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Synergistic effects of the dopaminergic and glutamatergic system on hippocampal volume in alcohol-dependent patients

I. Puls [a,1], J. Mohr [a,b,1], J. Wrase [a], J. Priller [a], J. Behr [a], W. Kitzrow [a], N. Makris [c], H.C. Breiter [c], K. Obermayer [b,d,1], A. Heinz [a,1,*]

[a] Department of Psychiatry and Psychotherapy, CCM, Charité University Medicine, Berlin, Germany
[b] Bernstein Center for Computational Neuroscience, Berlin, Germany
[c] Motivation and Emotion Neuroscience Collaboration, and Athinoula Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, USA
[d] School of Electrical Engineering and Computer Science, Berlin Institute of Technology, Berlin, Germany

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ABSTRACT

Several genes of the dopaminergic and glutamatergic neurotransmitter systems have been found to be associated with alcohol disease and related intermediate phenotypes. Here, we evaluated genetic variants of the catechol-*O*-methyltransferase (COMT) and the metabotropic glutamate receptor 3 (mGluR3) genes in alcohol-dependent patients and their association with volumetric measurements of brain structures. By combined analysis of imaging data and genotyping results, large numbers of variables are produced that overstrain conventional statistical methods based on tests for group differences. Limitations in assessment of epistatic effects and multiple testing problems are encountered. Therefore, we introduce a novel method for detecting associations between a set of genetic markers and phenotypical measurements based on machine learning techniques. Hippocampal volume was found to be associated with epistatic effects of the COMT–mGluR3 genes in alcohol-dependent patients but not in controls. These data are in line with prior studies supporting a role for dopamine–glutamate interaction in modulation of alcohol disease.

1. Introduction

Alcohol dependence is a widespread disorder, affecting about 10% of the population (Sher et al., 2005). Due to somatic, psychiatric and social consequences, a major economic impact is related to alcohol dependence. Well designed twin, adoption and family studies have shown that genetic factors play a considerable role for disease risk and symptom characteristics, with 40–60% of the risk variance explained by genetic influences (Goodwin, 1975; Kendler et al., 1994; Bierut et al., 2002; Koehnke, 2008). First degree relatives of alcohol-dependent patients display a three- to fourfold increased risk to develop the disorder, and there is a 55% or higher concordance rate in monozygotic twins compared to a 28% rate for dizygotic twins (Goodwin, 1975). Several linkage studies have been performed that gave evidence for many chromosomal loci, where – among others – glutamatergic and dopaminergic genes are located (Edenberg and Foroud, 2006).

Genetic association studies have shown a relation of alcohol dependence and/or alcohol-related traits with genes of the glutamatergic system, such as *N*-methyl-D-aspartate receptor channel 1 (NR1), *N*-methyl-D-aspartate receptor channel 2B (NR2B), glutamate transporter 1 (GLT1), glutamate receptor ionotropic kainate 3 (mGluR7), and clock genes such as Period 2 (PER2) affecting glutamate reuptake (Schumann et al., 2005; Spanagel et al., 2005) as well as genes of the dopaminergic neurotransmitter system, e.g. dopamine receptor D1 (DRD1), dopamine receptor D3 (DRD3), dopamine transporter (DAT) and catechol-*O*-methyltransferase (COMT) (Enoch et al., 2006; Bowirrat and Oscar-Berman, 2005; Dick and Bierut, 2006). Imaging studies have further supported the role of dopaminergic and glutamatergic alterations in the pathogenesis of alcohol dependence (Heinz et al., 2003; Wong et al., 2003).

However, genetic association studies in complex diseases often give inconsistent results, as it was also shown for the genes mentioned above (Bolos et al., 1990; Schumann et al., 2005; Hines

* Corresponding author at: Department of Psychiatry and Psychotherapy, Charité Campus Mitte, Charité University Medicine Berlin, Charitéplatz 1, D-10117 Berlin, Germany. Tel.: +49 30 450 517002; fax: +49 30 450 517921.
E-mail address: andreas.heinz@charite.de (A. Heinz).
[1] These authors contributed equally to this work.

