



King's Research Portal

DOI:

[10.1016/j.biopsycho.2016.05.002](https://doi.org/10.1016/j.biopsycho.2016.05.002)

Document Version

Peer reviewed version

[Link to publication record in King's Research Portal](#)

Citation for published version (APA):

Heinrich, A., Müller, K. U., Banaschewski, T., Barker, G. J., Bokde, A. L. W., Bromberg, U., Büchel, C., Conrod, P., Fauth-Bühler, M., Papadopoulos, D., Gallinat, J., Garavan, H., Gowland, P., Heinz, A., Ittermann, B., Mann, K., Martinot, J-L., Paus, T., Pausova, Z., ... Nees, F. (Accepted/In press). Prediction of alcohol drinking in adolescents: personality-traits, behavior, brain responses, and genetic variations in the context of reward sensitivity. *Biological Psychology*. <https://doi.org/10.1016/j.biopsycho.2016.05.002>

Citing this paper

Please note that where the full-text provided on King's Research Portal is the Author Accepted Manuscript or Post-Print version this may differ from the final Published version. If citing, it is advised that you check and use the publisher's definitive version for pagination, volume/issue, and date of publication details. And where the final published version is provided on the Research Portal, if citing you are again advised to check the publisher's website for any subsequent corrections.

General rights

Copyright and moral rights for the publications made accessible in the Research Portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognize and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the Research Portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the Research Portal

Take down policy

If you believe that this document breaches copyright please contact librarypure@kcl.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.

Accepted Manuscript

Title: Prediction of alcohol drinking in adolescents:
personality-traits, behavior, brain responses, and genetic
variations in the context of reward sensitivity

Author: Angela Heinrich Kathrin U. Müller Tobias
Banaschewski Gareth J. Barker Arun L.W. Bokde Uli
Bromberg Christian Büchel Patricia Conrod Mira
Fauth-Bühler Dimitri Papadopoulos Jürgen Gallinat Hugh
Garavan Penny Gowland Andreas Heinz Bernd Ittermann Karl
Mann Jean-Luc Martinot Tomáš Paus Zdenka Pausova
Michael Smolka Andreas Ströhle Marcella Rietschel Herta
Flor Gunter Schumann Frauke Nees



PII: S0301-0511(16)30181-8
DOI: <http://dx.doi.org/doi:10.1016/j.biopsycho.2016.05.002>
Reference: BIOPSY 7206

To appear in:

Received date: 22-9-2015
Revised date: 9-5-2016
Accepted date: 11-5-2016

Please cite this article as: Heinrich, Angela, Müller, Kathrin U., Banaschewski, Tobias, Barker, Gareth J., Bokde, Arun L.W., Bromberg, Uli, Büchel, Christian, Conrod, Patricia, Fauth-Bühler, Mira, Papadopoulos, Dimitri, Gallinat, Jürgen, Garavan, Hugh, Gowland, Penny, Heinz, Andreas, Ittermann, Bernd, Mann, Karl, Martinot, Jean-Luc, Paus, Tomáš, Pausova, Zdenka, Smolka, Michael, Ströhle, Andreas, Rietschel, Marcella, Flor, Herta, Schumann, Gunter, Nees, Frauke, Prediction of alcohol drinking in adolescents: personality-traits, behavior, brain responses, and genetic variations in the context of reward sensitivity. *Biological Psychology* <http://dx.doi.org/10.1016/j.biopsycho.2016.05.002>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Prediction of alcohol drinking in adolescents: personality-traits, behavior, brain responses, and genetic variations in the context of reward sensitivity

Angela Heinrich, PhD¹, Kathrin U. Müller, MS², Tobias Banaschewski, MD, PhD³, Gareth J. Barker, PhD⁴, Arun L. W. Bokde, PhD⁵, Uli Bromberg, PhD⁶, Christian Büchel, PhD⁶, Patricia Conrod, PhD^{4,7}, Mira Fauth-Bühler, PhD⁸, Dimitri Papadopoulos, PhD⁹, Jürgen Gallinat, MD²¹, Hugh Garavan, PhD^{11,12}, Penny Gowland, PhD¹³, Andreas Heinz, MD¹⁰, Bernd Ittermann, PhD¹⁴, Karl Mann, MD⁸, Jean-Luc Martinot, MD¹⁵, Tomáš Paus, MD^{16,17}, Zdenka Pausova, MD¹⁸, Michael Smolka, MD², Andreas Ströhle, MD¹⁰, Marcella Rietschel, MD¹⁹, Herta Flor, PhD^{1*}, Gunter Schumann, MD^{4,20*}, Frauke Nees, PhD^{1*}, and the IMAGEN consortium

*these authors contributed equally

¹Department of Cognitive and Clinical Neuroscience, Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany; ²Neuroimaging Center, Department of Psychiatry and Psychotherapy, Technische Universität Dresden, Germany; ³Department of Child and Adolescent Psychiatry and Psychotherapy, Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany; ⁴Institute of Psychiatry, Psychology & Neuroscience, King's College London, United Kingdom; ⁵Institute of Neuroscience and Discipline of Psychiatry, School of Medicine, Trinity College Dublin, Dublin, Ireland; ⁶Universitätsklinikum Hamburg Eppendorf, Hamburg, Germany; ⁷Department of Psychiatry, Université de Montreal, CHU Ste Justine Hospital, Canada; ⁸Department of Addictive Behavior and Addiction Medicine, Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany; ⁹Neurospin, Commissariat à l'Energie Atomique et aux Energies Alternatives, Paris, France; ²¹Department of Psychiatry and Psychotherapy, University Medical Center Hamburg-Eppendorf (UKE), Hamburg, Germany; ¹⁰Department of Psychiatry and Psychotherapy, Campus Charité Mitte, Charité – Universitätsmedizin Berlin, Germany; ¹¹Institute of Neuroscience, Trinity College Dublin, Dublin, Ireland; ¹²Departments of Psychiatry and Psychology, University of Vermont, USA; ¹³School of Physics and Astronomy, University of Nottingham, United Kingdom; ¹⁴Physikalisch-Technische Bundesanstalt (PTB), Braunschweig und Berlin, Germany; ¹⁵Institut National de la Santé et de la Recherche Médicale, INSERM Unit 1000 “Neuroimaging & Psychiatry”, Université Paris Sud, Orsay, and AP-HP Department of Adolescent Psychopathology and Medicine, Maison de Solenn, Université Paris Descartes, Paris, France; ¹⁶Rotman Research Institute, University of Toronto, Toronto, Canada; ¹⁷School of Psychology, University of Nottingham, United Kingdom; ¹⁸The Hospital for Sick Children, University of Toronto, Toronto, Canada; ¹⁹Department of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, Mannheim, Germany; ²⁰MRC Social, Genetic and Developmental Psychiatry (SGDP) Centre, London, United Kingdom;

Correspondence to:

Angela Heinrich, PhD, Department of Cognitive and Clinical Neuroscience, Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University; J 5, 68159 Mannheim, Germany, Tel: 0621-1703-6326, Fax: 0621-1703-6305, email: angela.heinrich@zi-mannheim.de

Highlights

- Prediction of alcohol consumption in 736 adolescents using longitudinal data
- Reward-related personality, behavior, brain responses, and genetic variations
- Personality is most important in explaining early alcohol consumption
- Personality and genetic variations are equally important in longitudinal prediction
- Genetic variations are most important for increase in alcohol consumption

Abstract

Adolescence is a time that can set the course of alcohol abuse later in life. Sensitivity to reward on multiple levels is a major factor in this development. We examined 736 adolescents from the IMAGEN longitudinal study for alcohol drinking during early (mean age = 14.37) and again later (mean age = 16.45) adolescence. Conducting structural equation modeling we evaluated the contribution of reward-related personality traits, behavior, brain responses and candidate genes. Personality seems to be most important in explaining alcohol drinking in early adolescence. However, genetic variations in *ANKK1* (rs1800497) and *HOMER1* (rs7713917) play an equal role in predicting alcohol drinking two years later and are most important in predicting the increase in alcohol consumption. We hypothesize that the initiation of alcohol use may be driven more strongly by personality while the transition to increased alcohol use is more genetically influenced.

Keywords: Prediction of alcohol consumption; adolescents; longitudinal study; personality; behavior; brain responses; genetic variations

Introduction

Adolescence is a phase with a high risk for first alcohol consumption and the transition to future alcohol abuse (e.g. Kim et al., 2011). The risk for prospective development of alcohol abuse increases the younger adolescents are when having their first drink (e.g. Behrendt et al., 2009). This suggests that the early identification of risk factors and subsequent interventions could best prevent alcohol abuse. Several factors contribute to the initiation of alcohol use. One aspect is the social environment with family and peer factors strongly influencing substance use initiation (e.g. Oxford et al., 2001). Besides these external factors, several individual aspects have been identified. Among them sensitivity to reward has been assumed to play a major role. In a previous study (Nees et al., 2012), we showed that neural responses to reward, reward-related personality traits, and reward-related behavioral data are correlated with alcohol consumption in early adolescence. This study suggested that personality correlates are more strongly related to alcohol drinking behavior than neural activation or behavior (Nees et al., 2012). However, in this study, we neither tested genetic effects nor modeled the development of alcohol drinking behavior over time. In the present study we therefore extended our previous findings using follow-up data assessed two years after the first study and we also analyzed the effects of candidate genetic variations in a considerably enlarged sample compared to our first study.

