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Dysbindin-1 genotype effects on emotional working memory

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Abstract

We combined functional imaging and genetics to investigate the behavioral and neural effects of a dysbindin-1 (*DTNBP1*) genotype associated with the expression level of this important synaptic protein, which has been implicated in schizophrenia. On a working memory (WM) task for emotional faces, participants with the genotype related to increased expression showed higher WM capacity for happy faces compared to the genotype related to lower expression. Activity in several task-related brain areas with known DTNBP1 expression was increased, including hippocampus, temporal and frontal cortex. Although these increases occurred across emotions, they were mostly observed in areas whose activity correlated with performance for happy faces. This suggests effects of variability in *DTNBP1* on emotion-specific WM capacity and region-specific task-related brain activation in humans. Synaptic effects of DTNBP1 implicate that altered dopaminergic and/or glutamatergic neurotransmission may be related to the increased WM capacity. The combination of imaging and genetics thus allows us to bridge the gap between the cellular/molecular and systems/behavioral level and extend the cognitive neuroscience approach to a comprehensive biology of cognition.

Keywords

dysbindin; genetic imaging; working memory; emotional faces; schizophrenia

Introduction

Inter-individual variability of cognitive skills is explained to a considerable degree by genetic factors (1, 2). The combination of molecular genetics and functional imaging allows for the effects of genetic variation in neurobiologically relevant proteins on neurophysiological responses in cognition- and emotion-related neural networks to be investigated in humans (3).

Recent evidence suggests that variability in the dysbindin-1(dystrobrevin-binding protein 1) gene (*DTNBP1*; OMIM 607145) contributes to interindividual variability of cognitive

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functions at both neurophysiological and behavioral levels in healthy individuals as well as in patients with schizophrenia (4-7). For example, 12% of variance in spatial WM performance in patients with schizophrenia were accounted by the C-A-T dysbindin-1 haplotype (4). At the molecular level, genetic variability markers in the *DTNBP1* gene, including SNP rs1047631 located in a 3′UTR (untranslated region) have been shown to index dysbindin-1 mRNA expression (8-10). The G-allele of SNP rs1047631 is associated with 17-19% increase of dysbindin-1 m-RNA levels (8, 9). Variability in non-protein coding sequences including UTRs has been proposed as a major source for interindividual differences of quantitative traits (11). Furthermore it has recently been reported that SNP rs1047631 is positioned within a microRNA binding site (12), which adds to the evidence that variability in this region is involved in gene regulation. Dysbindin-1 gene transcription has been observed in temporal neocortex, entorhinal cortex, orbitofrontal cortex, dorsolateral prefrontal cortex (DLPFC), amygdala and hippocampus of healthy adults, with higher abundance in gray than white matter (9). Reductions of dysbindin-1 mRNA in DLPFC (9), hippocampus (13, 14) and dysbindin-1 protein in glutamatergic pre-synapses of the hippocampus (13) have been reported in patients with schizophrenia. Glutamatergic synapses in these regions contribute to neuronal activity related to WM (15, 16). Therefore interindividual differences in dysbindin-1 protein levels at prefrontal and hippocampal synapses may contribute to interindividual variability in WM-related activity. Dysbindin-1 is involved in the regulation of neuroplasticity (17, 18) and has also been implicated as a candidate gene for schizophrenia (19). Dysbindin-1 directly interacts with 31 proteins involved in cell morphology, cellular development, intracellular and synaptic signalling at its different locations in synaptic vesicles, postsynaptic densities and microtubules (17, 18). Recently BLOC-1 (Biogenesis of lysosme-related organelles complex-1), a dysbindin-containing multi-protein complex, has been identified in the murine cerebral cortex, hippocampus and cerebellum (20). Furthermore this study revealed the developmental regulation of cortical dysbindin protein expression and neurite outgrowth defects in hippocampal neurons of BLOC-1-deficient mice (20). The relevance of *DTNBP1* for schizophrenia might be linked to the multiple interactions of dysbindin-1 with other proteins in neuronal inter-and intracellular signalling pathways, e.g. the PI3-kinase-PKB/Akt intracellular signalling pathway (21). Interestingly, the *Akt1* gene has been implicated in schizophrenia as well (22, 23). Lack of dysbindin synthesis in Sandy mouse, a dysbindin-1 knockout (24), has been found to affect the vesicle structure and kinetics of synaptic glutamatergic transmission of pyramidal neurons in the CA1 region of the hippocampus (25), to reduce evoked responses in prefrontal pyramidal neurons and to impair working memory performance (26). Furthermore, knockdown of dysbindin expression has been shown to affect the organization of actin filaments of the cytoskeleton and phosphorylation of c-Jun N-terminal kinase, which regulates neurite outgrowth (27). Increased dopamine turnover and reduced dopamine levels (28) in cortex and hippocampus have also been observed in Sandy mouse. In sum, there is converging evidence establishing a central role for dysbindin in the regulation of synaptic structure and function.

