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# Genome-wide association study of working memory brain activation

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ABSTRACT

In a population-based genome-wide association (GWA) study of *n*-back working memory task-related brain activation, we extracted the average percent BOLD signal change (2-back minus 0-back) from 46 regions-of-interest (ROIs) in functional MRI scans from 863 healthy twins and siblings. ROIs were obtained by creating spheres around group random effects analysis local maxima, and by thresholding a voxel-based heritability map of working memory brain activation at 50%. Quality control for test-retest reliability and heritability of ROI measures yielded 20 reliable ($r > 0.7$) and heritable ($h^2 > 20\%$) ROIs. For GWA analysis, the cohort was divided into a discovery ($n = 679$) and replication ($n = 97$) sample. No variants survived the stringent multiple-testing-corrected genome-wide significance threshold ($p < 4.5 \times 10^{-9}$), or were replicated ($p < 0.0016$), but several genes were identified that are worthy of further investigation. A search of 529,379 genomic markers resulted in discovery of 31 independent single nucleotide polymorphisms (SNPs) associated with BOLD signal change at a discovery level of $p < 1 \times 10^{-5}$. Two SNPs (rs7917410 and rs7672408) were associated at a significance level of $p < 1 \times 10^{-7}$. Only one, most strongly affecting BOLD signal change in the left supramarginal gyrus ($R^2 = 5.5\%$), had multiple SNPs associated at $p < 1 \times 10^{-5}$ in linkage disequilibrium with it, all located in and around the BANK1 gene. BANK1 encodes a B-cell-specific scaffold protein and has been shown to negatively regulate CD40-mediated AKT activation. AKT is part of the dopamine-signaling pathway, suggesting a mechanism for the involvement of BANK1 in the BOLD response to working memory. Variants identified here may be relevant to (the susceptibility to) common disorders affecting brain function.



## 1. Introduction

Working memory task-related brain activation is altered, showing mostly increased activation, in several neuropsychiatric disorders, such as schizophrenia (Bor et al., 2011; Callicott et al., 2003b), bipolar disorder (Drapier et al., 2008), and major depressive disorder (Matsuo et al., 2007), as well as the healthy, at-genetic-risk, siblings of patients for some of these disorders (e.g., Callicott et al., 2003a; Drapier et al., 2008; Winterer et al., 2003). These disorders are highly heritable, but their onset and trajectory are thought to be influenced by a large number of genetic polymorphisms, each with a small effect, as well as environmental factors. Because abnormal working memory (WM) brain activation is implicated in brain disorders, factors that influence the blood oxygen level–dependent (BOLD) response in human populations are of great interest. In our prior voxel-wise analyses of heritability, up to 65% (averaging ~33%) of the variation in WM task-related cerebral activation (Blokland et al., 2011) and up to 75% (averaging ~36%) of the variance in WM task-related cerebellar activation (Blokland et al., 2014) was attributed to genetic factors. While these studies showed that human brain function is under substantial genetic control, specific genetic variants influencing individual differences are largely unknown. Genes that contribute to brain function are important to identify, as several known examples confer protection or risk for brain disorders. Carriers of the 'disrupted in schizophrenia 1' (DISC1) risk haplotype, for example, have a fivefold increased risk for schizophrenia (Zhang et al., 2006).

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Candidate gene studies of WM brain function have provided mechanistic support for the implication of certain genetic variants associated with neuropsychiatric disorders. For example, healthy individuals carrying the ZNF804A schizophrenia and bipolar disorder risk allele (O'Donovan et al., 2008), showed changes in functional connectivity of the right dorsolateral prefrontal cortex (DLPFC) during WM that resembled those observed in schizophrenia (Esslinger et al., 2009). Their findings were partly replicated in an independent sample (Paulus et al., 2011). During rest and during an emotional task, a pattern of reduced inter-hemispheric prefrontal connectivity with increasing number of risk alleles similar to that during WM has been demonstrated, suggesting a state-independent influence of the gene variant on inter-hemispheric processing (Esslinger et al., 2011). Other studies have found genetic associations between the schizophrenia-risk gene neuroregulin1 (NRG1) (Li et al., 2006; Stefansson et al., 2003; Stefansson et al., 2002; Zhao et al., 2004) and WM brain activation in healthy individuals (Krug et al., 2008). While there were no effects of genetic status on behavioral task performance, the number of NRG1 risk alleles had a linear effect on hyperactivation of the superior frontal gyrus. Nicodemus et al. (2010b) showed similar inefficient processing in carriers of risk-associated genotypes, but in the DLPFC instead. Other candidate genes—mainly risk genes for schizophrenia and bipolar disorder—that have been associated with WM brain activation, include genes related to dopaminergic function, such as COMT (Bertolino et al., 2006a; Bertolino et al., 2006b; Egan et al., 2001; Pomarol-Clotet et al., 2010), the dopamine transporter gene (DAT1) (Bertolino et al., 2006a; Stollstorff et al., 2010; Tan et al., 2007), dopamine receptor genes DRD1, DRD2 and DRD4 (Bertolino et al., 2009; Bertolino et al., 2010; Herrmann et al., 2007; Tura et al., 2008); genes related to serotonergic function and glutamatergic action, such as MAOA, DAOA and GRM3 (Cerasa et al., 2008; Nixon et al., 2011; Tan et al., 2007); in addition to several genes involved in various other neuronal functions, such as CACNA1C (Bigos et al., 2010; Paulus et al., 2014), CYP2D6 (Stingl et al., 2012), AKT1 (Nicodemus et al., 2010b; Tan et al., 2008), and BDNF (Cerasa et al., 2010).

