Brief Report

The risk variant in ODZ4 for bipolar disorder impacts on amygdala activation during reward processing


Objectives: Bipolar disorder is a severe mood disorder, which normally begins during adolescence or early adulthood and has a heritability of up to 80%. The largest genome-wide association analysis of bipolar disorder recently identified a new genome-wide associated variant in ODZ4 (rs12576775). The aim of the present study was to further elucidate the role of this risk variant in the disease process using an imaging genetics approach. As increased amygdala and striatal responses during the processing of reward and emotion are characteristic for bipolar disorder patients, it was tested whether the risk variant has an influence on this endophenotype in healthy adolescents.

Methods: We examined the impact of the risk variant rs12576775 on functional magnetic resonance imaging data in an adolescent sample (N = 485). Differential activation between carriers of the risk allele (G-allele) and homozygous A-allele carriers in the amygdala and the striatum during a modification of the monetary incentive delay task (examining reward) and a face task (examining emotion) was analyzed.

Results: Carriers of the risk allele showed an increased blood oxygen level-dependent response in the amygdala during reward sensitivity (p = 0.05) and reward expectation (p < 0.05) but not during the face task. No significant group differences were found in the striatum during both reward and emotion processing.

Conclusion: Our results indicate that the ODZ4 risk variant influences reward processing in the amygdala. Alterations in the processing of emotion may have different underlying mechanisms and need to be further examined.

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Bipolar disorder is a severe mood disorder, mainly characterized by impairment of affect and by further functional detractions. It normally begins during adolescence or early adulthood and has an estimated heritability of up to 80% (1). Common variants with small effects and rare variants with stronger effects are both supposed to contribute to the disorder (2). Thus, many studies search to explore the genetic background on a molecular level, and the largest genome-wide association analysis of bipolar disorder (N = 63766) recently identified a new genome-wide associated variant in the intronic region of OZD4 (rs12576775) (3). OZD4 is located on chromosome 11 and encodes the teneurins. Cell surface signaling and neuronal pathfinding are expected to be the major functions of these genes. In order to further elucidate the role of this risk variant in the etiology of bipolar disorder, we used an imaging genetics approach. As bipolar disorder patients have been shown to display deficits in processing reward and emotion, we concentrated on these functions and the involved brain structures. Earlier studies have illustrated that patients with bipolar disorder show an increased blood oxygen level-dependent (BOLD) response in the amygdala during a reward task (4) and a dysfunction in a ventral–limbic brain network in response to emotional stimuli such as facial expressions (5). Another study showed a dysfunction of the balance of the ventral striatum and amygdala during computation of expected value in a guessing task (6). In this guessing task, subjects could gain or lose money by choosing cards. The gain-related part of the expected value was represented in the ventral striatum, whereas the loss-related part was represented in the amygdala. Thus, the authors hypothesized that prevalence of either part might contribute to emotional highs or lows. In our study, we sought to examine a possible impact of the ODZ4 risk variant on processing reward and emotion in the amygdala and the striatum in healthy adolescents. Such an endophenotype imaging genetics approach in healthy individuals has been proven to be successful in elucidating the role of risk variants as the results are not confounded by variables like medication or duration of the disorder (7).

Materials and methods

Subjects
A large sample of healthy adolescents was recruited from the general populations of Germany, the UK, Ireland, and France within the Imaging Genetics (IMAGEN) project (8). IMAGEN is a European research project that aims to detect biological and environmental factors that might have an influence on mental health in teenagers. The purpose of this project is to identify risks and develop better prevention strategies and therapies in the future. A detailed explanation of the study design can be found elsewhere (8). In the present study, a total of 485 healthy subjects (248 female) with a mean (± standard deviation) age of 14.26 (0.30) years (range: 13–15 years) were included. There was a partial overlap with samples used in previous studies (9, 10). Any mental disorder [as identified by the Development and Well-Being Assessment (11)], serious medical condition, known pregnancy, history of head trauma with unconsciousness, and contraindications for magnetic resonance imaging (MRI) were exclusion criteria for the present study. The respective local ethics committees approved the study, and it was conducted in accordance with the Declaration of Helsinki. Subjects and their legal guardians received a detailed explanation of the study, and written informed consent was obtained.