With its multiple effects on both neocortical and limbic areas, dysbindin is an ideal candidate protein for the regulation of emotion-cognition interactions. Influences of emotion on cognition have been documented in a wide range of domains, including attention, memory and reasoning (29). Here we investigated working memory of emotional faces, and thus memory in a specifically social context, because significant heritability estimates have been obtained for both face memory and emotion recognition (30). We were interested in genetic influences on emotional face WM because we had noted considerable interindividual variability of WM performance benefits for happy and angry compared to neutral faces (31-34). Performance benefits for angry faces were related to enhanced neural processing of angry compared to happy and neutral faces in prefrontal, temporal and subcortical areas (35). Because of dysbindin-1 expression in all of these areas, association of the SNP

rs1047631 with differences in dysbindin-1 expression, and dysbindin's role in both dopaminergic (36, 37) and glutamatergic neurotransmission (13, 18, 21), we hypothesized that genotypic differences for SNP rs1047631 in healthy volunteers contribute to individual differences in emotion effects on WM at the neurophysiological and behavioral level.

Materials and methods

Participants

56 participants (31 males, 52 right handed, age 31.8 ± 9.1 years, min 19 max 51 years, all European Caucasians) were recruited from the local community and through the Bangor University participant panel and were paid £25. Participants had no lifetime or family history of any psychiatric or neurological disease and normal or corrected to normal vision. They provided written informed consent prior to participation. The study was approved by the School's ethics committee in Bangor.

Stimuli

Six adult, male, grayscale Ekman face images each displaying neutral, happy and angry expressions were used. Each image covered approximately 1.43° × 1.36°. Scrambled grayscale face images selected at random were displayed to cover the face locations during encoding of fewer than 4 faces.

Working memory task for emotional faces

The behavioral paradigm has been tested in detail in previous studies (33, 35). In an event-related design (Figure 1) the influence of emotional expressions on visual WM capacity for faces and task-related brain activity was investigated through the manipulation of face expression (angry, happy, and neutral) and the number of faces to be remembered (load 1, 2, 3, 4). Each of the 12 conditions consisted of 4 match and 4 non-match trials. Trials were distributed over 4 runs with 48 trials each to minimize fatigue effects. Face expressions and face load varied randomly between trials and type of face expression was kept constant within one trial. Faces were presented at randomly alternating locations in a 2 × 2 matrix in the centre of the screen, and the centre of each image within the matrix was positioned at a visual angle of approximately 1.27° from fixation to ensure that the face display was foveal. In order to avoid eye movement artifacts, participants were asked to maintain fixation throughout each imaging session. All trials started with fixation towards a central cross on the display which served as baseline. This was followed by two seconds presentation of the memory array, a delay of one second, and the test face, where participants had to indicate a match or non-match response via the respective button. The between trials fixation interval jittered between 4500 - 6000 ms.

Acquisition and analysis of behavioral and imaging data

The task was generated and behavioral data recorded with the E-Prime software (Version 1.1, Psychology Software Tools, Inc.). Scanning was performed with a Philips 1.5T MRI whole-body scanner with a SENSE parallel head coil. Blood oxygenation level-dependent images were acquired by using a T2* weighted gradient echo planar sequence (TR = 2000 ms; TE = 40 ms; matrix size = 96 × 96; FOV =256 × 256 mm^2; voxel size =3 × 3 × 3 mm^3; 90° flip angle; 20 axial slices; 5 mm slice thickness). The first two volumes of each session were discarded to reduce possible T1 saturation effects. During each of the four working memory sessions 343 volumes were acquired. A high-resolution T1-weighted 3D anatomical MR data set was used for co-registration (TR/TE = 11.5/2.95ms; FA = 8°; coronal slice thickness = 1.3 mm; acquisition matrix 256 × 256; in-plane resolution 1 × 1 mm^2).

Working memory accuracy was assessed by calculating d′prime values (d′prime = z-transformed Hits − z-transformed False Alarms) for each of the 12 conditions and each subject. Working memory capacity for faces was measured by individual Cowan's K Max values for each emotion (Cowan's K Max = maximal K reached for this individual at any array size; Cowan's K values = array size * (Hits – FA)).

