other comorbidities- sedatives, stimulants, opiates, cocaine, hallucinogen, multiple drug dependency, etc.

[c] Missing values might lead to differences in total numbers

n.s not significant; ADHD, attention-deficit hyperactivity disorder; SE, standard error.

Supplementary Table S2: List of genes investigated

Gene Name	Symbol	Gene Bank Accession Number	Qiagen Primer Assay Cat. No.
18s ribosomal	**R18S**	V01270	QT 00199367
Beta-actin	**ACTB**	NM_001101	QT 00095431
Aminolevulinate delta synthase 1	**ALAS1**	NM_000688	QT 00073122
Glyceraldehydes-3-phosphate dehydrogenase	**GAPDH**	NM_002046	QT 00079247
Ribosomal protein L13a	**RPL13A**	NM_012423	QT 00089915
Peptidylprolyl isomerase A (cyclophilin A)	**PPIA**	NM_021130	QT 01669542
Solute carrier family 6 (Neurotransmitter transporter, dopamine member 3)	SLC6A3 (DAT1)	NM_001044	F: 5´CTC TGC GAG GCG TCT GTT 3´ R: 5´AGC TGG AGA AGG CGA TCA G 3´
Dopamine receptor D4	DRD4	NM_000797	QT00204316
Dopamine receptor D5	DRD5	NM_000798	QT00217651
Tryptophan Hydroxylase 1	TPH1	NM_004179	QT00045346
Solute carrier family 6 (neurotransmitter transporter, serotonin), member 4	SLC6A4 (SERT)	NM_001045	QT00058380
Synaptosomal-associated protein 25 kDa	SNAP25	NM_003081	QT00028546
Corticotropin-releasing factor binding protein	CRHBP	NM_001882	QT00019257

Bold- Reference genes

Supplementary Table S3: Genotype and allele frequencies for attention-deficit hyperactivity disorder (ADHD) patients and healthy controls.

Gene		**Genotype**			**HWE**		**Allele-frequencies**			***P***
					Chi²	***P***				
SNAP25		AA	AT	TT			A (%)	T (%)	A/T	
+2015 (rs6077690)	ADHD	17	57	33	.86	.35	91 (43)	123 (57)	.73	
	controls	9	19	6	.54	.46	37 (54)	31 (46)	1.19	.166
		AA	AG	GG			A	G	A/G	
+80609 (rs363006)	ADHD	4	32	71	.03	.87	40 (19)	174 (81)	.23	
	controls	0	6	28	.32	.57	6 (9)	62 94)	.10	.061
		AA	AC	CC			A	C	A/C	
-523 (rs6039769)	ADHD	9	48	50	.28	.59	66 (31)	148 (69)	.45	
	controls	6	17	11	.02	.897	29 (43)	39 (57)	.74	.079
SERT		SS	SL	LL			S	L	S/L	
(HTTLPR tri-allelic)	ADHD	20	51	36	.07	.797	91 (43)	123 (57)	.73	
	controls	6	18	10	.18	.668	30 (44)	38 (56)	.79	.888
TPH2 (rs4570625)		AA	AC	CC			A	C	A/C	
	ADHD	14	58	34	1.92	.165	86 (40)	126 (60)	.68	
	controls	6	16	12	.03	.868	28 (41)	40 (59)	.70	.929
DAT1 (3'UTR VNTR)		9/9	9/10	10/10			9R	10R	9/10	
	ADHD	3	40	63	1.29	.255	46 (22)	166 (78)	.28	.103
	controls	4	14	16	.12	.729	22 (32)	46 (68)	.48	
DRD4 (Exon 3 VNTR)		other	7/other	7/7			other	7	7/other	
	ADHD	69	34	5	.09	.758	172 (80)	44 (20)	3.91	.295
	controls	26	8	1	.16	.693	60 ()	10	6.00	

Hardy-Weinberg-Equilibrium (HWE) was tested with a Chi² procedure. For comparison of allele frequencies between groups, we used Fisher's exact test. SNAP25, synaptosomal-associated protein 25 kDa; SERT, serotonin transporter; TPH2, tryptophan hydroxylase 2; DAT1, dopamine transporter (SLC6A3); DRD4, dopamine receptor D4.