Reward processing associated with alcohol use can be examined using several individual interrelated traits including the levels of personality, behavior, brain responses, and genetic variations. Reward

sensitivity on all these connected levels has been shown to be related to the initiation of drinking or the development of alcohol abuse. To build up on the model of our first study and thus determine the additional influence of genetic factors and changes in alcohol consumption over time, we used all previously examined variables, i.e. the variables representing reward-related personality traits, reward-related behavior, and brain responses to reward were the same as in our first study (Nees et al., 2012). Likewise, we used the total score of the Alcohol Use Disorders Identification Test (AUDIT; Saunders et al., 1993) as outcome variable to account for the fact that even lower levels of alcohol consumption during adolescence might indicate hazardous drinking (e.g. Chung et al., 2000). For personality, sensation seeking, novelty seeking, impulsivity and extraversion are the most important components describing reward-related personality and have been found to be strongly associated with early initiation of hazardous alcohol use and as predictors for later alcohol abuse (e.g. Ayer et al., 2011; Cloninger, 1987; Hittner and Swickert, 2006). On a behavioral level, risk taking has been related to increased alcohol use as well as impaired control over alcohol use (Leeman et al., 2012; MacPherson et al., 2010). Here, the increased preference of a smaller immediate reward towards a larger delayed reward (de Wit, 2009) clearly shows its connection with reward sensitivity. An increased responsiveness in the brain reward system is also associated with reward sensitivity (Hahn et al., 2009) which makes the involved structures essential for the investigation of reward sensitivity in the context of alcohol consumption. The anterior cingulate cortex, the ventral pallidum, the ventral striatum, the orbitofrontal cortex, and the dopaminergic midbrain neurons are key structures of this network, and the amygdala, thalamus, orbital prefrontal cortex and the hippocampus are also involved in the regulation of reward (Haber and Knutson, 2010). We analyzed brain responses to a Monetary Incentive Delay (MID) task (Knutson et al., 2001). We included regions of interest in our analysis which have been shown to be associated with reward sensitivity during an MID task on the one hand and with alcohol consumption on the other hand. Hence, we included the striatum, amygdala, nucleus accumbens, nucleus caudatus, thalamus, insula, putamen, cerebellar vermis, and the prefrontal cortex which all met these criteria (Gilman et al., 2012; Haber and Knutson, 2010; Hahn et al., 2009; Nees et al., 2012; Oberlin et al., 2012) and have been shown to be valid predictors in our previous study (Nees et al., 2012).

Genetic factors play an important role in the development of alcohol abuse and several genetic risk factors for alcoholism have been identified (e.g. Morozova et al., 2012). Regarding our aim to establish a model on alcohol consumption specifically in the context of reward sensitivity, it has to be considered that reward sensitivity per se is also genetically influenced (Nees et al., 2013; Richter et al., 2013; Rietschel et al., 2010; Stacey et al., 2012). This suggests that the appropriate genetic factors might also contribute to alcohol drinking behavior via modifying reward sensitivity. To determine the effects of various domains including genetic variations on any mental symptom, specific hypotheses on single chosen variables are mandatory to exclude the risk of false positive results. Thus, one selection criterion was that the single nucleotide polymorphisms (SNPs) to be included in our analysis had to be associated with reward sensitivity. The second criterion for the selected SNPs was that they should be associated with alcohol use or addiction not only in our sample but in patient studies to ensure the clinical relevance of the selected SNPs. This led to the following candidate genes and specific SNPs: *ANKK1* (rs1800497), *RASGRF2* (rs26907), and a regulatory region of *HOMER1* (rs7713917) (Richter et al., 2013; Rietschel et al., 2010; Stacey et al., 2012). *ANKK1* encodes a protein belonging to a protein kinase family, which is involved in signal transduction and may influence dopaminergic signaling in the striatum (Klein et al., 2007; Neville et al., 2004). As striatal dopamine responses to salient alcohol cues may be an inherited risk factor for alcoholism (Oberlin et al., 2013)

this directly implies *ANKK1* in the formation of alcoholism. The TaqIA SNP (rs1800497) in *ANKK1* is one of the most widely examined genetic variations in mental disorders and its TaqIA A1 polymorphism is associated with alcoholism and other addiction disorders (Ponce et al., 2009). An imaging genetics study has shown that a variation in rs1800497 interacts with motivation in a reward flanker task (Richter et al., 2013), indicating a link between *ANKK1* and reward deficiency. Homer proteins regulate extracellular glutamate levels in cortical-limbic brain regions as well as signal transduction, synaptogenesis and receptor trafficking (Szumlinski et al., 2006). Homer isoforms seem to influence the processing of reward anticipation; especially carriers of the A-allele of rs7713917 (located in a regulatory region of *HOMER1*) seem to be at a higher risk for a dysregulation of cognitive and motivational processes by influencing prefrontal activity during anticipation of reward (Rietschel et al., 2010). *HOMER1* has also been explicitly implicated in the formation of alcoholism (Szumlinski et al., 2006). *RASGRF2* encodes a protein that mediates Ca^{2+} -dependent activation of the extracellular signal-regulated kinases pathway and has been shown to be associated with reward sensitivity and alcohol intake via a hypothesized regulation of mesolimbic dopamine neuron activity (Stacey et al., 2012).

The first aim of the present study was to expand previous findings on the relative contribution of reward-related variables on the levels of personality, behavior, and brain responses to alcohol drinking behavior now additionally examining candidate genetic factors. Secondly, we conducted follow-up analyses on alcohol drinking behavior two years later and established predictive models with longitudinal data identifying risk factors for the subsequent development of hazardous alcohol drinking. By the use of factor analyses we developed different categories (personality, behavior, brain responses, and candidate genetic variations). Using structural equation modeling these categories were correlated with alcohol drinking behavior at early adolescence and we established a predictive model for alcohol drinking behavior at late adolescence, and a model on increase of alcohol consumption between these time points.

Methods

Participants

Within the IMAGEN study (Schumann et al., 2010) adolescents were recruited from the general public in Germany, the United Kingdom, Ireland, and France. Exclusion criteria were serious medical conditions, pregnancy, previous head trauma with unconsciousness, and any contra indications for functional magnetic resonance imaging (fMRI) examinations. We analyzed the association of personality variables, behavioral data, fMRI data and candidate genetic variants of adolescents (mean age = 14.37 years, sd = 0.68 years; henceforth named early adolescence) and AUDIT score at the same time point and again two years later in the same adolescents (mean age = 16.4, years, sd = 0.51 years; henceforth named late adolescence). Out of the IMAGEN sample 736 (389 female) subjects provided complete data sets on all relevant variables and thus were included in the analysis. This sample includes 185 participants of our previous study (Nees et al., 2012) who provided complete data sets in the follow up examination. The newly added subjects (N=551) showed the same results as in our previous study with personality being the most important factor explaining alcohol drinking behavior in early adolescence (see supplemental Figure S1). The study was approved by the local ethics committees and was conducted in accordance with the declaration of Helsinki of 1975 as revised in 1983. After complete description of the study to the subjects and their parents, written informed consent was obtained.

Personality and behavioral data acquisition

The Temperament and Character Inventory-Revised (TCI-R (Cloninger et al., 1991)), the Substance Use Risk Profile Scale (SURPS (Woicik et al., 2009)), and the Neuroticism-Extraversion-Openness Five Factor Inventory (NEO (Costa and McCrae, 1997)) were employed to examine novelty seeking, sensation seeking, impulsivity, and extraversion. These subscales describe reward-related personality traits and are strongly associated with early initiation of hazardous alcohol use and as predictors for later alcohol abuse (e.g. Ayer et al., 2011; Cloninger, 1987; Hittner and Swickert, 2006). Furthermore, risk adjustment, risk taking, and delay aversion were surveyed using the Cambridge Gambling Task (CGT) from the Cambridge Cognition Neuropsychological Test Automated Battery (CANTAB; Cambridge Cognition). Altering numbers of red and blue boxes were presented on a screen and adolescents had to guess whether a yellow token was hidden in either a blue or a red box. We reduced the time between stakes from 5 to 2 s to make it more interesting for adolescents. The tests were completed with the help of trained research assistants within a laboratory.

Alcohol use

Alcohol use and alcohol-related problems were examined by the Alcohol Use Disorders Identification Test (AUDIT; Saunders et al., 1993). We applied the total score of the AUDIT. Whilst 142 female and 143 male participants scored 0 at early adolescence, meaning that they had never used alcohol before, no participant scored 0 two years later (see Figure 1).

fMRI – paradigm and proof of principle

To examine the processing of reward, we used a modified version of the monetary incentive delay (MID) task adapted from Knutson *et al.* (Knutson et al., 2001), which resulted in stable brain and behavioral response patterns in adolescents and as a measure of reward sensitivity, also in the context of alcohol consumption (e.g. Nees et al., 2012; Nymberg et al., 2014; Schneider et al., 2012). In this task the participants had to hit a square randomly appearing on a screen and could win points if successful. The number of points which could be won was indicated beforehand by either a triangle (no points), or a circle with one line (two points), or a circle with two lines (ten points). These three conditions consisted of 22 trials each with an adjusted duration to ensure hit rates of 66%. At the end of the sessions, the participants received one candy for every five points, which was found by a pilot study to be rewarding. Visual Basic 2005, NET Framework Version 2.0, and the visual and response grip system from Nordic Neuro Lab (NordicNeuroLab AS, Bergen, Norway) were used for presentation and recording.