Imaging data analysis was performed using the BrainVoyager 1.9.10 software (Braininnovation, Maastricht, The Netherlands). Functional images were co-registered with the structural 3D image, spatially normalized to the Talairach system and resampled at a voxel size of 1 × 1 × 1 mm^3, resulting in 218 z-normalized volume time course files (vtcs), (six runs could not be used because of motion artifacts; head motion > 3 mm or chance performance; FA mean > 0.5). Functional images were scan time corrected using sinc interpolation, 3D motion corrected using trilinear interpolation, spatially smoothed (8 mm Gaussian kernel), and temporally high pass filtered (3 cycles per time course). The 218 design matrix files (rtcs) for the general linear model (GLM) analysis incorporated predictors for each of the 12 conditions for all correct trials, one separate predictor for all error trials and 6 predictors derived from the head motion correction for each subject. All but the motion predictors were convolved with a two-gamma haemodynamic reference function.

Based on these vtcs and rtcs from all subjects we computed a random-effect general linear model (RFX-GLM) to obtain beta values per subject and condition at each voxel. These were used as dependent variable to compute a second-level RFX-within-subject two-factors ANOVA with the within subject factors emotion (3 levels) and load (4 levels) to generate functional whole-brain 3D maps for the contrasts angry minus neutral and happy minus neutral faces. In order to reduce the probability of false negatives while still reducing false positives, we corrected for multiple comparisons by using cluster-size thresholding (38, 39) for which we set a corrected significance threshold of $p < .05$. Cluster thresholds were set at 200 voxels and calculated using Brainvoyager QX Cluster-level Statistical Threshold estimator based on a Monte Carlo simulation with 1000 iterations. For each of these clusters an RFX-GLM region of interest (ROI) analysis was computed to extract beta values representing the mean activity over the entire cluster for all 12 task conditions (including only correct trials) per subject for subsequent correlation with behavioral data and statistical analysis in combination with the genetic data.

Finally we tested whether activity in regions affected by overall task performance overlapped with activity in regions affected by genotype. Whole brain maps including individual scores for global performance (z-transformed mean of hits across all 12 conditions minus z-transformed mean of false alarms across all 12 conditions) as covariate were computed for both emotion contrasts (angry-neutral and happy-neutral), and correlations between this performance score and the respective contrast were visualised at a threshold of $r(54) = .26$ ($p<.05$). Each correlation map was overlaid with the respective original contrast map. For regions with overlapping activity beta values were extracted for subsequent statistical analysis for genotype effects.

Genotyping

Genomic DNA was extracted from venous EDTA blood samples, using Invisorb® Blood Giga Kit (Invitek GmbH, Germany). The DNA sequence fragment containing SNP rs1047631 was PCR-amplified (5′-GGT TTG GCT ACA GTC AGC TCT T-3′ and 5′-AGG ACA GCG ACT CTT AAA TTG G-3′, annealing temperature 60°C; 36 cycles, amplification fragments length 444bp). Genotypes were discriminated by digesting PCR-amplified gene products with restriction nuclease BsaA I (New England BioLabs, USA) at 37°C for 4.5 hours. The genotype fragments (*GG* genotype 121bp and 321bp; *AA*-genotype

442bp; *GA* genotype 121bp, 321bp and 442bp) were separated via electrophoresis on a 2% agarose gel supplemented with ethidium bromide (Promega, UK) and visualized under UV-light.

Statistical analysis

a) Analysis of genetic data—Hardy-Weinberg-Equilibrium was checked with χ^2-test (α-level .05; DF = 2), χ^2-test (α-level .05; DF =1) and independent-samples t-test (2-tailed) were used to test whether genotype groups differed on confounding factors.

b) Genotype effects on WM-capacity—We performed independent-samples (*GA* versus *AA*) t-tests (2-tailed) for d′prime mean differences (angry-neutral, happy-neutral, angry-happy and angry&happy-neutral) and maximal Cowan's K values (all 3 emotions) to assess *DTNBP1* genotype effects on working memory accuracy differences (angry-neutral, happy-neutral, angry-happy and angry&happy-neutral) as well as on the individual working memory capacity for each emotion.

c) Correlations between brain activity and WM-performance—Beta value measures (beta means for angry, happy and neutral faces averaged across the four loads and beta mean differences between angry and neutral as well as happy and neutral) from each of the brain regions significantly activated for the angry-neutral and happy-neutral contrasts were tested for correlation (Pearson's correlation coefficient, 2-tailed) with behavioral measures (d′prime mean values for angry, happy and neutral faces averaged across all loads and the d′prime mean differences between angry or happy and neutral), in order to determine task-performance relevant brain regions. Correlations were used as a filter to select those regions for the analysis of dysbindin-1 genotype effect which were active in relation to working memory performance for angry and/or happy faces.

d) Genotype effects on brain activity—Only brain regions where activity significantly correlated with task-performance were analyzed for genotype effects. Mixed ANOVAs with two within-subjects factors (emotion: angry, happy, neutral and load: 1 to 4) and one between-subjects factor (*DTNBP1* genotype: *GA*, *AA*) were calculated to assess genotype effects on brain activation. We then tested the genotype effect for each emotion (averaged across load) separately and for the difference between angry or happy and neutral using independent-samples t-test (2-tailed).

