A non-synonymous polymorphism in galactose mutarotase (GALM) is associated with serotonin transporter binding potential in the human thalamus: Results of a genome-wide association study

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Positron emission tomography (PET) imaging using the tracer [11]C]DASB is a sensitive and noninvasive technique for measuring brain serotonin transporter (5-HTT) levels in vivo. Previously, Cannon et al. found that 5-HTT levels were increased in thalamus, striatum, insular and cingulate cortices in unmedicated, depressed subjects with major depressive disorder or bipolar disorder. To explore this potential biomarker for mood disorders, we undertook a proof-of-principle genome-wide association study.

5-HTT levels, measured as [11]C]DASB binding potential (BPND), were assessed in six brain regions-of-interest (ROIs). Healthy (n=22) and unmedicated participants diagnosed with bipolar disorder (n=16) or major depressive disorder (n=17), aged 18 to 48 years (Mean ± SD, 34 ± 9 yr) underwent PET scanning with [11]C]DASB. Subject ascertainment, PET scanning, and analysis were described previously. DNA was extracted from peripheral blood and genotyped on the Illumina Human-1 SNP array. Genotypes were called with...
BeadStudio v3.0. Data were filtered for minor allele frequencies < 1%, missing genotype rates > 8%, Hardy-Weinberg Equilibrium p-values <0.05, gender mismatch, and hidden relatedness. A total of 93,427 SNPs passed all quality control filters for all of the ROIs tested. Association analysis was performed by linear regression in PLINK\(^4\) (v1.02), with 3 covariates to control for ancestry. Genomic Control lambda values were all <1.05, indicating good control of confounding factors.

Overall, 5 SNPs representing 5 different genes met our FDR<0.1 criterion for being declared of interest: rs6741892 in \(GALM\), rs390704 near \(FRY\), rs7161217 near \(TTLL5\), rs583241 near \(CPLX4\), and rs7095106 near \(CASC2\). The strongest association was between thalamic \([^{11}C]DASB-BP\) and a coding SNP in the gene \(GALM\) (rs6741892, \(p=4.67\times10^{-8}\); FDR=0.004). This result meets the threshold of genome-wide significance under typical frequentist assumptions, but not if corrected for 6 ROIs. Rs6741892 accounted for about 50% of the variance in \([^{11}C]DASB-BP\) in thalamus. Carriers of the “TT” genotype showed the highest \([^{11}C]DASB-BP\).

Since the T-allele frequency differed by self-reported ancestry (10/13 in African-Americans, 3/13 in whites, and 0/7 in Hispanics), we also performed the analysis within each ancestry group, then combined the p-values by meta-analysis (META 5.3, \url{http://userpage.fuberlin.de/~health/meta_e.htm}), with similar results. We further confirmed this finding in a voxel-based analysis (Fig 1).

Nominally-significant associations were also observed between rs6741892 and \([^{11}C]DASB-BP\) in dorsal cingulate cortex (DCC; \(p=4.03\times10^{-2}\)) and insula (\(p=1.14\times10^{-3}\)). No SNPs near the gene encoding the 5-HTT (\(SLC6A4\)) showed significant association in this sample, consistent with one prior study\(^5\), but we may have missed a true association due to lack of power. Rs6741892 was also individually genotyped using a modification of the 5′ nuclease (Taqman) assay, with similar results.

Consistent with our prior findings\(^2-3\), we found a significant main effect of diagnosis on \([^{11}C]DASB-BP\) measured in thalamus (\(F(2, 52) =7.07, p<0.002\)) due to significantly higher thalamic \([^{11}C]DASB-BP\) in participants with mood disorders. However, the overall association results were not dependent on diagnosis: rs6741892 was also associated with \([^{11}C]DASB-BP\) in healthy participants.

Supportive evidence of association with rs6741892 was obtained from an independent replication sample of 51 European-ancestry subjects (16 females aged 43±20 yr, 35 males aged 34±18 yr) ascertained in Denmark, using TaqMan. Imaging and analysis were as described\(^6\). Significant associations were observed between rs6741892 and \([^{11}C]DASB-BP\) in DCC (\(p=3.5\times10^{-3}\)) and insula (\(p=4.9\times10^{-3}\)), but not in thalamus, although \([^{11}C]DASB-BP\) is highly correlated across subcortical structures\(^7\). Combined analysis with META 5.3 supported association between rs6741892 and \([^{11}C]DASB-BP\) in all 3 regions of interest (DCC, \(p=9.18\times10^{-4}\); insula, \(p=3.25\times10^{-5}\); and thalamus, \(p=4.6\times10^{-6}\)).

\(GALM\) encodes galactose mutarotase, which catalyzes the conversion of beta-D-galactose to alpha-D-galactose\(^8\), important in carbohydrate metabolism and the production of complex oligosaccharides. Galactose mutarotase might affect regional neurophysiology, leading to
local increases in serotonin release and in membrane trafficking of 5-HTT\textsuperscript{9}, thereby increasing $^{[1]}$C\textsuperscript{[1]}]DASB-BP\textsubscript{ND}. Galactose mutarotase may also play a role in N-glycosylation, which is important for surface expression of 5-HTT\textsuperscript{10}.

To our knowledge, this is the first genome-wide association study of brain 5-HTT. These preliminary results suggest that neuroimaging phenotypes could represent informative targets for a GWAS, even in relatively small samples. Further studies are needed to confirm these findings and determine the underlying biological mechanisms.

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Figure 1. Map of t-values from voxel-wise analysis of rs6741892, overlaid on a sample axial MRI slice at the level of the medial thalamus (z=6 mm). Bilaterally, T-allele carriers (n=13) have greater serotonin-transporter binding potential than AA homozygotes (n=42). The color bar indicates the range of t-values displayed (max t = 6.56, df=53, p=2.3 × 10⁻⁸).