Supplementary Table S4: Association of each genotype with diagnostic status

	Chi²	***P***
SNAP25		
+2015 (rs6077690)	3.22	0.200
+80609 (rs363006)	3.65	0.161
-523 (rs6039769)	3.45	0.178
SERT (HTTLPR tri-allelic)	0.30	0.859
TPH2 (rs4570625)	0.72	0.697
DAT1 (3'UTR VNTR)	4.89	0.087
DRD4 (Exon 3 VNTR)	1.30	0.523

Annotation: SNAP25, synaptosomal-associated protein 25 kDa; SERT, serotonin transporter; TPH2, tryptophan hydroxylase 2; DAT1, dopamine transporter (SLC6A3); DRD4, dopamine receptor D4.

Supplementary Table S4: Correlations between age and gene expression

	Correlation coefficient	***p*-value**
SLC6A3	- 0.087	0.323
DRD4	- 0.042	0.623
DRD5	- 0.035	0.687
TPH1	- 0.174	0.039
SERT	0.140	0.105
SNAP25	0.136	0.115
CRHBP	- 0.071	0.412

Annotation: SNAP25, synaptosomal-associated protein 25 kDa; SERT, serotonin transporter; TPH1, tryptophan hydroxylase 1; SLC6A3, dopamine transporter (DAT); DRD4, dopamine receptor D4; DRD5, dopamine receptor D5; CRHBP, corticotropin-releasing factor binding protein

Supplementary Table S5: Binary logistic regression model for SLC6A3, DRD5, TPH1 and SNAP25

	***P*-value**	**OR (95% CI)**	**Correct classification**	**Total AUC**
TPH1	0.001	1.11 (1.04–1.18)	74.8 %	0.917
DRD5	0.010	1.28 (1.06–1.53)	82.4 %	
SNAP25	0.013	1.06 (1.01–1.12)	83.2 %	
SLC6A3	0.049	1.06 (1.00–1.11)	86.6 %	

Annotation: AUC, area under the curve; OR, odds ration; SNAP25, synaptosomal-associated protein 25 kDa; TPH1, tryptophan hydroxylase 1; SLC6A3, dopamine transporter (DAT); DRD5, dopamine receptor D5.