Results

Dysbindin-1 genotype

The frequency for the G-allele of the *DTNBP1* SNP rs1047631 was 0.12 with the genotypes distributed according to Hardy-Weinberg equilibrium. There were no individuals homozygous for the G-allele. Participants in the *GA* (N = 13) and *AA* (N = 43) groups showed no significant difference of age, years of education, gender or handedness (Tab. S1).

Dysbindin-1 genotype affects working memory performance for happy faces

When we pooled the angry and happy compared to the neutral condition, there was no significant (p = .44) effect of the DTNBP1 genotype on WM accuracy (d′prime difference). The difference between angry and happy likewise was not affected significantly (p =.14) by the genotype. Both genotype groups showed better WM accuracy for angry compared to neutral faces (d′prime difference angry minus neutral for *GA* group M = 0.35, SE = 0.22; for *AA* group M = 0.22, SE = 0.10), but there was no difference of this angry benefit between groups. Conversely, for happy vs. neutral faces only the *GA* group had significantly

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better WM accuracy (d′prime difference happy minus neutral for *GA* group $M = 0.38$, $SE = 0.19$; for *AA* group $M = -0.02$, $SE = 0.09$). This group difference was significant at $t(54) = 2.08$, $p < .05$, representing a medium effect $r = .27$ (7% of variance explained) of the *DTNBP1* genotype (Figure 2a).

The K max, an estimate of WM capacity, was also higher for happy faces in the *GA* group ($M = 2.70$, $SE = 0.18$) than in the *AA* group ($M = 2.29$, $SE = 0.10$), $t(54) = 1.97$, $p = .05$ representing a medium effect ($r = .26$) of genotype on the maximal number of happy faces held in WM (Figure 2b).

When we added participant gender as a factor to our analysis of DTNBP1 genotype effects on WM performance (d′prime and Kmax) we found neither an influence of gender nor any interaction between DTNBP1 genotype, gender and type of emotion with all p's >.1.

Imaging data

We sought to unravel why working memory performance for happy but not for angry faces was significantly improved in participants heterozygous for the G-allele. First we identified brain regions with significantly higher activity during WM for angry compared to neutral and happy compared to neutral faces, based on the performance benefit for these emotions. Second we tested those brain regions for significant correlations between WM-related activity and WM-accuracy for angry, happy or neutral faces. Third, we analyzed the activity in regions with significant activity-accuracy correlations for modulation by dysbindin-1 genotype.

1. Neural correlates of working memory for angry and happy faces—There was no significant interaction between the factors load and emotion, and we thus report planned whole-brain contrasts (angry-neutral and happy-neutral) with emotions pooled across loads applying a cluster threshold correction for multiple comparisons of 200 voxels at $p < .05$. Higher activation for angry faces compared to neutral faces was observed in the left and right insula, right superior temporal sulcus (STS), right and left inferior temporal gyrus (ITG), left and right globus pallidus (GP), right orbital-frontal cortex (OFC), left and right ventrolateral prefrontal cortex (VLPFC), right dorsolateral premotor cortex (DLPC), right middle frontal gyrus (MFG), right caudate nucleus (CN), right amygdala extended, left hippocampus, left and right fusiform face area (FFA), lower part of the right intra-parietal sulcus (IPS), right inferior parietal lobe (IPL), right and left occipital cortex (OC), right and left occipital face area (OFA, (40), and left substantia innominata (SI) (Fig. 3a, Tab. S2a). Higher activation for happy compared to neutral faces was observed in the left and right OC, left and right OFA, left insula, left SI, right VLPFC, right and left inferior frontal gyrus, right OFC, right inferior and middle temporal gyrus, right and left amygdala, left FFA, and left entorhinal cortex (Fig. 3b, Tab. S2b).

2. Activity-performance correlations of working memory for angry and happy faces—Increased activity correlated significantly with better WM accuracy in 16 brain regions activated for the angry-neutral contrast and the happy-neutral contrast (Tab.1). All regions with significant activity-accuracy correlations for happy faces showed correlations between mean activity and mean accuracy for happy faces, and additionally for the difference between happy and neutral faces in the STS, ITG and FFA of the right hemisphere. For angry faces, only the right IPL, left OFA and bilateral ITG showed significant correlations between mean activity and mean accuracy, with all remaining activity-accuracy correlations for angry faces referring to the difference between angry and neutral faces. WM accuracy-activity correlations for emotions and emotion contrasts thus differed between brain regions.

3.Effect of dysbindin-1 genotype on task-related brain activity measures—Of the above areas that showed both higher activities for emotional compared to neutral faces and correlations of activation levels with performance, only FFA, ITG, OFC, OC and OFA of the right hemisphere and the left hippocampus showed a significant dysbindin-1 genotype effect. In all brain regions the genotype effect was produced by enhanced activity for the *GA* compared to the *AA* group (Fig. 4a-f).

