Genetic Variation in the Atrial Natriuretic Peptide Transcription Factor *GATA4* Modulates Amygdala Responsiveness in Alcohol Dependence

Anne Jorde, Patrick Bach, Stephanie H. Witt, Kathleen Becker, Iris Reinhard, Sabine Vollstädt-Klein, Martina Kirsch, Derik Hermann, Katrin Charlet, Anne Beck, Lioba Wimmer, Josef Frank, Jens Treutlein, Rainer Spanagel, Karl Mann, Henrik Walter, Andreas Heinz, Marcella Rietschel, and Falk Kiefer

Background: Two genome-wide association studies recently showed alcohol dependence to be associated with a single-nucleotide polymorphism (rs13273672) located on a gene (*GATA4*) that encodes a transcription factor of atrial natriuretic peptide (ANP). A growing body of evidence suggests that ANP might be involved in the symptomology of alcohol dependence. This study examined whether reactivity to alcohol cues in the ANP target region amygdala, a key area implicated in addictive behavior, differs depending on the *GATA4* genotype of a patient. We also investigated potential associations between these differences in amygdala activation and relapse behavior.

Methods: Eighty-one abstinent, alcohol-dependent patients completed a functional magnetic resonance imaging cue-reactivity task in a 3-Tesla scanner and provided blood samples for DNA extraction.

Results: The results showed significantly lower alcohol-cue-induced activations in G-allele carriers as compared with AA-homozygotes in the bilateral amygdala. A survival analysis revealed that a stronger alcohol-specific amygdala response predicted a lowered risk for relapse to heavy drinking in the AA-homozygotes, whereas this effect could not be observed in G-allele carriers.

Conclusions: These results illuminate potential underlying mechanisms of the involvement of the *GATA4* gene in the etiology of alcohol dependence via its influence on ANP and amygdala processing.

Key Words: Alcohol dependence, ANP, cue-reactivity, fMRI, *GATA4*, imaging genetics

Although alcohol dependence is one of the leading risk factors for global burden of disease (2), optimal treatment options are lacking. Because genetic factors contribute an estimated 40%–60% of the variance in the likeliness of individuals to develop alcohol dependence (3), identifying these susceptibility genes might provide a new way to identify treatment targets. Genome-wide association studies (GWAS) have already successfully identified variants implicated in the genetic susceptibility to alcohol dependence (4–6). The "imaging genetics" approach might also be able to contribute to better identifying targets for treatment. By examining associations between susceptibility genes and quantitative, intermediate phenotypes, imaging genetics could deepen our understanding of the underlying biological mechanisms behind the genetic influences on alcohol dependence (7).

From the Department of Addictive Behavior and Addiction Medicine (AJ, PB, KB, SV-K, MK, DH, KM, FK); Department of Genetic Epidemiology in Psychiatry (SHW, JF, JT, MR); Department of Biostatistics (IR); Institute of Psychopharmacology (RS); Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University; Division of Mind and Brain Research (HW); Department of Psychiatry and Psychotherapy (KC, AB, AH), Charité—Universitätsmedizin, Berlin, Germany; and the Department of Psychiatry (LW), University Clinic Bonn, Bonn, Germany.

Address correspondence to Anne Jorde, M.Res., Department of Addictive Behavior and Addiction Medicine, Central Institute of Mental Health, Medical Faculty Mannheim, Heidelberg University, Square J5, D-68159 Mannheim, Germany; E-mail: anne.jorde@zi-mannheim.de.

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One recently identified susceptibility gene for alcohol dependence is the GATA binding protein 4 (*GATA4*) gene, a transcription factor regulating the transcription of the atrial natriuretic peptide (ANP) (8,9). A recent GWAS and its corresponding follow-up study identified an association between a single-nucleotide polymorphism (SNP) (rs13273672) located intronically in the *GATA4* gene on chromosome 8 and alcohol dependence (6). This finding was replicated in a subsequent GWAS (4). Similarly, a recent candidate association study found *GATA4* to be associated with alcohol dependence on the gene level (10). Moreover, analyzing the treatment response of 324 alcohol-dependent patients 3 months after completing treatment, we showed variations in SNP rs13273672 to be significant predictors of time to first relapse to heavy drinking (11). These results suggest that this SNP might be involved not only in the development of alcohol dependence but also in its maintenance, further underlining its clinical significance.