fMRI – examination

Scanning was executed at the IMAGEN sites with 3T whole body MRI systems with a 12 channel head coil [Siemens AG (Munich, Germany), Philips Healthcare (Best, the Netherlands), General Electric Healthcare (Chalfont StGiles, Great Britain), and Bruker Biospin (Billerica, MA, USA)]. Forty slices (2.4 mm, 1-mm gap) were obtained using a T2*-weighted gradient echo planar imaging sequence (EPI) and the following image parameters: repetition time (TR) = 2200 msec; echo time (TE) = 30 msec; in-plane voxel size = 64 * 64 pixels over a 21.8 cm field of view. The plane of acquisition was sloped to the anterior-posterior commissure line (rostral > caudal). A 3D magnetization prepared gradient echo sequence (MPRAGE) of the whole brain was acquired for anatomical information with parameters based on the Alzheimer's Disease Neuroimaging Initiative (ADNI) protocol

(<http://adni.loni.usc.edu/methods/mri-analysis/mri-acquisition/>). Parameters compatible with all scanners were applied to guarantee comparability of data (Schumann et al., 2010).

fMRI – data analysis

Data were analyzed using Statistical Parametric Mapping (SPM8, Wellcome Department of Imaging Neuroscience, University College London, UK). Individual analyses included slice time correction, spatially realignment correcting for head movement, and non-linearly warping on the Montreal Neurological Institute space via a norm echo planar imaging template relying on the average of the mean images of 400 adolescents. This norm template image (53*63*46 voxels) was applied to all functional T2* data and voxels were resampled at a resolution of 3*3*3 mm. Functional data were smoothed for group analysis with an isotropic Gaussian kernel (5 mm full-width at half-maximum). Within the first-level statistics regressors were modeled. We distinguished between reward magnitudes of no win, small win, and big win as subject-specific regressors in the MID task and modeled reward anticipation as predictor variable. Modeling took place using the general linear model on a voxel by voxel basis with an autoregressive noise model against a design matrix. Estimated movement was implied by 18 additional columns (three translational, three rotations, three quadratic and three cubic translations, three translations shifted 1 TR before, and three translations shifted 1 TR later). The second-level analysis included a non-sphericity correction to address the problem of non-independent data within subjects and error variance heterogeneity. We analyzed weighted mean blood oxygenation level dependent signal change in regions of interest (ROIs) with probabilistic anatomical masks (Nielsen and Hansen, 2002) thresholded with a fractional intensity of ≥ 0.5 . We followed the established literature on reward processing (e.g. Knutson et al., 2001), which suggests the analyses of the weighted mean BOLD signal change of designated regions of interests for the anticipation of big versus small win as a measure of reward sensitivity. We decided to use the contrast big versus small win over big versus no win, because we aimed at examining brain responses when subjects are engaged in a rewarding context, i.e. when they are faced with different reward magnitudes instead of without reward. This second situation includes some components of punishment, which was not the focus of the present study (cf. Nees et al., 2012).

Deoxyribonucleic acid (DNA) extraction and genotyping

DNA extraction was operated in a semi-automated manner (Nees et al., 2013; Schumann et al., 2010). For genome-wide genotyping of ~600 000 autosomal SNPs the Illumina Quad 610 chip (Illumina, San Diego, CA, USA) was employed. DNA purification was performed by the Centre National de Génotypage in Paris, France. DNA was extracted from whole-blood samples (~10mL) and preserved in BD Vacutainer EDTA tubes (Becton, Dickinson and Company) using the Genra Puregene Blood Kit (QIAGEN) according to the manufacturer's instructions. See Table 1 for demographic details. None of the genetic variants showed significant differences in genotypes for age or sex ($p > 0.5$).

Exploratory factor analysis

We chose a two-step approach (Factor analysis / Structural equation modeling) to compare our findings to our previous study which used this procedure (Nees et al., 2012). The exploratory factor analysis aimed on the one hand to confirm the a priori hypothesized constructs personality, behavior, brain responses, and candidate genetic variations, and to exclude that variables within one category

have stronger associations with variables out of another category. This would cause problems in the subsequent structural equation modeling by not representing separated constructs. On the other hand we aimed to test how many factors are constituted by the included SNPs. Thus, we implemented variables that have previously shown to be involved in reward processing (Gilman et al., 2012; Nees et al., 2012; Nees et al., 2013; Oberlin et al., 2012; Richter et al., 2013; Rietschel et al., 2010; Stacey et al., 2012): Personality (novelty seeking, impulsivity, extraversion, sensation seeking), behavior (risk adjustment, delay aversion, risk taking), brain responses (reward anticipation in the striatum, amygdala, nucleus accumbens, nucleus caudatus, thalamus, insula, putamen, cerebellar vermis, and prefrontal cortex), and genetic variation (rs7713917, rs26907, rs1800497). An exploratory factor analysis was conducted including all participants (N=736) using IBM SPSS Statistics 20 to obtain underlying latent variables. To be sure not to enforce the formation of the hypothesized four domains personality, behavior, brain responses, and genetic variations, the chosen variables could load freely to any latent variable, i.e. variables were not forced to load by domain. The analyses comprised orthogonal rotation and the Kaiser Criterion with Eigen values > 1 as well as the Scree test were used to determine the number of latent variables.

Structural equation modeling

We performed structural equation modeling using IBM SPSS AMOS 21 to determine the impact of the latent variables (ROIs for brain responses, personality, behavior, and genetic variations) on AUDIT score. We used three models: one with AUDIT at early adolescence score as outcome variable (basic model), one with AUDIT score two years later (prediction model), and one model involved the difference in the AUDIT scores between early and late adolescence to uncover relations that might specifically determine the increase of alcohol consumption. In addition, we tested mediational models, examining the indirect influence of genetic variations via personality, behavior, and brain responses to reward on AUDIT score for all three models. We tested the influence of the applied genetic variations on the other latent variables separately as well as simultaneously as we had no a priori hypotheses about the possible pathways in our models. This approach was chosen because one could assume that genetic variations might not directly influence drinking behavior but through altering behavioral, neural, and / or personality-related traits.

Inclusion criteria for model fit were goodness of fit indices ≥ 0.9 and a root mean square error of approximation (RMSEA) not significantly exceeding 0.05. When modeling was impossible because single variables did not explain sufficient variance in AUDIT score, these variables were removed from the model. This had to be done in one step for rs26907, striatum, nucleus accumbens, and thalamus. A new factor analysis without the omitted variables was conducted to ensure validity of the latent variables. We report the final factor analysis in the results section.

Usually the effects of single genetic variations are quite small and large sample sizes are needed to detect them. Therefore, we initially conducted our analysis with the entire sample to be able to detect small effects. Nevertheless, in neuroimaging in mental disorders the problem of inflated predictions has been raised (Whelan and Garavan, 2014). Although our sample seems to be large enough to exclude inflated predictions, the question has come up whether an overestimation of effects had occurred. Therefore, we randomly chose half of our sample and repeated the analyses. We chose this validation approach as the best way to avoid multiple testing. In order to test for center effects of our multi-center study, we applied the “dropping one site” approach for fMRI multi-center studies (Friedman et al., 2008). Using this approach, we repeated the analyses three times,

each time leaving out one randomly chosen examination site. To test for sex effects, we conducted the models separately for male and female adolescents.

Results

Sample

A detailed sample description and the distribution of AUDIT scores can be found in Table 1 and Figure 1. Female and male participants did not significantly differ in age and genotype distribution (all $p > 0.05$), thus, the data of all participants are presented together. At the first examination time point (named early adolescence in the models) participants had a mean age of 14.43 years ($sd = 0.41$, range: 12.91-16.02). About two years later they were reassessed again at a mean age of 16.45 years ($sd = 0.47$, range: 14.64-18.23). As the most significant change in AUDIT scores occurred around a score of 4 (Figure 1) and as it has previously been shown that much lower cut off values for AUDIT scores indicating hazardous drinking should be applied for adolescents than for adults (Chung et al., 2000) we contrasted adolescents with an AUDIT score of 4 or lower with adolescents scoring higher in order to classify the results (Table 1). Of the 736 participants, 448 scored 4 or lower at the second study time point. Significant differences between adolescents scoring 4 or lower and those who score higher occurred in extraversion, impulsivity, sensation seeking, novelty seeking, and risk taking (Table 1).

Factor analysis

The factor analysis revealed four latent variables explaining 54.85% of variance with the following factor loadings: Factor 1 (insula: 0.925, putamen: 0.918, caudate nucleus: 0.856, amygdala: 0.758, cerebellar vermis: 0.729, prefrontal cortex: 0.628), factor 2 (novelty seeking: 0.825, impulsivity: 0.661, extraversion: 0.602, sensation seeking: 0.512), factor 3 (risk adjustment: 0.701, delay aversion: 0.657, risk taking: 0.651), and factor 4 (rs7713917: 0.792, rs1800497: 0.584). None of the variables had significant cross-loadings on multiple factors. Due to readability we will refer to these factors as ROIs, personality, behavior, and candidate genes in our models, although these constructs are much more complex than the used nomenclature would imply (e.g. Dick et al., 2010; Smith et al., 2009).

