

Glutamate concentration in the anterior cingulate cortex in alcohol dependence: association with alcohol withdrawal and exploration of contribution from glutamatergic candidate genes

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Glutamate-mediated neuronal excitability is found to be increased during alcohol withdrawal in animal studies. The present study aimed to corroborate whether, in humans, the level of glutamate measured by magnetic resonance spectroscopy is increased during withdrawal in the anterior cingulate cortex (ACC), a brain region important for the reward circuitry. It furthermore aimed to test whether glutamatergic candidate genes play a role in this pathophysiological state.

Two previous magnetic resonance spectroscopy studies suggest that the glutamate-to-creatine ratio (Glu/Cr) is increased during early abstinence in the ACC. These two studies included only a few individuals: (i) $n = 33$ alcohol-dependent patients ($n = 15$ with acamprosate treatment, and $n = 18$ with placebo) measured at days 4 and 25 of abstinence (Umhau *et al.*, 2010), and (ii) $n = 13$ at least 2-week-abstinent alcohol-dependent patients and $n = 18$ controls (Lee *et al.*, 2007). Umhau *et al.* (2010) found a significant decrease in Glu/Cr over time in the acamprosate group and a nonsignificant trend of an increase in Glu/Cr in the placebo group. Lee *et al.* (2007) reported increased Glu/Cr in patients compared with controls. We

previously showed an increase in Glu, referenced to unsuppressed water signal (Hermann *et al.*, 2012; see Supplementary Text, Supplemental digital content 1, <http://links.lww.com/PG/A205>, for further information).

The present study comprised 62 cases and 57 controls (see Supplementary Information, Supplemental digital content 1, <http://links.lww.com/PG/A205>, for details). Glutamate concentrations were assessed using Glu/Cr, as a comparatively large region of interest was analyzed, and Glu/Cr better corrects for the large proportion of cerebrospinal fluid in large measurement areas than the reference to a water signal.

In cases, Glu/Cr in the ACC was significantly increased compared with controls [$t(116.74) = -2.27$; $P = 0.025$]. This association was based on the absolute Glu_{corrected} level [$t(101.13) = 2.81$; $P = 0.006$], but not on the Cr_{corrected} level [$t(117.00) = 1.11$; $P = 0.27$] (both: ratio to water, corrected for cerebrospinal fluid content in the voxel), indicating that in our study, Glu is the main neurometabolite driving the Glu/Cr association signal.

In the genetic analysis, 216 genes related to the glutamate system (rat ‘glutamate chip’, Supplementary Information, Supplemental digital content 1, <http://links.lww.com/PG/A205>) were tested for association with glutamate levels. No association was significant after correction for multiple testing. The most significant glutamatergic candidate gene in the 62 cases was GATA-binding protein 4 (*GATA4*) (gene-based test, $P_{\text{uncorrected}} = 0.0029$; Supplementary Tables S2–S4, Supplemental digital content 1, <http://links.lww.com/PG/A205>).

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com/PG/A205). *GATA4* influences the neuroendocrine regulation of stress by the atrial natriuretic peptide and in a recently published large genome-wide association study of neuroticism in over 329 000 individuals (Luciano *et al.*, 2018), *GATA4* was found to be located in a genome-wide significant region ($P=2.18 \times 10^{-24}$). Neuroticism, a personality trait characterized by negative emotionality, is considered a risk factor for psychiatric disorders including alcohol dependence. Furthermore, *GATA4* was among the top findings in two independent GWAS on alcohol dependence (see Supplementary text, Supplemental digital content 1, <http://links.lww.com/PG/A205>, for further information), as well as in a genetic study on relapse risk (Kiefer *et al.*, 2011).

In conclusion, in the largest sample to date, we corroborated that alcohol withdrawal is associated with increased Glu/Cr in the ACC. Although our top finding *GATA4* is plausible, it should be considered that it was not significant after correction for multiple testing.

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Conflicts of interest

There are no conflicts of interest.

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