In the left hippocampus (Fig. 4b, Tab. S3b) and right OC (Fig. 4c, Tab. S3c), the *GA* group showed significantly higher activity than the *AA* group for all face categories. In the right FFA (Fig. 4a, Tab. S3a), right OFA (Fig. 4f, Tab. S3f) and right ITG (Fig. 4e, Tab. S3e), activity for angry and happy but not for neutral faces was significantly higher in the *GA* versus *AA* group. In the right OFC (Fig. 4d, Tab. S3d), activity for angry and neutral faces but not for happy faces was significantly enhanced in the *GA* versus *AA* group.

4. Relationship between overall task performance and dysbindin-1 genotype on task-related brain activity—A whole brain correlation analysis between the global performance and brain activity for each emotion contrast (angry-neutral/ happy-neutral) at $p < .05$ and cluster threshold 200 voxels (and even without applying the cluster threshold) revealed no overlap with the respective original emotion contrast maps (Fig. S5) except in the right and left inferior frontal sulcus region for the happy-neutral maps. ANOVAs revealed no significant DTNBP1 effect on activity in the right ($p = .49$ and left ($p = .74$) DLPFC in agreement with our initial analysis that revealed no genotype effects in both these regions.

Discussion

We report a dysbindin-1 genotype effect on WM performance for emotional faces that is also reflected in enhanced task-related brain activity. Participants heterozygous for the G-allele (the *GA* group) compared to homozygous A-allele carriers (the *AA* group) for SNP rs1047631 showed higher WM accuracy for happy compared to neutral faces and also higher individual maximal WM capacity for happy faces. At the neurophysiological level we found enhanced activity for happy faces in the right FFA, left hippocampus, right OC, right OFA and right ITG in the *GA* compared to the *AA* group. The *GA* group also showed increased activity for angry faces in these regions and additionally in the right OFC. Except for the occipital cortex for which expression data is still unavailable, these brain areas match with those where dysbindin-1 mRNA (8, 9) and protein expression have been reported (13, 18). The G-allele of SNP rs1047631 has been associated with a 17-19% mRNA expression increase in prefrontal and temporal areas (8, 9). All these brain regions except the right FFA, right OFA and right ITG also showed higher activity for neutral faces in *GA*-genotype carriers. This suggests an effect of the *GA* genotype on WM-related brain activity in regions likely to express dysbindin-1.

Neural correlates of working memory for angry and happy faces

Irrespective of the genotype effect we identified brain regions with enhanced activity for angry or happy compared to neutral faces to test whether those regions contribute to WM performance for angry or happy compared to neutral faces. Correlations between WM performance and WM-related brain activity were significant in STS, FFA, OFC, OC, OFA, amygdala extended, hippocampus, ITG, GP, IPS, IPL and CN, regions repeatedly reported in fMRI studies of emotional face processing (41, 42) and face WM (34, 43, 44). In addition electrophysiological evidence points to face and/or face expression processing neurons in the STS, OFC, FFA, ITG and the amygdala (45), adding to the plausibility of brain areas

with emotion effects in the present study. All significant correlations were positive, linking higher activation with better task performance.

Although the *DTNBP1* genotype affected brain activity for all emotion conditions, at the behavioral level it only showed a significant effect on WM for happy faces. Interestingly in the FFA, ITG, OC and OFA of the right hemisphere, the significant enhancement of activity for happy faces in the GA group compared to the AA group was combined with a positive correlation of performance and activity for happy faces. Conversely, for angry faces we found a correlation between activity and performance and significantly increased activity for angry faces associated with the GA genotype only in the right ITG. The reason for the selective enhancement of WM capacity for happy faces may thus lie in the genotype-associated increases in activity and the positive effects of increased activity on task performance in these visual areas for happy faces.

Several previous studies of genotype effects on neural activity have observed activity changes that did not translate into performance differences (46-48). This observation suggests that the small neurochemical changes brought about by most functional polymorphisms need to influence performance-related neural activity in a critical number of regions within the task-related neural network before they will significantly alter behavioral performance.