The involvement of *GATA4* in the etiology of alcohol dependence is likely mediated by its effects on ANP expression and the influences of ANP on central nervous system (CNS) functioning. Accordingly, *GATA4* is expressed in various cells of the human CNS (12,13), and SNP rs13273672 has been linked to altered expression of ANP (11), with G-allele carriers exhibiting a reduced variability of ANP concentration (11).

On the molecular level, ANP exerts its effects on the CNS mainly through natriuretic peptide-A (NPR-A) (guanylyl cyclase A) and natriuretic peptide-B (NPR-B) (guanylyl cyclase B) receptors (14,15). The NPR-A and NPR-B messenger RNA (mRNA)-expressing cells have been found in the amygdaloid nuclei as well as other parts of the CNS, including the hypothalamus, circumventricular organs, hippocampus, cerebellum, striatum, olfactory bulb, and spinal cord (15–17). Binding of natriuretic peptides to NPR-A stimulates guanylyl A, which produces cyclic guanosinemonophosphate.

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Increasing cyclic guanosinemonophosphate levels, in turn, activates cyclic nucleotide gated channels and protein kinase G, which increases the intracellular calcium level thereby altering cell potential and transmitter release (16,18). Binding of ANP to natriuretic peptide receptors has been found to inhibit noradrenergic and dopaminergic neurotransmission (16). Additionally, natriuretic peptides reduce the release of corticotrophin-releasing hormone (CRH) receptors with an effect on the hypothalamic-pituitary-adrenal axis as well as the amygdala, which also expresses CRH receptors (19–21).

Clinical studies showed detoxified alcoholic patients with low ANP plasma levels to report increased levels of anxiety and craving (22). Similarly, ANP mRNA expression in alcoholic patients has been found to be higher, whereas promoter-related DNA methylation of ANP, which correlates with self-reported craving, has been found to be lower than in nonalcoholic subjects (23). Atrial natriuretic peptide also seems to have anxiolytic effects (24,25). In rodents, peripheral ANP application reduced alcohol withdrawal-induced anxiety (14), and local infusion of ANP into the central nucleus of the amygdala attenuated anxiety in rats (24,26). Taken together, these studies suggest that lowered ANP levels contribute to the dysregulation of the stress and anxiogenic systems of the brain that is commonly observed in alcohol dependence (14,22,27).

The amygdala plays an integral part in these systems (28). Receptors for natriuretic peptides and ANP mRNA as well as receptors for CRH found to be affected by ANP are expressed in the amygdala (15,16,19–21,29). It has been implicated in a range of addiction-relevant processes, including withdrawal-like negative affect, stress-induced alcohol intake, craving, and the evaluation of negative aspects of drug stimuli (30,31).

We hypothesized that genetic variation in *GATA4* rs13273672 contributes to inter-individual variability in alcohol-related neural functioning in the amygdala. In this first exploratory imaging genetics study on *GATA4,* we tested this hypothesis by examining cue-reactivity, a well-established intermediate phenotype in addiction research, with a cue-reactivity task shown to elicit amygdala activity in the functional magnetic resonance imaging (fMRI) scanner (32,33). We explored the potential effects of rs13273672 on alcohol-cue-induced brain functioning by comparing alcohol-dependent patients with different *GATA4* genotypes. Furthermore, we examined possible associations between amygdala activity, relapse risk, and measures of depressiveness, anxiety, and alcohol dependence. In accordance with previous findings (11), we expected G-allele carriers to show an increased relapse risk.[1]