These candidate gene studies have been somewhat helpful in improving our understanding of the neurobiology underlying neuropsychiatric disorders, but the genes they studied only explain a small proportion (4–10% variance explained; (Bertolino et al., 2009; Egan et al., 2001; Munafò et al., 2008)) of the heritability of brain activation (up to 65%, averaging 33%; Blokland et al., 2011) and/or of the disorders themselves. Furthermore, these studies were generally limited by small sample sizes, and the findings would be more credible if verified in larger samples. Genome-wide association (GWA) studies using quantitative traits relevant to brain function or disorders have the potential to improve our understanding of the etiology of these processes even further by identifying genes whose relationship with the phenotype has not previously been hypothesized. Using GWA analysis of WM performance, a genetic polymorphism within SCN1A (encoding a subunit of the type I voltage-gated sodium channel) was replicated in three independent populations ($n = 1699$) (Papassotiropoulos et al., 2011). In a subsequent candidate gene fMRI study, SCN1A allele-dependent activation differences during an $n$-back WM task were detected (Papassotiropoulos et al., 2011). However, very few GWA studies have been carried out that use brain activation as a quantitative phenotype. To the best of our knowledge only two GWA studies have investigated brain activation in response to a WM task. Potkin et al. (2009b) studied activation (mean BOLD signal in the DLPFC) during the Sternberg Item Recognition Paradigm in $n = 64$ schizophrenia patients and $n = 74$ matched controls, and identified 6 genes or chromosomal regions involved in neurodevelopment and response to stress (ROBO1-ROBO2, TNIK, CTXN3-SLC12A2 POU3F2, TRAF, and GPC1) with single nucleotide polymorphisms (SNPs) significant at $p < 10^{-6}$ for the interaction between BOLD response and schizophrenia diagnosis. Potkin et al. (2009a) extended this study to $n = 82$ schizophrenia patients and $n = 91$ controls and identified 2 different genes worthy of further study, RSRC1 and ARHGAP18. However, these 2 studies were carried out in a patient-control sample. It would be of great interest to identify gene variants associated with brain function in healthy individuals as this may improve our understanding of normal brain function.

Here, we used an unbiased genome-wide search to identify common genetic variants associated with variations in the fMRI BOLD response to an $n$-back WM task in a healthy young adult twin-sibling cohort, the Queensland Twin Imaging Study ($n = 679$). We incorporated prior knowledge from a voxel-wise study about the total genetic influence on the BOLD response to WM (Blokland et al., 2011), to reduce the number of phenotypes being tested to a manageable number, while maximizing the quality of the functional quantitative trait. A few studies have attempted to carry out voxel-wise GWA analyses on imaging phenotypes (e.g. Hibar et al., 2011; Stein et al., 2010), but with the enormous number of statistical tests performed in voxel-wise GWA analyses (≤200,000 voxels × ≤1,000,000 SNPs), and the stringent multiple testing corrections needed to account for this, it is almost impossible to find significant results. Additionally, in a previous ROI-based study on the heritability of WM brain activation (Blokland et al., 2008), we discovered that using mean BOLD signal across anatomically defined ROIs might obscure the genetic variance, as it is possible that not the entire anatomical region is reliably activated and heritable. Here we carried out several strict quality control steps on the functional phenotype before proceeding to GWA analyses. We enforced a genome-wide statistical threshold, and used two independent samples, a large discovery sample of young adult twins and siblings ($n = 679$) and a smaller replication sample ($n = 97$), to verify any associations and help diagnose false-positive findings.

## 2. Materials and methods

### 2.1. Participants

This study uses data from participants in the Queensland Twin Imaging Study (QTIMS). Most of these twins and siblings, between 16 and 30 years of age, had previously participated in the Brisbane Adolescent Twin study (Wright and Martin, 2004), so measures of cognitive functioning, birth information, and parental socio-economic status (SES) were available for most participants, in addition to the imaging data (details described elsewhere; Blokland et al., 2011). Of $n = 2645$ individuals that were initially approached for the study by letter, 520 declined participation (19.7%), 677 were unable to participate (e.g. moved out of state) or could not be contacted by phone for further screening (25.6%). Prior to inclusion in QTIMS, twins were assessed for handedness using the Annett Handedness Questionnaire (6 questions) (Annett, 1970; Wright and Martin, 2004), and screened (by self-report) for their suitability for imaging. Of $n = 1420$ individuals that had been screened for inclusion in the imaging study at the time this paper was written, 2.2% were excluded because they were left-handed, and a further 22.5% were excluded because they had a history of significant medical, psychiatric or neurological conditions, including head injuries, MRI contra-indicators, a current or past diagnosis of substance abuse, or current use of medication that could affect cognition.

A sample of $n = 1070$ twins and singleton siblings met the inclusion criteria and $n = 1060$ (99%) completed the study. Subsequently, 43 individuals were excluded from analysis due to head motion or abnormal findings on their structural scans, 70 individuals were excluded due to head motion on their functional scans, and an additional 84 individuals were excluded due to insufficient task performance (<40% accuracy on 0-back condition; <30% accuracy on 2-back condition). After excluding these individuals, $n = 863$ twins and siblings remained for analysis. As data acquisition was ongoing when we published our heritability studies, a subset of the current sample was included in our previous voxel-wise studies on the heritability of WM brain activation in the cerebrum (285 of 863, 33.0%) and the cerebellum (353 of 863, 40.9%) (Blokland et al., 2014; Blokland et al., 2011).