Link with schizophrenia

The G-allele of SNP rs1047631 is included in a putative protective haplotype for schizophrenia that also comprises the G-allele of marker rs3213207 and T-allele of marker rs760761, which both were shown to be under-transmitted in patients with schizophrenia (8). This haplotype has been strongly associated with high *DTNBP1* expression (8). The combination of the T-allele of SNP rs2619538, and the A-allele of rs3213207 with the A-allele of rs1047631 has been demonstrated to maximize the frequency difference (5.2%) between patients with schizophrenia and healthy controls (8). The relative expression of the A-allele of SNP rs1047631 has been found to be more reduced in the presence than in the absence of this T-A-A risk haplotype (8). Even in the absence of this risk haplotype, interindividual variability of relative DTNBP1 expression has been observed, demonstrating that this risk haplotype can account for some but not all variation in DTNBP1 expression (8). Furthermore the low expression A-allele has been shown to be in phase with several previously identified risk haplotypes (8). The alleles T and A of SNP rs2619538 and rs3213207 from the T-A-A risk haplotype are also included in the C-A-T haplotype associated with schizophrenia (49) which has been linked to reduced bilateral occipital response during low-level visual processing in patients with schizophrenia (5). Schizophrenia patients and control participants carrying the T-allele of rs1018381, which is a tagging SNP for another dysbindin-1 haplotype linked to schizophrenia, showed significantly worse general cognitive ability (6). Interestingly, in this sample the T-allele was in complete linkage disequilibrium with the A-allele of rs1047631, the risk allele of the polymorphism investigated in the present study (6). Taken together these findings suggest that SNP rs1047631 is probably non-independent of other markers that also index variability in DTNBP1 gene expression, variability at the neurophysiological and the behavioral level, as well as the genetic risk for schizophrenia. Thus, future studies of neural and behavioral effects of DTNBP1 variability should look at the entire haplotypes rather than individual SNPs.

Although the associations between variability in the dysbindin gene and schizophrenia are still tentative, they are interesting in light of the reported reductions of *DTNBP1* mRNA and expression in the substantia nigra, hippocampus and PFC of patients with schizophrenia (9, 13, 14), which may be related to hypodopaminergic states of these regions, negative

symptoms and cognitive impairments in schizophrenia (28). Underexpression of dysbindin may thus also contribute to the well-documented deficits in emotion processing in schizophrenia (50, 51).

Neurobiological mechanisms for *DTNBP1* effects

How then can changes in *DTNBP1* expression affect neuronal functioning? Up-regulation of *DTNBP1* protein expression in cultured cortical neurons induced expression of the pre-synaptic proteins SNAP25 (SNAP25 is one component of SNARE protein complex, involved in intracellular vesicle trafficking and neurotransmitter release) and synapsin I (synaptic vesicle-associated, cytoskelatal protein) resulting in enhanced exocytotic glutamate release (21). Higher *DTNBP1* expression also promoted neuronal function and survival via the phosphorylation of Akt protein (protein kinase B, PKB) mediated by activation of the phosphatidylinositide 3-kinase (PI3K) pathway. The down-regulation of dysbindin-1 protein resulted in the opposite effects on glutamate release, protein expression and neuronal survival (21). In neurons of the midbrain, knockdown of dysbindin-1 increased dopamine release and SNAP25 protein expression, while up-regulation of dysbindin-1 showed no significant effect on SNAP25 protein expression (36).

These combined findings suggest a region and transmitter-system dependent role of *DTNBP1* expression. Thus a critical reduction of *DTNBP1* might reduce glutamatergic as well as dopaminergic signaling and SNAP25 expression in regions such as orbital frontal cortex and hippocampus while increasing dopaminergic signaling and SNAP25 expression in the midbrain. With respect to our results in healthy volunteers this suggests that the reduced task-related activity that we observed in regions such as hippocampus and orbital-frontal cortex in carriers of the genotype associated with reduced *DTNBP1* expression may be linked to reduced and/ or less efficient glutamatergic and dopaminergic signaling in these areas. Considering the reciprocal connections between these regions (52), dopamine signaling in orbital frontal-cortex could affect hippocampal-prefrontal synaptic transmission and dopaminergic neurons in midbrain could be modulated by PFC and hippocampus.

Nevertheless the high percentage of carriers with the dysbindin-1 genotype associated with low expression suggests some advantage of reduced dysbindin-1 levels. These may be linked to its role as activator of the PI3K-PKB pathway with ensuing effects on cell growth, cell division, cell differentiation, cell migration, and cell survival (53).

Investigation of genetically-driven interindividual variability in cognitive functions with genetic imaging – potentials and limitations