Structural equation modeling

Using the latent variables ROIs, personality, behavior, and candidate genes as predicting factors, three models could be established on alcohol drinking behavior as measured by AUDIT score at early adolescence, late adolescence and on the increase (difference of scores at these two time points) (see Figures 2-4). For the model explaining alcohol drinking behavior at early adolescence ($R^2 = 0.13$), reward-related personality traits were the most important factor with a standardized regression weight of 0.35 (Figure 2). In predicting alcohol drinking behavior two years later ($R^2 = 0.14$), reward-related personality traits and the candidate genetic variations contributed almost equally with standardized regression weights of 0.26 and 0.27, respectively (Figure 3). Within the model predicting the increase in AUDIT score ($R^2 = 0.11$), the contribution of the factor containing rs7713917 and rs1800497 was most important with a standardized regression weight of 0.33 (Figure 4). Overall, there was a very good model fit with squared multiple correlations of 0.13, 0.14, and 0.11, goodness of fit indices were 0.944 and the RMSEA did not significantly exceed 0.05 ($p > 0.05$). The mediational models (examining the indirect influence of genetic variations via personality, behavior, and brain responses) failed to reach the inclusion criteria for model fit.

The models conducted separately for male and female adolescents showed comparable effects (see supplemental Table S1).

Split sample analyses and multi-center effects examination

Conducting the same analyses as described above with the randomly selected half of the sample revealed reward-related personality traits as most important factor on alcohol drinking at early adolescence (standardized regression weights for ROIs = 0.03, personality = 0.38, behavior = 0.02, candidate genetic variations = 0.05; R^2 AUDIT = 0.15), which decreased at late adolescence, while genetic variations in rs1800497 and rs7713917 became more powerful (standardized regression weights for ROIs = 0.06, personality = 0.30, behavior = 0.04, candidate genetic variations = 0.17; R^2 AUDIT = 0.16). In explaining the increase in AUDIT scores, candidate genetic variations were the most important factor (standardized regression weights for ROIs = 0.04, personality = 0.04, behavior = 0.02, candidate genetic variations = 0.21; R^2 AUDIT = 0.05).

The “dropping one site” tests confirmed the above reported relationships between the three models in terms of maintaining comparable standardized regression weights.

Discussion

We aimed to correlate various risk variables with alcohol drinking behavior at early adolescence, to predict alcohol drinking behavior two years later, and the increase in alcohol drinking between these two time points using reward-related personality traits, behavior, brain responses to reward, and reward-related genetic variations. Using genetic variations as a predictor for alcohol consumption is novel as is the comprehensive prediction with longitudinal data. In all three models, reward-related behavior and brain responses to reward seem to constitute the least important factors. The most remarkable result is the shifting contribution of reward-related personality traits and genetic variations in rs7713917 and rs1800497. At early adolescence, novelty seeking, impulsivity, extraversion, and sensation seeking build the most important factor in explaining alcohol drinking behavior (Figure 2). This is consistent with previous findings (Nees et al., 2012) and could now be extended to a larger sample. For the prediction of alcohol drinking behavior at late adolescence using longitudinal data the above mentioned personality traits and genetic variations in *ANKK1* and a regulatory region in *HOMER1* played an almost equal role (Figure 3). Considering the increase in AUDIT score from early to late adolescence (Figure 4), genetic variations in *ANKK1* and a regulatory region on *HOMER1* is the most important factor. At first sight it seems surprising that the contribution of these factors varies over this small amount of time. However, these data are in line with adoption studies (e.g. Cadoret et al., 1996) which have shown a far more important role of genetic factors in the transition from drug use to abuse than in drug use itself. Whereas the initiation of alcohol use may be driven by personality traits, which are associated with choosing certain peers and environments, the further development of alcohol misuse might be more strongly influenced by genetic factors. Accordingly, several studies reported that the influence of alcohol-specific genetic risk factors increased slowly through mid-adulthood while the environmental moderation was more pronounced in early and mid-adolescence than in later periods (Kendler et al., 2011). It is likely that our sample can be used for the prediction of alcohol misuse, as 386 (reflecting 52.4%) of the participants at late adolescence score 4 and higher in the AUDIT, which indicates harmful alcohol use for adolescents (Chung et al., 2000). Thus, with the model on increase of AUDIT score we identified the risk of transition to (more) hazardous alcohol use between early and later adolescence for

adolescents who did not show a high AUDIT score in early adolescence or who's AUDIT score further increased from an already risky stage.

The genetic variations analyzed in this study only represent a very small part of the overall genetic variations. We chose those candidate genes based on strong a priori hypotheses. One of the implied SNPs failed to predict alcohol drinking behavior in our model (rs26907). Probably, this variation is not involved in altering drinking behavior regarding reward sensitivity. Another explanation might be that the three initially implemented SNPs do not have comparable effects, i.e. rs26907 could still have an impact on alcohol drinking behavior, but via pathways that differ from the other two candidate genes. Although rs1800497 and rs7713917 form one latent variable, it has to be noted that their standardized regression weights of 0.1 and 0.2 for the latent variable "genetics" are quite small. This is likely a consequence of their location on two different chromosomes (11 and 5, respectively) resulting in a largely independent inheritance. Nevertheless, to ensure not to over-estimate the impact of single genetic variations, we chose the approach of building one category "candidate genetic variations" rather than analyzing the impact of each distinct SNP separately.

The factor "candidate genetic variations" seems not to influence alcohol drinking behavior via the factors personality, behavior, or brain responses in our model. These three factors are built on specific variables (novelty seeking, impulsivity, extraversion, and sensation seeking; risk adjustment, delay aversion, and risk taking; BOLD signal change for the anticipation of big versus small win in the insula, putamen, caudate nucleus, amygdala, cerebellar vermis, and prefrontal cortex), derived from previous work. However, they did not represent all the pathways that influence drinking behavior. Therefore, it is possible that the genetic variations analyzed in this study might impact on drinking behavior via other pathways which are not accounted for in our models. This hypothesis is strengthened by the fact that our mediational models, which aimed to examine the influence of the genetic variations via personality, behavior, and brain responses on AUDIT score, failed to reach the inclusion criteria for model fit. Although the present study implicates a strong influence of rs1800497 and rs7713917 on alcohol drinking behavior it has to be prospectively examined via which routes, particularly on a molecular level, their genetic influence becomes manifest. Especially those pathways including D1 and D2 receptors regulating reward would be of great interest for further studies as they have been shown to enhance the brain's reactivity to drug cues (Volkow and Morales, 2015) especially in adolescents (Perreault et al., 2014). The previously mentioned points emphasize the fact that our findings of genetic and personality-related effects have to be considered within the context of our specific model. With our model we cannot determine a particular impact of a distinct variable on alcohol consumption, but highlight the relationship between the implemented factors. Furthermore, although we have accounted for possible center effects using a "dropping one site" approach for multi-center studies (Friedman et al., 2008), it cannot be completely ruled out that the effect is not of the same magnitude in the different assessment sites of our multi-center project. One further limitation of this study is that we did not assess hormone status. Although our models do not significantly differ between male and female participants (see supplemental Table S1), it has to be considered that some adolescent typical behaviors are influenced by gonadal steroids (e.g. Joinson et al., 2012; Temple et al., 2014; Varlinskaya et al., 2013). Regarding the processing of reward, emotion, and (social) stress, also in the context of learning and memory, sex differences have been shown during the past years (e.g. Andreano and Cahill, 2009; Cahill, 2006; Caldu and Dreher, 2007; Hamann and Canli, 2004; Kajantie and Phillips, 2006; Kudielka and Kirschbaum, 2005). Hence, in the context of our models, the association between ovarian hormone level and structural changes of the brain

across the menstrual cycle (Sakaki and Mather, 2012) as well as the influence of *HOMER1* on the steroidogenic function of the adrenal glands (Grinevich et al., 2011), where gonadal steroids are synthesized in adolescents (Kiezun et al., 2015) could be of interest, for example. However, as the influences of gonadal hormones on alcohol intake and alcohol preference have been shown to be relatively modest (Varlinskaya et al., 2013) and as our models did not show sex-specific differences, we hypothesize that the mechanisms leading to an increased consumption might be comparable for boys and girls.

Our study strengthens the hypothesis that reward sensitivity plays a major role in the development of alcohol abuse, especially on the levels of personality (novelty seeking, extraversion, sensation seeking, and impulsivity) as well as genetic susceptibility.

Taken together, we highlighted that personality and candidate genetic variations are most important in predicting alcohol use and that the impact of these two factors develops differentially over the years of adolescence. This model is unique in that it includes longitudinal data of adolescents on reward sensitivity-related factors on the levels of personality, behavior, brain responses, and candidate genetic variations. Future research should focus on the potential for prevention strategies in this context.

Funding and Disclosure

This work was supported by the European Union-funded FP6 Integrated Project IMAGEN (LSHM-CT-2007-037286), the FP7 project IMAGEMEND, the Innovative Medicine Initiative Project EU-AIMS (115300-2), and Medical Research Council Programme Grant (93558), as well as the Swedish funding agency FORMAS. Further support was provided by the Bundesministerium für Bildung und Forschung (NGFN Plus; FKZ: 01GS08152, grant # 01EV0711, and the project AERIAL grant # 01EE1406C) and the Deutsche Forschungsgemeinschaft (Reinhart-Koselleck Award SP 383/5-1 and grant # HE 7288/2-1). Further support was provided by an ANR grant (project AF12-NEUR0008-01 - WM2NA), a grant from the Mission Interministérielle de Lutte contre la Drogue et la Toxicomanie (MILDT), a grant from the Fondation de France, and a grant from the Fondation pour la Recherche Médicale. A. Heinrich receives funding from the Olympia-Morata-Program of the University of Heidelberg.