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et al., 2005). Reasons for the ambiguity are manifold, including genetic and allelic heterogeneity of the disease, interactive gene–gene effects, contributing external factors and comparatively small sample size (Buckland, 2001). Over the last years, the concept of intermediate phenotypes has been promoted, which tries to dissect complex psychiatric diseases such as alcohol dependence into more basic neurobiological components (Enoch et al., 2003; Hines et al., 2005). Parameters such as electrophysiological measures (Reischies et al., 2005) or structural and functional imaging data (Hariri et al., 2002; Breiter and Gasic, 2004; Heinz et al., 2005; Pezawas et al., 2005) have shown to be reliable tools for genetic association studies in alcoholism and other psychiatric disorders. Several studies have evaluated structural brain alterations associated with alcohol dependence (Hommer, 2003; Pfefferbaum, 2004; Spampanato et al., 2005). Among subcortical and cortical regions found to be different in alcoholics, hippocampal volume reductions have been reported to exceed the general decrease in cerebral volume found in alcohol-dependent individuals (Agartz et al., 1999; Beresford et al., 2006; Mechtcheriakov et al., 2007). These alterations raise the question whether hippocampal volume may represent a stable or "trait" marker of the disorder that already shows alterations in a presymptomatic stage, or if the hippocampus is more susceptible towards alcohol-toxic influences compared to other brain areas.

A pitfall for the approach of "imaging genomics" concerns the large number of variables that is produced by the combination of genetic data and imaging parameters. Conventional statistical methods that are based on tests for group differences are overstrained by multiple testing issues, potentially producing false positive results. Inclusion of different models of allelic interactions (dominant or linear) further increases the number of tests performed. The necessity for multiple testing corrections leads to a severe loss in statistical power. Also, potential epistatic gene–gene interactions, as they most likely occur in vivo, cannot be reliably dissected. Whereas epistasis was initially used to depict the suppression of a phenotypic characteristic of one gene by another, we refer with epistatic effects to the more recent denotation describing common gene–gene interactions of both additive and multiplicative nature. New statistical methods are needed that are able to deal with these small but high dimensional datasets and are capable to test not only for the impact of single genes but also evaluate epistatic interactions (Marchini et al., 2005; Chapman and Clayton, 2007; Zhang and Liu, 2007). We propose a novel method for detecting associations between a set of genetic markers and phenotypical measurements based on techniques from the area of machine learning, which we refer to as "machine learning genotype–phenotype analysis" (MLGPA).

In the following study this statistical approach was applied to evaluate effects of group II metabotropic glutamate receptor 3 (mGluR3) genetic variants and their interaction with COMT variations in alcohol-dependent patients and matched controls. COMT was chosen for its potential role in the development of alcohol dependence as indicated by several human studies (Kauhanen et al., 2000; Oroszi and Goldman, 2004). mGluRs play a vital role in synaptic plasticity (Bortolotto et al., 1999), and an essential role for mGluR3 in LTD (long-term depression) and a modulatory role for mGluR3 in LTP (long-term potentiation) have been found. In addition, it has been suggested that activation of mGluRs modulates excitation and inhibition of dopaminergic mesencephalic neurons (Meltzer et al., 1997; Shen and Johnson, 1997; Campusano et al., 2002; Bustos et al., 2004). Recently, significant statistical epistasis between COMT and mGluR3 has been shown (Nicodemus et al., 2007): several genetic variants of the mGluR3 gene increased the risk of schizophrenia conferred by COMT-SNPs, whereas mGluR3 itself did not have an influence on disease risk. Also, epistatic effects of COMT and mGluR3 on working memory function in healthy subjects have been found (Tan et al., 2007). Based on these findings, COMT–mGluR3 interactions were evaluated for their effects on volumetric measurements of brain structures in alcohol-dependent patients and controls.