Genetic imaging holds the potential to detect genetic effects that influence interindividual differences at the neural network level. It is encouraging that despite the generally small size of genetic effects we found statistically significant associations between a single marker for variability in the dysbindin-1 gene, brain activity and performance measures of a complex WM task for emotional faces. The size of our sample was large enough to detect significant effects at both behavioural and neural network level. The power to detect the DTNBP1 genotype effect on WM performance (d′prime) for the difference between happy and neutral was 48.1%. At the neural network level power to detect the genotype effect for example in the right occipital cortex was 79.2% for angry, 82.4% for happy and 69.7% for neutral, which conforms to suggestions that brain activation measures are more sensitive to gene effects than behavioural measures. The effect sizes are comparable to previous genetic imaging work (54) and a single variant in a single gene is certainly at best a small contributor to the overall interindividual variability in neurophysiological and behavioral measures of a complex trait (55). Cognitive traits are modulated by multiple interacting genetic (56), epigenetic (57) and environmental factors (58). Indeed interindividual

variability in the relative allelic expression for SNP rs1047631 has been shown, indicating additional cis/trans-acting, epigenetic or environmental influences (8, 10) on the regulation of the turnover, translation and subcellular localization of dysbindin-1 mRNA. We were particularly interested in SNPs within 3'UTRs because of their potential significance for gene regulation by microRNAs, as assumed for SNP rs1047631 (12). The translational repression of synaptic proteins by miRNAs has been shown to regulate dendritic growth (59-61). In this way changes in regulative mRNA sequences could mediate genetically-driven neurophysiological changes with effects on cognitive functions as well as being a target of neuronal activity-dependent regulators with effects on gene expression. Genetic imaging can contribute to our understanding of the functions of non-coding sequences by investigating the effects of their variations on complex traits like cognition. However, the conclusions of this study, like of any genetic imaging study, would be strengthened by replication in an independent sample.

The genotype selected for the present study may be paradigmatic of a new trend in the investigation of gene regulation, especially the role of regulative non-coding sequences, and their influence on interindividual differences in complex cognitive traits. Our results suggest that variability in a non-coding sequence of *DTNBP1* contributes to individual differences in emotional working memory and together with previous findings support a role of dysbindin-1 in enhancing synaptic function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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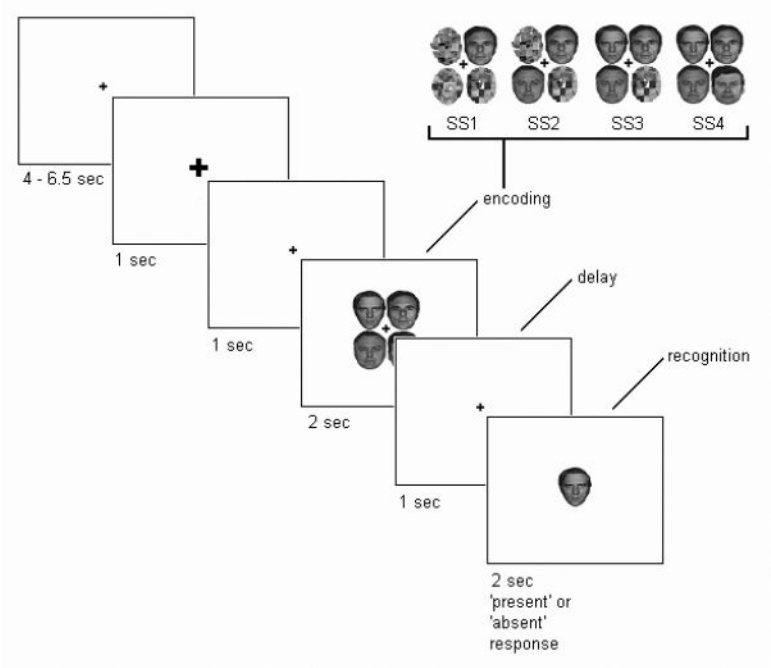

Figure 1. The working memory paradigm
Trial structure of the working memory task for emotional faces.

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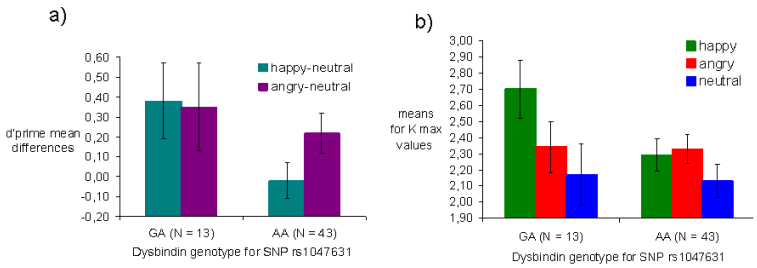

Figure 2. Effect of dysbindin-1 genotype on emotional face WM-performance

a) The d′prime (accuracy) mean difference between happy and neutral faces was significantly bigger ($p < .05$, 2-tailed) in the *GA* group than *AA* group. Genotype groups differed not significantly for the d′prime difference between angry and neutral faces. Error bars display standard error of the mean. **b)** The K max mean values (WM-capacity) were higher ($p = .05$, 2-tailed) for happy but not for angry or neutral faces in participants with *GA*- compared to *AA*-genotype. Error bars display standard error of the mean.