T. Banaschewski served in an advisory or consultancy role for Actelion, Hexal Pharma, Lilly, Medice, Novartis, Oxford outcomes, PCM scientific, Shire and Viforpharma. He received conference support or speaker's fee from Janssen McNeil, Lilly, Medice, Novartis, and Shire. He has been involved in clinical trials conducted by Shire and Viforpharma. During the past three years, GB has received honoraria for teaching from General Electric Medical Systems, and acted as a consultant for IXICO. JG has received research funding from the German Federal Ministry of Education and Research, AstraZeneca, Eli Lilly & Co, Janssen-Cilag, and Bristol-Myers Squibb; and has received speakers' fees from AstraZeneca, Janssen-Cilag, and Bristol-Myers Squibb. A. Heinz has received research funding from the German Research Foundation and the Bernstein Center for Computational Neuroscience Berlin (German Federal Ministry of Education and Research), Eli Lilly & Co., Janssen-Cilag, and Bristol-Myers Squibb; and has received speakers' honoraria from Janssen-Cilag, Johnson & Johnson, Eli Lilly & Co., Pfizer, and Servier. A. Ströhle received research funding from the German Federal Ministry of Education and Research, the European Commission (FP6), speaker honoraria from AstraZeneca, Boehringer Ingelheim, Bristol-Myers Squibb, Eli Lilly & Co, Lundbeck, Pfizer, Wyeth and UCB. He was

a consultant for Actelion. Educational grants were given by the Stifterverband für die Deutsche Wissenschaft, the Berlin Brandenburgische Akademie der Wissenschaften, the Boehringer Ingelheim Fonds, the Eli Lilly International Foundation, Janssen-Cilag, Pfizer and Eli Lilly & Co. However, the present work is unrelated to the above grants and relationships. K. Mann received honorary and travel grants from Lundbeck A/S, Copenhagen.

The authors report no financial relationships with commercial interests in relation to this study.

References

- Andreano, J.M., Cahill, L., 2009. Sex influences on the neurobiology of learning and memory. *Learn Mem* 16, 248-266.
- Ayer, L., Rettew, D., Althoff, R.R., Willemsen, G., Ligthart, L., Hudziak, J.J., Boomsma, D.I., 2011. Adolescent personality profiles, neighborhood income, and young adult alcohol use: a longitudinal study. *Addict Behav* 36, 1301-1304.
- Behrendt, S., Wittchen, H.U., Hofler, M., Lieb, R., Beesdo, K., 2009. Transitions from first substance use to substance use disorders in adolescence: is early onset associated with a rapid escalation? *Drug Alcohol Depend* 99, 68-78.
- Cadore, R.J., Yates, W.R., Troughton, E., Woodworth, G., Stewart, M.A., 1996. An adoption study of drug abuse/dependency in females. *Compr Psychiatry* 37, 88-94.
- Cahill, L., 2006. Why sex matters for neuroscience. *Nat Rev Neurosci* 7, 477-484.
- Caldu, X., Dreher, J.C., 2007. Hormonal and genetic influences on processing reward and social information. *Ann N Y Acad Sci* 1118, 43-73.
- Chung, T., Colby, S.M., Barnett, N.P., Rohsenow, D.J., Spirito, A., Monti, P.M., 2000. Screening adolescents for problem drinking: performance of brief screens against DSM-IV alcohol diagnoses. *J Stud Alcohol* 61, 579-587.
- Cloninger, C.R., 1987. Neurogenetic adaptive mechanisms in alcoholism. *Science* 236, 410-416.
- Cloninger, C.R., Przybeck, T.R., Svrakic, D.M., 1991. The Tridimensional Personality Questionnaire: U.S. normative data. *Psychol Rep* 69, 1047-1057.
- Costa, P.T., Jr., McCrae, R.R., 1997. Stability and change in personality assessment: the revised NEO Personality Inventory in the year 2000. *J Pers Assess* 68, 86-94.
- de Wit, H., 2009. Impulsivity as a determinant and consequence of drug use: a review of underlying processes. *Addict Biol* 14, 22-31.
- Dick, D.M., Smith, G., Olausson, P., Mitchell, S.H., Leeman, R.F., O'Malley, S.S., Sher, K., 2010. Understanding the construct of impulsivity and its relationship to alcohol use disorders. *Addict Biol* 15, 217-226.
- Friedman, L., Stern, H., Brown, G.G., Mathalon, D.H., Turner, J., Glover, G.H., Gollub, R.L., Lauriello, J., Lim, K.O., Cannon, T., Greve, D.N., Bockholt, H.J., Belger, A., Mueller, B., Doty, M.J., He, J., Wells, W., Smyth, P., Pieper, S., Kim, S., Kubicki, M., Vangel, M., Potkin, S.G., 2008. Test-retest and between-site reliability in a multicenter fMRI study. *Hum Brain Mapp* 29, 958-972.
- Gilman, J.M., Smith, A.R., Ramchandani, V.A., Momenan, R., Hommer, D.W., 2012. The effect of intravenous alcohol on the neural correlates of risky decision making in healthy social drinkers. *Addict Biol* 17, 465-478.

- Grinevich, V., Jezova, D., Gambaryan, S., Illarionova, A., Kollaker, A., Seeburg, P.H., Schwarz, M.K., 2011. Hypertrophy and altered activity of the adrenal cortex in Homer 1 knockout mice. *Horm Metab Res* 43, 551-556.
- Haber, S.N., Knutson, B., 2010. The reward circuit: linking primate anatomy and human imaging. *Neuropsychopharmacology* 35, 4-26.
- Hahn, T., Dresler, T., Ehli, A.C., Plichta, M.M., Heinz, S., Polak, T., Lesch, K.P., Breuer, F., Jakob, P.M., Fallgatter, A.J., 2009. Neural response to reward anticipation is modulated by Gray's impulsivity. *Neuroimage* 46, 1148-1153.
- Hamann, S., Canli, T., 2004. Individual differences in emotion processing. *Curr Opin Neurobiol* 14, 233-238.
- Hittner, J.B., Swickert, R., 2006. Sensation seeking and alcohol use: a meta-analytic review. *Addict Behav* 31, 1383-1401.
- Joinson, C., Heron, J., Araya, R., Paus, T., Croudace, T., Rubin, C., Marcus, M., Lewis, G., 2012. Association between pubertal development and depressive symptoms in girls from a UK cohort. *Psychol Med* 42, 2579-2589.
- Kajantie, E., Phillips, D.I., 2006. The effects of sex and hormonal status on the physiological response to acute psychosocial stress. *Psychoneuroendocrinology* 31, 151-178.
- Kendler, K.S., Gardner, C., Dick, D.M., 2011. Predicting alcohol consumption in adolescence from alcohol-specific and general externalizing genetic risk factors, key environmental exposures and their interaction. *Psychol Med* 41, 1507-1516.
- Kiezun, J., Kaminska, B., Jankowski, J., Dusza, L., 2015. Concentrations of the adrenocorticotropic hormone, corticosterone and sex steroid hormones and the expression of the androgen receptor in the pituitary and adrenal glands of male turkeys (*Meleagris gallopavo*) during growth and development. *Gen Comp Endocrinol* 217-218, 62-70.
- Kim, Y., Zerwas, S., Trace, S.E., Sullivan, P.F., 2011. Schizophrenia genetics: where next? *Schizophr Bull* 37, 456-463.
- Klein, T.A., Neumann, J., Reuter, M., Hennig, J., von Cramon, D.Y., Ullsperger, M., 2007. Genetically determined differences in learning from errors. *Science* 318, 1642-1645.
- Knutson, B., Adams, C.M., Fong, G.W., Hommer, D., 2001. Anticipation of increasing monetary reward selectively recruits nucleus accumbens. *J Neurosci* 21, RC159.
- Kudielka, B.M., Kirschbaum, C., 2005. Sex differences in HPA axis responses to stress: a review. *Biol Psychol* 69, 113-132.
- Leeman, R.F., Patock-Peckham, J.A., Potenza, M.N., 2012. Impaired control over alcohol use: An under-addressed risk factor for problem drinking in young adults? *Exp Clin Psychopharmacol* 20, 92-106.

MacPherson, L., Magidson, J.F., Reynolds, E.K., Kahler, C.W., Lejuez, C.W., 2010. Changes in sensation seeking and risk-taking propensity predict increases in alcohol use among early adolescents. *Alcohol Clin Exp Res* 34, 1400-1408.

Morozova, T.V., Goldman, D., Mackay, T.F., Anholt, R.R., 2012. The genetic basis of alcoholism: multiple phenotypes, many genes, complex networks. *Genome Biol* 13, 239.

Nees, F., Tzschoppe, J., Patrick, C.J., Vollstadt-Klein, S., Steiner, S., Poustka, L., Banaschewski, T., Barker, G.J., Buchel, C., Conrod, P.J., Garavan, H., Heinz, A., Gallinat, J., Lathrop, M., Mann, K., Artiges, E., Paus, T., Poline, J.B., Robbins, T.W., Rietschel, M., Smolka, M.N., Spanagel, R., Struve, M., Loth, E., Schumann, G., Flor, H., 2012. Determinants of early alcohol use in healthy adolescents: the differential contribution of neuroimaging and psychological factors. *Neuropsychopharmacology* 37, 986-995.

Nees, F., Witt, S.H., Lourdasamy, A., Vollstadt-Klein, S., Steiner, S., Poustka, L., Banaschewski, T., Barker, G.J., Buchel, C., Conrod, P.J., Frank, J., Gallinat, J., Garavan, H., Heinz, A., Ittermann, B., Loth, E., Mann, K., Artiges, E., Paus, T., Pausova, Z., Smolka, M.N., Struve, M., Schumann, G., Rietschel, M., Flor, H., 2013. Genetic risk for nicotine dependence in the cholinergic system and activation of the brain reward system in healthy adolescents. *Neuropsychopharmacology* 38, 2081-2089.