Suppl Figure S1

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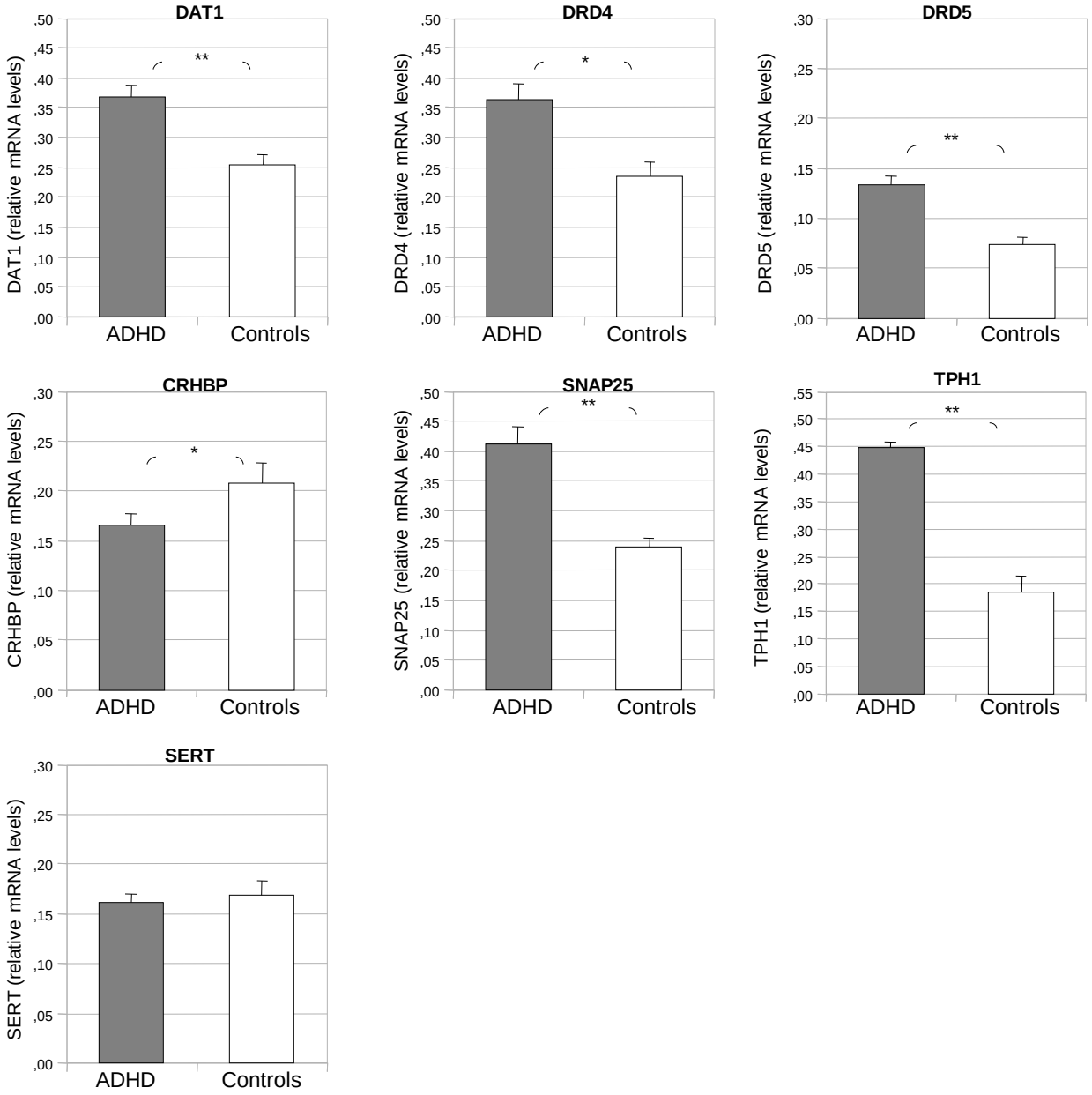

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Suppl Figure S2

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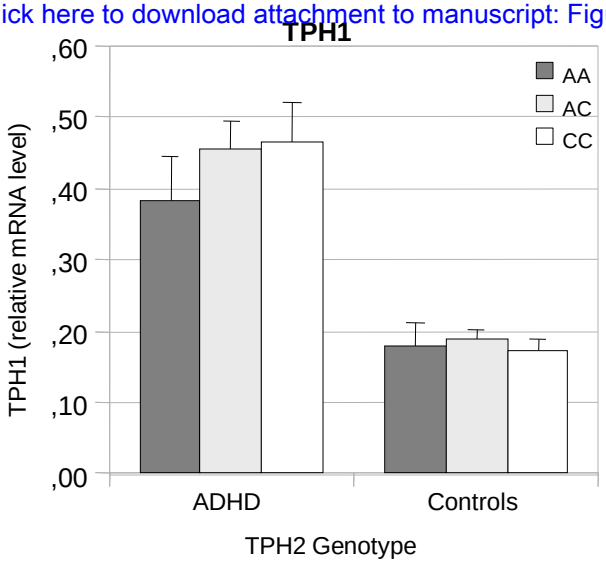