2. Methods

2.1. Patients

We included 38 patients of Central European descendent (31 male, 7 female, mean age 41 ± 7, range 26–57 years) diagnosed with alcohol dependence according to ICD-10 and DSM-IV. The severity of alcohol dependence was assessed with the Alcohol Dependence Scale (Skinner and Horn, 1984) and the amount of lifetime alcohol intake was measured with the Life Time Drinking History (Skinner and Sheu, 1982). Patients had no previous substance dependence or current substance abuse other than alcoholism which was confirmed by random breath and urine drug testing. All multi-drug abusers were excluded prior to study enrolment. Detoxification was undertaken according to general medical practice using benzodiazepines or chlormethiazole not more than 5 days. Medication was stopped at least 1 week prior to MRI scan. 41 age matched healthy volunteers of Central European descendent were included as controls (26 male, 15 female; mean age 39 ± 8, range 25–61 years). Standardized clinical assessment with the Structured Clinical Interviews I and II (First et al., 1997; First et al., 2001) was performed to exclude other axis I psychiatric disorders (and axis II disorders in healthy volunteers). All individuals included into the study were free from any continuous medications and severe somatic disorders, including major neurological and hepatic complications in alcohol-dependent patients. All patients and controls gave fully informed written consent prior to their participation. The study was approved by the local ethics committee and was performed in accordance with the Declaration of Helsinki (1964).

2.2. Imaging data

Structural imaging was performed using a 1.5T clinical whole-body MRI (Magnetom VISION; Siemens, Erlangen, Germany) that was equipped with a standard quadrate head coil. A morphological 3D T1-weighted MPRAGE (magnetization prepared rapid gradient echo) image data set (1 mm × 1 mm × 1 mm voxel size, FOV 256 mm, 162 slices, TR = 11.4 ms, TE = 4.4 ms, $a = 12^{\circ}$) covering the whole head was acquired. Images were analyzed at the Massachusetts General Hospital as part of the Phenotype–Genotype Project in Addiction and Mood Disorder. A brief synopsis of procedures is provided here following those published previously (Makris et al., 2004). Image positions were normalized by imposing a standard three-dimensional coordinate system on each three-dimensional image. Gray-white matter segmentation was performed using a semi-automated intensity contour mapping algorithm for cerebral exterior definition and signal intensity histogram distributions for demarcation of gray-white matter borders. The cerebral regions investigated in our study are depicted in Fig. 1. Targeted brain regions were segmented as individual structures using a contour line and manual editing following probabilistically weighted landmarks, cross-referencing of lateral, coronal, and sagittal views, and anatomist supervision. Landmarks and definitions for the targeted brain regions have been well defined elsewhere (Caviness et al., 1996; Makris et al., 2004). Segmentation was performed by two BA level MR technicians blinded to subject diagnosis and group status, and with a randomly ordered sequence of subjects. Intra-rater and inter-rater reliabilities were assessed via percent common voxel assignments (PCVA) (Seidman et al., 2002). Reliabilities were between 85.9 and 90.1. These values are consistent with intra-rater and inter-rater reliabilities reported in previous studies (Goldstein et al., 2002; Seidman et al., 2002; Makris et al., 2004). To rule out gross volumetric effects, total head circumference was calculated for all subjects. Group differences were not significant for this measure ($p > 0.05$).

2.3. Genetic analysis

For genetic analysis, 30 ml of EDTA blood were collected from each individual. Eight single nucleotide polymorphisms (SNPs) distributed over the mGluR3 gene were genotyped (for SNP details see Table 1). SNPs were selected for potential functional relevance, location on physical and genetic maps, and minor allele frequency based on the University of California Santa Cruz (UCSC) Human Genome Browser, the National Center of Biotechnology Information (NCBI) database and Applied Biosystems (ABI) SNP Browser software. Two SNPs were removed after genotyping due to limited minor allele frequency (Table 1). For assessment of gene–gene interactions, three SNPs of the COMT gene were chosen (rs2097603, rs4680 [Val^{158}Met], rs165599) (Table 1) according to prior publications (Meyer-Lindenberg et al., 2006; Nicodemus et al., 2007). Primers were designed for amplification of relevant genomic regions by polymerase chain reaction (PCR); products were cut by allele specific restriction enzymes and visualized after gel electrophoresis. Primer information and specific assay conditions are available on request.