Neville, M.J., Johnstone, E.C., Walton, R.T., 2004. Identification and characterization of ANKK1: a novel kinase gene closely linked to DRD2 on chromosome band 11q23.1. *Hum Mutat* 23, 540-545.

Nielsen, F.A., Hansen, L.K., 2002. Modeling of activation data in the BrainMap database: detection of outliers. *Hum Brain Mapp* 15, 146-156.

Nymberg, C., Banaschewski, T., Bokde, A.L., Buchel, C., Conrod, P., Flor, H., Frouin, V., Garavan, H., Gowland, P., Heinz, A., Ittermann, B., Mann, K., Martinot, J.L., Nees, F., Paus, T., Pausova, Z., Rietschel, M., Robbins, T.W., Smolka, M.N., Strohle, A., Schumann, G., Klingberg, T., 2014. DRD2/ANKK1 Polymorphism Modulates the Effect of Ventral Striatal Activation on Working Memory Performance. *Neuropsychopharmacology*.

Oberlin, B.G., Dzemidzic, M., Bragulat, V., Lehigh, C.A., Talavage, T., O'Connor, S.J., Kareken, D.A., 2012. Limbic responses to reward cues correlate with antisocial trait density in heavy drinkers. *Neuroimage* 60, 644-652.

Oberlin, B.G., Dzemidzic, M., Tran, S.M., Soeurt, C.M., Albrecht, D.S., Yoder, K.K., Kareken, D.A., 2013. Beer flavor provokes striatal dopamine release in male drinkers: mediation by family history of alcoholism. *Neuropsychopharmacology* 38, 1617-1624.

Oxford, M.L., Harachi, T.W., Catalano, R.F., Abbott, R.D., 2001. Preadolescent predictors of substance initiation: a test of both the direct and mediated effect of family social control factors on deviant peer associations and substance initiation. *Am J Drug Alcohol Abuse* 27, 599-616.

Perreault, M.L., O'Dowd, B.F., George, S.R., 2014. Dopamine D(1)-D(2) receptor heteromer regulates signaling cascades involved in addiction: potential relevance to adolescent drug susceptibility. *Dev Neurosci* 36, 287-296.

Ponce, G., Perez-Gonzalez, R., Aragues, M., Palomo, T., Rodriguez-Jimenez, R., Jimenez-Arriero, M.A., Hoenicka, J., 2009. The ANKK1 kinase gene and psychiatric disorders. *Neurotox Res* 16, 50-59.

Richter, A., Richter, S., Barman, A., Soch, J., Klein, M., Assmann, A., Libeau, C., Behnisch, G., Wustenberg, T., Seidenbecher, C.I., Schott, B.H., 2013. Motivational salience and genetic variability of dopamine D2 receptor expression interact in the modulation of interference processing. *Front Hum Neurosci* 7, 250.

Rietschel, M., Mattheisen, M., Frank, J., Treutlein, J., Degenhardt, F., Breuer, R., Steffens, M., Mier, D., Esslinger, C., Walter, H., Kirsch, P., Erk, S., Schnell, K., Herms, S., Wichmann, H.E., Schreiber, S., Jockel, K.H., Strohmaier, J., Roeske, D., Haenisch, B., Gross, M., Hoefels, S., Lucae, S., Binder, E.B., Wienker, T.F., Schulze, T.G., Schmal, C., Zimmer, A., Juraeva, D., Brors, B., Bettecken, T., Meyer-Lindenberg, A., Muller-Myhsok, B., Maier, W., Nothen, M.M., Cichon, S., 2010. Genome-wide association-, replication-, and neuroimaging study implicates HOMER1 in the etiology of major depression. *Biol Psychiatry* 68, 578-585.

Sakaki, M., Mather, M., 2012. How reward and emotional stimuli induce different reactions across the menstrual cycle. *Soc Personal Psychol Compass* 6, 1-17.

Saunders, J.B., Aasland, O.G., Babor, T.F., de la Fuente, J.R., Grant, M., 1993. Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO Collaborative Project on Early Detection of Persons with Harmful Alcohol Consumption--II. *Addiction* 88, 791-804.

Schneider, S., Peters, J., Bromberg, U., Brassen, S., Miedl, S.F., Banaschewski, T., Barker, G.J., Conrod, P., Flor, H., Garavan, H., Heinz, A., Ittermann, B., Lathrop, M., Loth, E., Mann, K., Martinot, J.L., Nees, F., Paus, T., Rietschel, M., Robbins, T.W., Smolka, M.N., Spanagel, R., Strohle, A., Struve, M., Schumann, G., Buchel, C., 2012. Risk taking and the adolescent reward system: a potential common link to substance abuse. *Am J Psychiatry* 169, 39-46.

Schumann, G., Loth, E., Banaschewski, T., Barbot, A., Barker, G., Buchel, C., Conrod, P.J., Dalley, J.W., Flor, H., Gallinat, J., Garavan, H., Heinz, A., Ittermann, B., Lathrop, M., Mallik, C., Mann, K., Martinot, J.L., Paus, T., Poline, J.B., Robbins, T.W., Rietschel, M., Reed, L., Smolka, M., Spanagel, R., Speiser, C., Stephens, D.N., Strohle, A., Struve, M., 2010. The IMAGEN study: reinforcement-related behaviour in normal brain function and psychopathology. *Mol Psychiatry* 15, 1128-1139.

Smith, G.T., McCarthy, D.M., Zapsolski, T.C., 2009. On the value of homogeneous constructs for construct validation, theory testing, and the description of psychopathology. *Psychol Assess* 21, 272-284.

Stacey, D., Bilbao, A., Maroteaux, M., Jia, T., Easton, A.C., Longueville, S., Nymberg, C., Banaschewski, T., Barker, G.J., Buchel, C., Carvalho, F., Conrod, P.J., Desrivieres, S., Fauth-Buhler, M., Fernandez-Medarde, A., Flor, H., Gallinat, J., Garavan, H., Bokde, A.L., Heinz, A., Ittermann, B., Lathrop, M., Lawrence, C., Loth, E., Lourdasamy, A., Mann, K.F., Martinot, J.L., Nees, F., Palkovits, M., Paus, T., Pausova, Z., Rietschel, M., Ruggeri, B., Santos, E., Smolka, M.N., Staehlin, O., Jarvelin, M.R., Elliott, P., Sommer, W.H., Mamelì, M., Müller, C.P., Spanagel, R., Girault, J.A., Schumann, G., 2012. RASGRF2 regulates alcohol-induced reinforcement by influencing mesolimbic dopamine neuron activity and dopamine release. *Proc Natl Acad Sci U S A* 109, 21128-21133.

Szumliński, K.K., Kalivas, P.W., Worley, P.F., 2006. Homer proteins: implications for neuropsychiatric disorders. *Curr Opin Neurobiol* 16, 251-257.

Temple, J.L., Ziegler, A.M., Graczyk, A., Bendlin, A., Sion, T., Vattana, K., 2014. Cardiovascular responses to caffeine by gender and pubertal stage. *Pediatrics* 134, e112-119.

Varlinskaya, E.I., Vetter-O'Hagen, C.S., Spear, L.P., 2013. Puberty and gonadal hormones: role in adolescent-typical behavioral alterations. *Horm Behav* 64, 343-349.

Volkow, N.D., Morales, M., 2015. The Brain on Drugs: From Reward to Addiction. *Cell* 162, 712-725.

Whelan, R., Garavan, H., 2014. When optimism hurts: inflated predictions in psychiatric neuroimaging. *Biol Psychiatry* 75, 746-748.

Woicik, P.A., Stewart, S.H., Pihl, R.O., Conrod, P.J., 2009. The Substance Use Risk Profile Scale: a scale measuring traits linked to reinforcement-specific substance use profiles. *Addict Behav* 34, 1042-1055.

Figure captions:

Figure 1 “Alcohol Use Disorders Identification Test scores”:

Distribution of the Alcohol Use Disorders Identification Test (AUDIT) score at the first time point of examination (grey bars; mean age 14.43) and at the follow up examination (black bars; mean age 16.45).

Figure 2 “Model on early alcohol drinking behavior”:

Structural equation model on the impact of Regions of interest (ROIs), personality, behavior, and candidate genetic variations on the Alcohol Use Disorders Identification Test (AUDIT) score at early adolescence. Numbers above arrows show standardized regression weights, numbers above rectangles represent squared multiple correlations. Goodness of fit (GFI) = 0.944; Adjusted GFI = 0.924; parsimonious GFI = 0.701; comparative fit index = 0.942. The root mean squared error of approximation was 0.055 and did not significantly exceed 0.05 ($P_{close} = 0.096$). The p-value of χ^2 reached the 0.001 level, which is often seen in large samples.