Deoxyribonucleic acid (DNA) extraction and genotyping
To ensure high quality and sufficient quantity, DNA extraction was semiautomated (8). The Illumina Quad 610 chip (Illumina, San Diego, CA, USA) was used for genome-wide genotyping of ~600000 autosomal single nucleotide polymorphisms (SNPs). For rs12576775: 347 subjects were homozygous, and 12 were homozygous G-allele carriers (in Hardy–Weinberg equilibrium: p = 1.00). Due to the small number of GG-allele carriers, the GG- and GA-carriers were included in one group. The two groups were not significantly different in age, sex, and intelligence (Hawik-R) (12).

Functional MRI paradigms

Monetary incentive delay (MID) task. In a modified version of the MID task from Knutson et al. (13), subjects had to quickly press a button in order to hit a square. Each time subjects hit the square, they could gain points. A figure on the screen showed the number of points which could be gained (triangle = no points; circle with one line = two points; circle with three lines = ten points). Each of the three conditions was composed of 22 trials. To ensure that subjects hit the target in 66% of all trials, the duration of the target display was...
Face task. A sequence of video clips (of two to five seconds’ duration) of human faces appeared on the screen and subjects were asked to look at them. Faces started from a neutral expression and turned either to an angry or ambiguous expression without particular emotional content (14). Non-biological control stimuli of expanding and contracting concentric circles with varying contrasts were displayed on the screen in between clips. Four to seven video clips, with the control video clip in between clips, built a block. All clips displayed either angry or ambiguous faces. Five blocks of ambiguous faces and five blocks of angry faces were presented in counterbalanced order. Altogether, ten 18-sec blocks were presented.

MRI acquisition. At each of the IMAGEN assessment sites, scanning was conducted with 3T whole body MRI systems [Siemens AG (Munich, Germany), Philips Healthcare (Best, the Netherlands), General Electric Healthcare (Chalfont St Giles, Great Britain), Bruker Biospin (Billericia, MA, USA)]. Forty slices were acquired in descending order (2.4 mm, 1-mm gap) using a gradient-echo T2*-weighted sequence (EPI) and the following image parameters: repetition time (TR) = 2200 msec; echo time (TE) = 30 msec; in-plane resolution of 64 × 64 pixels. The plane of acquisition was tilted to the anterior–posterior commissure line (rostral > caudal). For anatomical reference, a three-dimensional magnetization prepared gradient echo sequence (MPRAGE) of the whole brain was obtained with TR = 6.8 msec and TE = 3.2 msec, in accordance with the Alzheimer’s Disease Neuroimaging Initiative (ADNI) protocol (http://www.loni.ucla.edu/ADNI/Cores/index.shtml To ensure comparability of MRI data, the image-acquisition techniques involved use of a set of parameters that were compatible with all scanners (8).

Data analysis

Statistical parametric mapping (SPM8; Wellcome Department of Imaging Neuroscience, University College London, London, UK) was used for the analysis of the functional magnetic resonance imaging (fMRI) data. Individual data were slice time corrected, spatially realigned to correct for head movement, and non-linearly warped on the Montreal Neurological Institute space using a custom EPI template based on an average of the mean images of 400 adolescents. This custom template image (53 × 63 × 46 voxels) was subsequently applied to all functional T2* data and voxels were resampled at a resolution of 3 × 3 × 3 mm. An isotropic Gaussian kernel for group analysis (5-mm full-width at half-maximum) was used to smooth the functional data. First-level statistics involved modeling reward anticipation and reward feedback as predictor variables within the context of the general linear model on a voxel-by-voxel basis with an autoregressive noise model against a design matrix. Estimated movement was added to the design matrix in the form of 18 additional columns (three translational, three rotations, three quadratic, and three cubic translations, three translations shifted one TR before, and three translations shifted one TR later). For the MID task, we differentiated between reward magnitudes of no win, small win, and big win as subject-specific regressors of interest, and included study center as a covariate in the fMRI analysis. For the face task, each face was modeled on its separate regressor.