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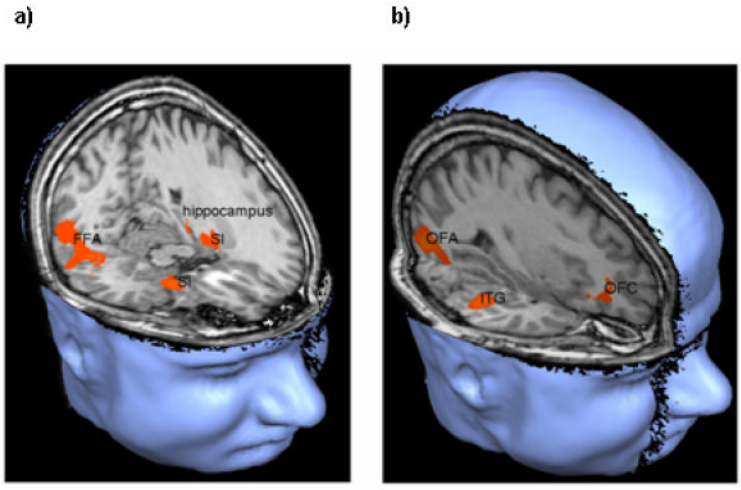

Figure 3. Effect of dysbindin-1 genotype on brain activity for angry and happy faces

a) Higher activation for angry than neutral faces in the right fusiform face area (FFA), left hippocampus, right and left substantia innominata (IS). **b)** Higher activation for happy than neutral faces in the right occipital face area (OFA), right inferior temporal gyrus (ITG) and right orbital frontal cortex (OFC), corrected $p < .05$ (2-tailed) and cluster-threshold 200 voxels.

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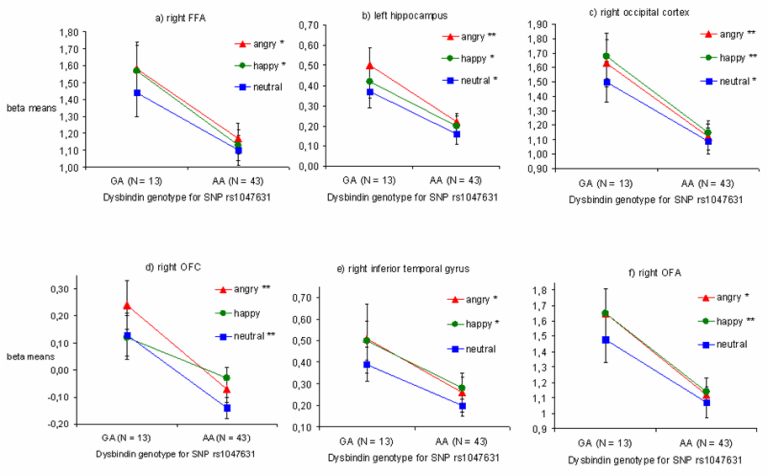

Figure 4. Effect of dysbindin-1 genotype on localised brain activity

Effect of dysbindin-1 genotype on beta means for angry, happy and neutral faces (* $p < .05$, ** $p < .01$, *** $p < .001$; t-tests (2-tailed)) **a)** the right fusiform face area, **b)** left hippocampus, **c)** right occipital cortex, **d)** right orbital frontal cortex, **e)** right inferior temporal gyrus and **f)** right occipital face area. Error bars display standard error of the mean.

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Tab.1

Brain regions where activity significantly correlated with behavioural WM-performance.

Brain region	d′prime mean by condition & mean beta values by condition	R^2	*p*	Brain region	d′prime mean difference between conditions & beta mean difference between conditions	R^2	*p*
Right FFA	happy	.08	.040	Right amygdala extended	angry & neutral	.09	.027
Right GP	happy	.07	.042	Right CN	angry & neutral	.10	.021
Right IPL	angry	.08	.038	Right FFA	angry & neutral	.23	< .001
	neutral	.10	.016		happy & neutral	.11	.012
Right IPS	happy	.11	.011	Left FFA	angry & neutral	.13	.006
	neutral	.10	.021				
Right ITG	happy	.13	.006	Left hippocampus	angry & neutral	.08	.030
	angry	.08	.033				
Left ITG	happy	.10	.015	Right IPL	angry & neutral	.24	< .001
	angry	.07	.044				
	neutral	.14	.005				
Right OC	happy	.07	.044	Right ITG	angry & neutral	.07	.049
					happy & neutral	.10	.016
Right OFA	happy	.07	.046	Left OC	angry & neutral	.08	.037
	neutral	.09	.024				
Left OFA	angry	.09	.022	Right OFA	angry & neutral	.14	.005
	happy	.08	.039				
Right OFC	happy	.09	.022	Left OFA	angry & neutral	.10	.019
Right STS	happy	.13	.007	Right STS	angry & neutral	.15	.003
					happy & neutral	.17	.001