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GATA4 variant interaction with brain limbic structure and relapse risk: A voxel-based morphometry study

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Abstract
Atrial natriuretic peptide (ANP) receptors are highly expressed in the amygdala, caudate and hypothalamus. GATA4 gene encodes a transcription factor of ANP associated with the pathophysiology of alcohol dependence. We have previously demonstrated that the GATA4 single nucleotide polymorphism (SNP) rs13273672 revealed stronger alcohol-specific amygdala activation associated with lowered relapse risk to heavy drinking at 90 days in the AA-homozygotes. Our understanding however with respect to GATA4 variation on gray matter (GM) regional amygdala, caudate and hypothalamus volume is limited. We investigated GM differences specific to GATA4 and hypothesized that GM alterations will be predictive of heavy relapse. Eighty-three recently detoxified alcohol dependent patients were included.

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Neuroimaging data was analyzed using Voxel Based Morphometry (VBM). The main effects of GM volume and genotype as well as their interaction effect on time to heavy relapse (60 and 90 days) were analyzed using cox regression. Significant higher GM volume was found for the AA-genotype group compared with AG/GG-genotype in the hypothalamus and caudate. A significant interaction was revealed between caudate and amygdala GM volume and GATA4 genotype on time to heavy relapse. The interaction was expressed by means of higher GM in the AA genotype group to be associated with reduced risk to relapse whereas in the AG/GG group higher GM was associated with increased risk to relapse. This is the first report on GM regional volume alterations specific to GATA4 genotype [(SNP) rs13273672] and its association with relapse in alcohol dependence. Current findings further support the role of GATA4 in alcoholism.

1. Introduction

Relapse prevention and the effectiveness of different pharmacological interventions, remain key challenges in the treatment of alcohol dependence (Spanagel et al., 2010). Evidence suggests that alcoholism is manifested through environmental factors interacting with genetics (Mayfield et al., 2008). Genome-wide association studies (GWAS) have underlined the role of the GATA binding protein *4* (GATA4) in alcohol dependence and of the single nucleotide polymorphism (SNP) rs13273672, located in the GATA4 gene (Edenberg et al., 2010; Treutlein et al., 2009). GATA4 acts as regulatory mechanism of plasma levels of several peptide hormones such as the atrial natriuretic peptide (ANP) (Grepin et al., 1994). The investigation of hormonal ANP levels during alcohol withdrawal has important clinical and therapeutic implications (Kovacs, 2000). ANP is involved in homeostatic balance of bodily fluid and blood pressure (Kangawa et al., 1984). It is also a neuropeptide with binding sites in the hypothalamus, the caudate, and the amygdala (Cao and Yang, 2008); regions regulating emotion and anxiety responses. Stress is of particular interest due to its involvement in the development and prolongation of addiction (Kovacs, 2003).

In the event of stress inflation, drug craving increases with stress-induced responses to predict the amount of later drug abuse (Sinha et al., 2006). ANP release in the brain during withdrawal regulates anxiety and stress suggesting its anxiolytic effects in the hypothalamic-pituitary-adrenal (HPA) axis (Antoni et al., 1992). It inhibits secretion of corticotrophin-releasing hormone (CRH), adrenocorticotrophic hormone (ACTH) and cortisol reducing anxiety and stress (Antoni et al., 1992). ANP is therefore a relevant component in the neuroendocrine stress regulation via HPA axis response inhibition (Kiefer and Wiedemann, 2004). In mice, ANP intra-cerebra ventricular injections reduced hyper-excitability of withdrawal like symptoms, whereas injections of an antiserum against ANP had the opposite effect (Kovacs, 2000, 2003). Corresponding human studies demonstrate similar effects of ANP on alcohol dependence and provide evidence of increased anxiety levels and elevated craving to alcohol stimuli in detoxified alcohol patients who had low ANP plasma levels (Kiefer et al., 2002).

Further findings demonstrate a link between the SNP rs13273672 and alcohol relapse at 90 days and higher ANP plasma levels to be accompanied by decreased relapse risk within the 3 month pharmacological treatment period (Kiefer et al., 2011). This finding was true only for the *AA* gene allele carries (Kiefer et al., 2011). In a recent functional magnetic resonance imaging (fMRI) study findings showed the influence of the GATA4 genotype on amygdala activation and alcohol relapse in alcohol dependence (Jorde et al., 2014). Findings revealed significantly lower activation in *AG/GG*-genotype carriers compared to *AA* homozygotes in bilateral amygdala when viewing images of alcohol specific content compared to neutral images. Interestingly, stronger alcohol-specific amygdala activation was significantly associated with lower relapse risk at 90 days in the *AA*-genotype group (Jorde et al., 2014). Taken together, the above findings indicate dysregulation of the stress response system due to altered ANP concentration levels linked to alcohol-related pathophysiology (Kiefer et al., 2002; Kovacs, 2000, 2003); namely withdrawal severity and relapse.

Prolonged alcohol abuse and dependence is known to alter brain gray matter (GM) (Buhler and Mann, 2011), especially in subcortical limbic areas seemingly more sensitive to alcohol's detrimental effects (Makris et al., 2008; Sullivan et al., 2005; Wrase et al., 2008). GM deficits in subcortical brain limbic structures could subsidize relapse and alcohol abuse continuation (Jernigan et al., 1991; Sullivan et al., 2005; Wrase et al., 2008). Regions with high expression of ANP receptor binding sites are mainly limbic including the amygdala, caudate and hypothalamus (Cao and Yang, 2008; Herman et al., 1996; Kawata et al., 1985; Raidoo et al., 1998; Tanaka et al., 1984). However, limited data is available describing how amygdala, caudate and hypothalamus GM volume could relate to GATA4 genotype and heavy relapse in alcohol dependence. Therefore, in the present report GM volume differences or alterations specific to GATA4 genotype were examined and specifically the effects of rs13273672 SNP on amygdala, caudate and hypothalamus. We tested the hypothesis that GM volume differences existed specific to GATA4 genotype and examined a differential effect of GM volume by GATA4 variant on time to heavy relapse within 90 days (Jorde et al., 2014; Kiefer et al., 2011) but also 60 days after study inclusion (Cornelius et al., 2003).

2. Experimental procedures

2.1. Participants

Data in this report have not been collected anew but rather parts of it with a focus on genotype variation and brain function in the amygdala have been previously published (Jorde et al., 2014) including 81