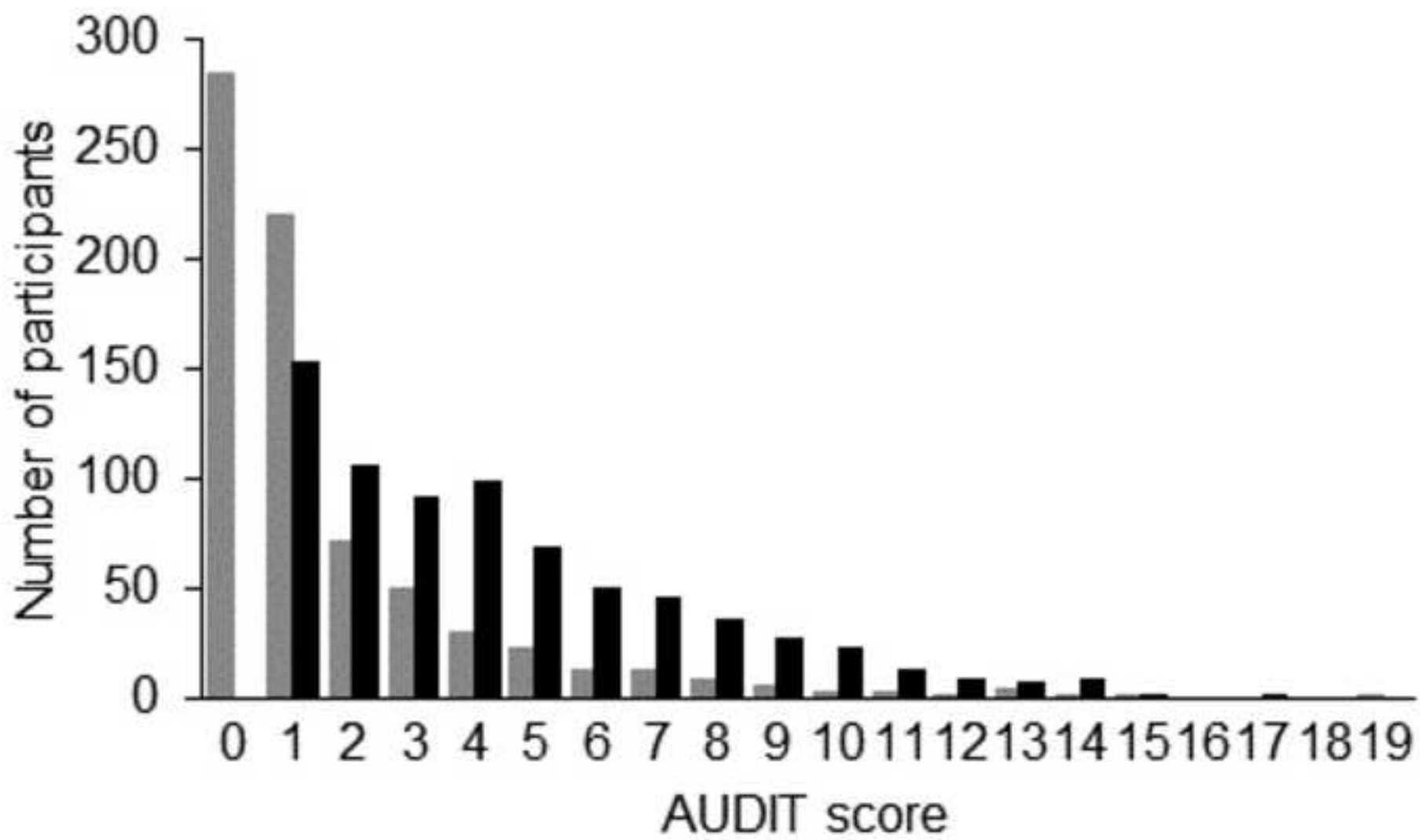
Figure 3 “Predictive model on later alcohol drinking behavior”:

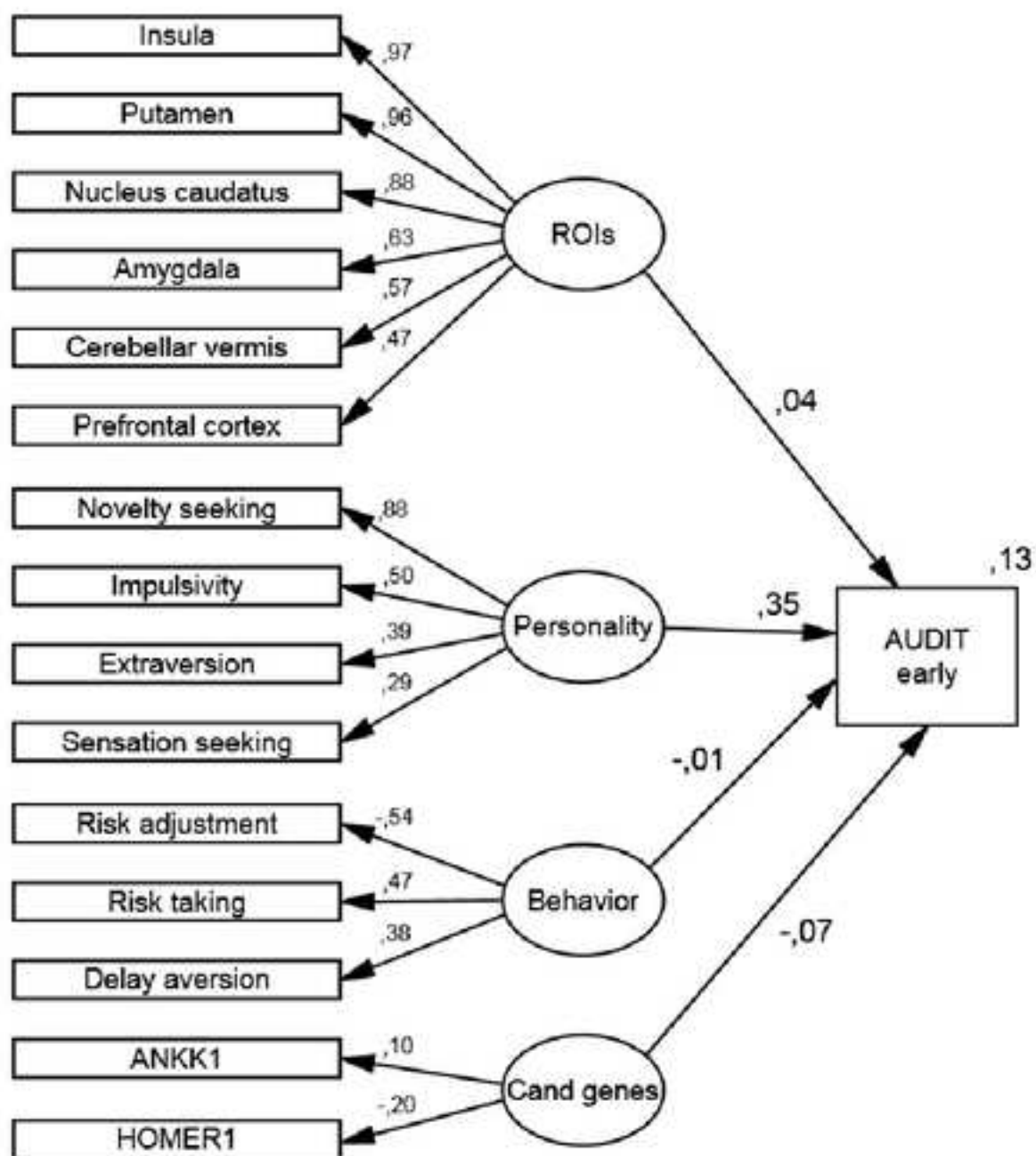
Structural equation model on the impact of Regions of interest (ROIs), personality, behavior, and candidate genetic variations on the Alcohol Use Disorders Identification Test (AUDIT) score at later adolescence. Numbers above arrows show standardized regression weights, numbers above rectangles represent squared multiple correlations. Goodness of fit (GFI) = 0.944; Adjusted GFI = 0.925; parsimonious GFI = 0.701; comparative fit index = 0.942. The root mean squared error of approximation was 0.055 and did not significantly exceed 0.05 ($P_{close} = 0.108$). The p-value of χ^2 reached the 0.001 level, which is often seen in large samples.