Methods and Materials

Participants

Eighty-one abstinent, alcohol-dependent patients (57 men) took part in this study. They were recruited from the day clinic and inpatient wards of the Department of Addictive Behavior and Addiction Medicine at the Central Institute of Mental Health (Mannheim, Germany). Participants were eligible for participation in the study if they were between 18 and 65 years of age, fulfilled the diagnostic criteria for alcohol dependence according to the DSM-IV, were right-handed, had completed medically supervised detoxification, and had been abstinent for at least 3 and at most 28 days. Exclusion criteria were the presence of an Axis-I disorder within the previous 12 months (other than alcohol and nicotine dependence), positive drug-test results, current use of psychotropic or anticonvulsive medications, epilepsy, suicidal tendency, pregnancy, neurological or severe physiological illness, and various other MRI exclusion criteria (e.g., metal implants, pacemakers, tattoos). All patients received sessions of health education, supportive therapy, and cognitive-behavioral therapy (36). All participants provided written informed consent in accordance with the Declaration of Helsinki. The study was approved by the ethics committee of Heidelberg University.

Materials and Procedure

fMRI, Assessment of Cue Reactivity, and Data Acquisition. The fMRI cue-reactivity paradigms have found widespread application and have been shown to distinguish between patient and control cohorts (37). The task used in the present study has been validated previously in alcoholic patients (38,39) and social drinkers (40). Task and scanning parameters were identical to the ones used previously by our research group (38–40). Patients were presented with 12 blocks containing a total of 60 alcohol-related images and 9 blocks containing a total of 45 neutral images from the International Affective Picture System set (41). Each block consisted of five pictures, which were presented for 4 sec each. Blocks were preceded by a 10-sec resting period. After each block the participants were asked to rate the intensity of their craving for alcohol on a visual-analogue scale ranging from 0 ("no craving at all") to 100 ("extremely intense craving"). The order of the picture categories (i.e., alcohol-related vs. neutral) was pseudo-randomized, whereas the order of the individual pictures was randomized for each participant. The total task duration was 12 min. Scanning was performed with a 3-Tesla whole-body tomograph (MAGNETOM Trio, TIM Technology, Siemens, Erlangen, Germany). Imaging parameters and information on stimulus presentation software are provided in Supplement 1.

Treatment Outcome and Clinical Variables. All patients took part in a semi-structured interview assessing demographic information and drinking history [Form90 (42)]. Furthermore, they completed a range of self-report questionnaires after the fMRI scan, including the Alcohol Dependence Scale (43), the Beck Depression Inventory (44), the Spielberger State-Trait-Anxiety Inventory (45), and the Obsessive-Compulsive Drinking Scale (46). Withdrawal symptoms were assessed before fMRI scanning with the Clinical Institute Withdrawal Assessment scale (CIWA-A) (47).

Follow-up information was available for 48 patients for a total period of approximately 3 months after the fMRI scan (the remaining 33 patients had taken part during a pilot phase of the study, during which follow-up information was not obtained). These patients were contacted by telephone at monthly intervals and were asked to provide information with regard to their drinking status, date of any relapse, amount of alcohol consumed, and use of any relapse prevention medication employing a semi-structured interview incorporating the Alcohol Timeline Follow-back (48). In line with Kiefer *et al.* (11), we used time to relapse to heavy drinking within 90 days after fMRI scanning as our outcome criterion, with heavy drinking defined as alcohol consumption of

[1]The hypothalamus also plays an important role in the stress response to alcohol stimuli and in withdrawal-related negative affect in alcohol-dependent individuals (31). It expresses ANP and NPR mRNA as well as receptors for CRH found to be affected by ANP (19–21). Given these facts, it could be speculated that alcohol-related neural functioning in the hypothalamus might also be affected by genetic variations in *GATA4*. However, its small size and inhomogeneous structure as well as its unequal distribution of NPRs might make it difficult to detect this putative effect with our method (16,17,34,35). Nevertheless, secondary analyses on *GATA4*-genotpye dependent differences in alcohol-cue-induced hypothalamus activity are provided in Supplement 1.