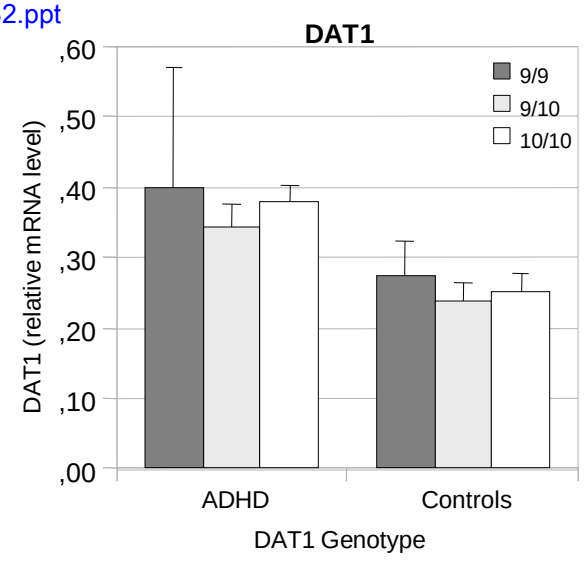

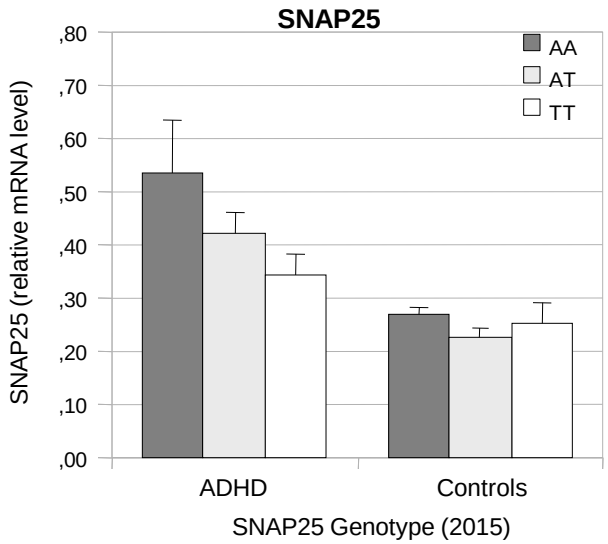

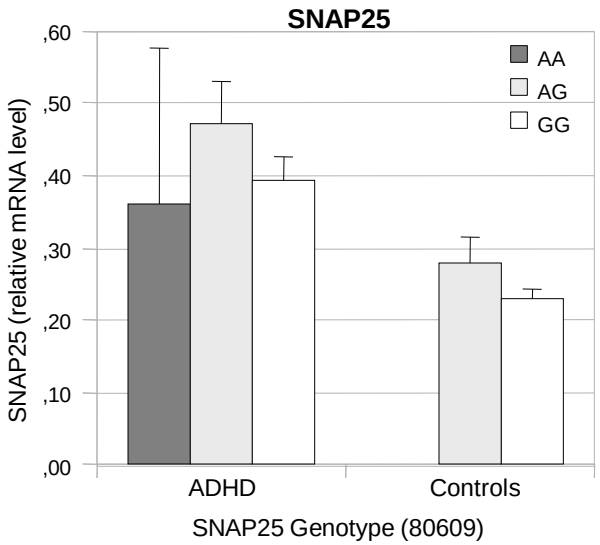

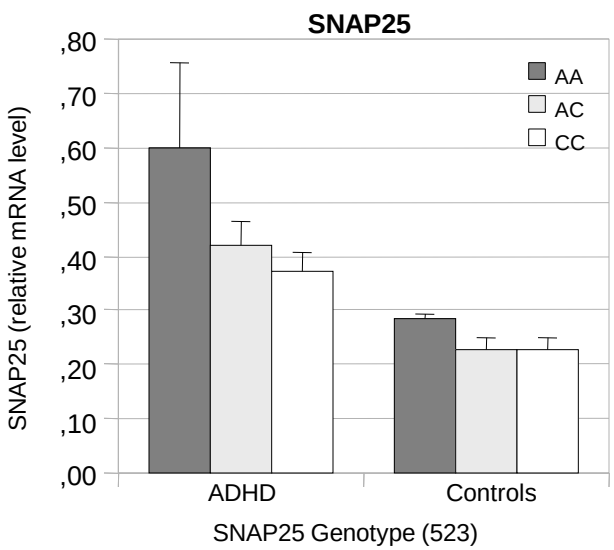

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