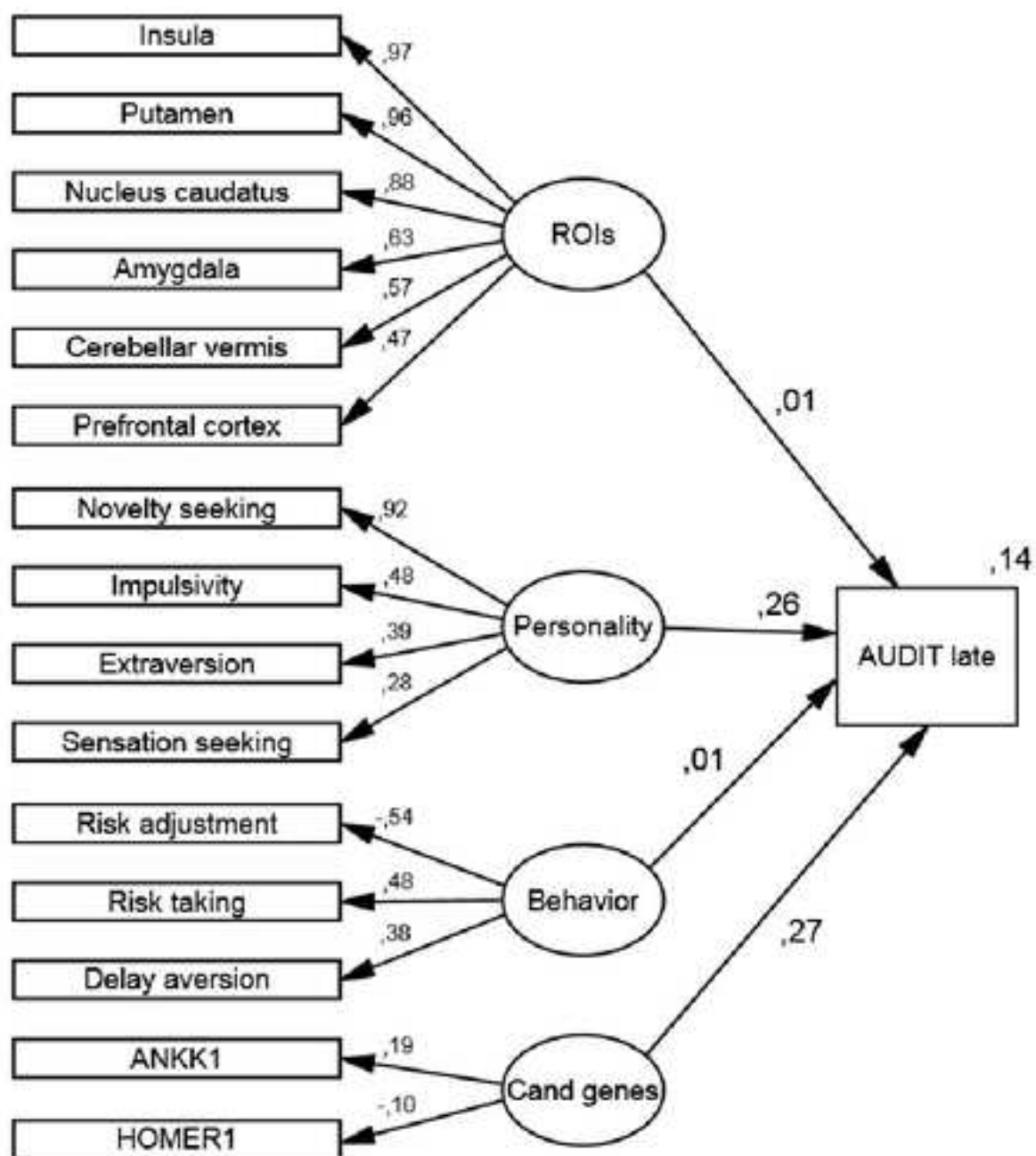
Figure 4 “Model on increase of AUDIT score from early to later adolescence”:

Structural equation model on the impact of Regions of interest (ROIs), personality, behavior, and candidate genetic variations on the increase in Alcohol Use Disorders Identification Test (AUDIT) score from early to later adolescence. Numbers above arrows show standardized regression weights, numbers above rectangles represent squared multiple correlations. Goodness of fit (GFI) = 0.944; Adjusted GFI = 0.924; parsimonious GFI = 0.701; comparative fit index = 0.941. The root mean squared error of approximation was 0.055 and did not significantly exceed 0.05 ($P_{close} = 0.088$). The p-value of χ^2 reached the 0.001 level, which is often seen in large samples.

Figure 1







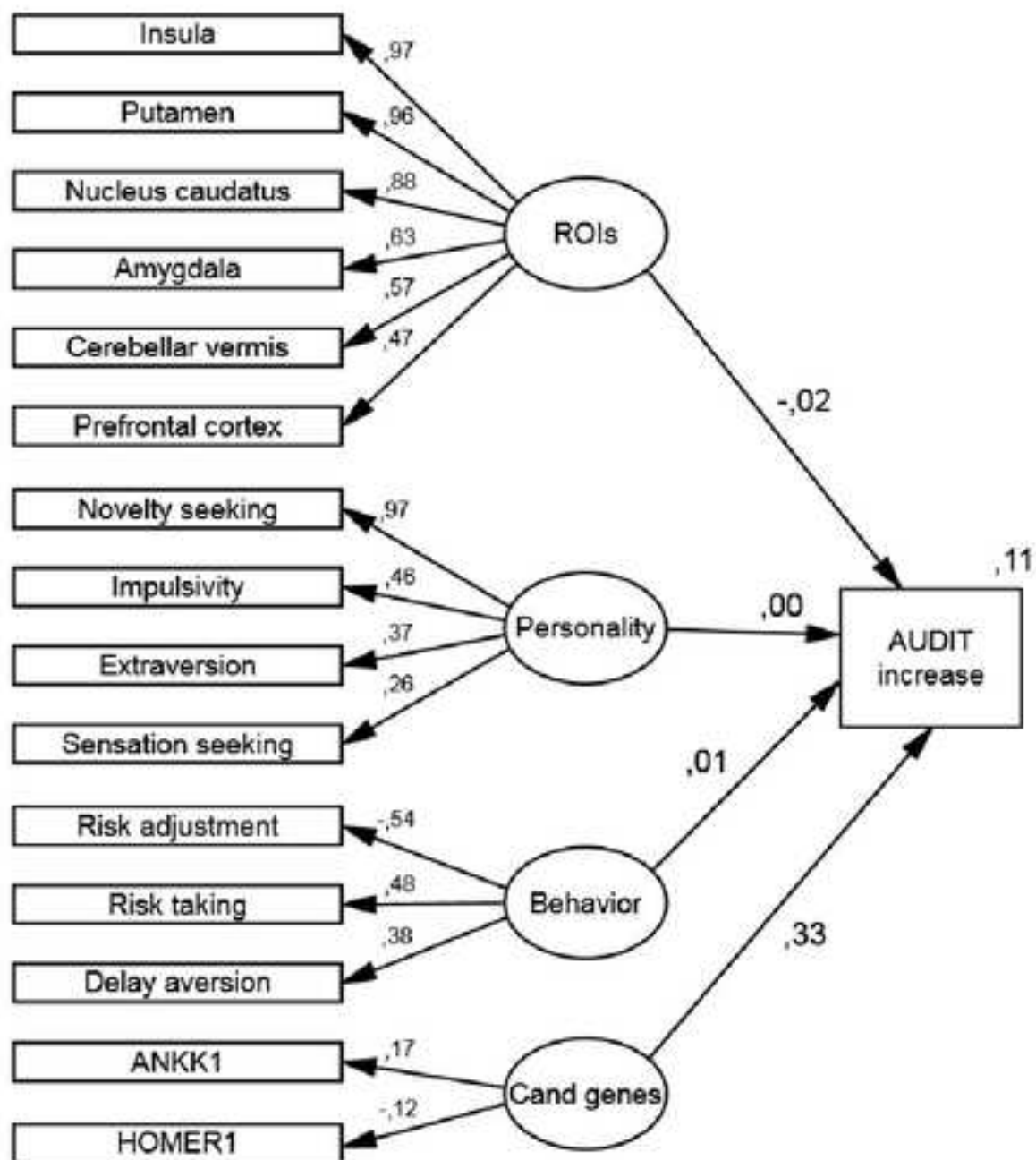


Table 1: Sample description and variable distribution

Variable	Entire sample (N = 736)	AUDIT score late ≤ 4 (N = 448)	AUDIT score late > 4 (N = 288)	p- value
Age 1 st time point	14.43	14.42	14.43	0.78
(SD, range)	(0.41, 12.91-16.02)	(0.40, 12.91-15.45)	(0.42, 12.97-16.02)	
Age 2 nd time point	16.45	16.43	16.48	0.16
(SD, range)	(0.47, 14.64-18.23)	(0.46, 14.98-18.23)	(0.48, 14.64-18.03)	
<i>ANKK1</i> , rs1800497	460 (GG), 30 (AA), 246 (AG)	282 (GG), 16 (AA), 150 (AG)	178 (GG), 14 (AA), 96 (AG)	0.69
<i>HOMER1</i> , rs7713917	288 (AA), 114 (GG), 334 (GA)	174 (AA), 65 (GG), 209 (GA)	114 (AA), 49 (GG), 125 (GA)	0.57
Extraversion	2.51	2.46	2.58	<0.01
(SD, range)	(0.46, 0.25-3.67)	(0.46, 0.25-3.67)	(0.45, 1.08-3.50)	
Impulsivity	2.41	2.37	2.46	<0.01
(SD, range)	(0.42, 1.00-3.80)	(0.43, 1.00-3.80)	(0.41, 1.40-3.80)	
Sensation seeking	2.78	2.73	2.85	<0.01
(SD, range)	(0.52, 1.00-4.00)	(0.53, 1.00-4.00)	(0.50, 1.60-4.00)	
Novelty seeking	112.43	110.19	115.92	<0.01
(SD, range)	(12.55, 74-146)	(12.23, 77-146)	(12.27, 74-146)	
Delay aversion	0.24	0.24	0.23	0.34
(SD, range)	(0.14, -0.14-0.78)	(0.15, -0.12-0.78)	(0.14, -0.14-0.68)	
Risk adjustment	1.64	1.62	1.67	0.55
(SD, range)	(1.00, -0.86-4.98)	(1.00, -0.86-4.98)	(1.01, -0.83-4.93)	
Risk taking	0.53	0.52	0.54	0.05
(SD, range)	(0.14, 0.05-0.88)	(0.14, 0.05-0.87)	(0.13, 0.09-0.88)	

SD = standard deviation; age in years; genotype distribution as total number; psychometric and neuropsychological data as mean; risk adjustment, risk taking, and delay aversion measured by CGT (CANTAB, Cambridge Cognition); sensation seeking and impulsivity according to Substance Use Risk Profile Scale (Woicik et al., 2009); novelty seeking derived from Temperament and Character

Inventory - Revised (Cloninger et al., 1991); extraversion from NEO-Five Factor Inventory (Costa and McCrae, 1997); p-values for the difference between early AUDIT score and late AUDIT score, calculated by the use of t-tests and chi-square tests (SNPs)