First-level analyses were followed by second-level random effects analyses in which individual contrast images were included, using the full flexible procedure of SPM8. A non-sphericity correction addressed the problems of non-independent data within subjects and error variance heterogeneity. We chose a significance level of p ≤ 0.05, family-wise error (FWE) corrected. In accordance with the literature (15), the analyses focused on a region of interest (ROI) approach using ROIs from Wake Forest University PickAtlas v3.0.3 (16, 17) and probabilistic anatomical masks (18) that were thresholded with a fractional intensity of ≥ 0.5. In accordance with Yacubian et al. (6), we concentrated on the amygdala and the striatum as core structures relating to bipolar disorder. As Yacubian et al. (6) described effects in conjunction with reward and expectation, we chose the appropriate contrasts. The ROIs and contrasts were as follows: (i) MID task: striatum and amygdala BOLD response during anticipation and feedback of reward: (a) anticipation of win versus no win, (b) feedback of win versus no win [always hit trials; i.e., subjects met the time criterion (we refer to this contrast as reward sensitivity)], and (c) feedback of hit win versus feedback of missed win (we refer to this contrast as reward expectation); and (ii) face task: striatum and amygdala BOLD response during the viewing of angry versus neutral faces. Whole-brain analyses of each task revealed no significant differences between the allele groups. Using SPM8 we conducted two-sample t-tests for each ROI
Risk variant and amygdala during reward

Fig. 1. Increased amygdala activity in rs12576775 GG homozygotes and AG heterozygotes compared to AA homozygotes corresponding to (A) reward sensitivity during the feedback phase \([T = 2.45; p = 0.05 \text{ (FWE corrected for ROI); } x = -21, y = -4, z = -17; k = 29 \text{ voxels}]\) and (B) reward expectation during the feedback phase \([T = 2.38; p < 0.05 \text{ (FWE corrected for ROI); } x = 24, y = 2, z = -23; 6 \text{ voxels}].\) FWE = family-wise error; ROI = region of interest.

and contrast with the homozygous A-allele carriers as one group and G-risk allele carriers as one group. To evaluate if the tasks robustly lead to the BOLD response in the amygdala and the striatum, we analyzed the main effects of the BOLD response for all subjects irrespective of genotype. This was done by one-sample \(t\)-tests both in whole brain and in the amygdala and striatum during relevant contrasts of the MID task and the face task (See Supplementary Material).

Results

In order to test possible influences on reward and emotion, we analyzed the impact of the \(ODZ4\) rs12576775 SNP (G/A alleles) on amygdala and striatum activation of 485 healthy adolescents. There were no significant behavioral differences between the two genotypes in the reaction times of the MID. Carriers of the risk variant showed a significantly increased BOLD response in the amygdala during two components of the reward processing task (MID) (Fig. 1): reward sensitivity (\(F_{\text{WECorrected}} = 0.05\), win versus no win in the feedback phase; see also Materials and Methods) and reward expectation (\(F_{\text{WECorrected}} < 0.05\), win versus missed time criterion in the feedback phase; see also Materials and Methods). No significant differences between the two groups were found in the amygdala and the striatum during the face task. Analysis of the main effects of the MID and face task on all subjects, regardless of genotype, revealed significant BOLD responses to reward and face processing. The anticipation phase and the feedback phase of the MID task, as well as the neutral faces condition and the angry faces condition of the face task, showed a significant BOLD response in the amygdala and the striatum, whereas the control condition did not show significant activation (See Supplementary Material).

Discussion

Patients with bipolar disorder show altered processing of reward and emotion (4, 5). We demonstrated that carriers of the risk allele in \(ODZ4\) display an increased BOLD response in the amygdala during reward processing. There were no significant group differences in emotion processing (face task) but robust activation in the whole group was shown during the viewing of faces and not in the control condition with concentric circles (See Supplementary Material). This suggests that the face task is a functioning paradigm for the analysis of face processing but that the risk variant does not change the processing of emotional stimuli, like faces. This is interesting, as Yacubian et al. (6) demonstrated that an imbalance of amygdala and striatum activation could contribute to mood disorders. These authors showed that the gain-related part of the expected value in a guessing task was represented in the ventral striatum, whereas the loss-related part was represented in the amygdala. Yacubian and colleagues (6) proposed that the higher involvement of either the amygdala or the ventral striatum might render expectations more positive or negative. In the present study, we demonstrated that carriers of the risk allele in \(ODZ4\) (rs12576775) more strongly involve the amygdala in reward processing. Following the hypothesis of Yacubian et al. (6), we suggest that the risk variant in \(ODZ4\) might influence the amygdala in a potential complex network of factors contributing to an imbalance between the amygdala and striatum in mood disorders. Thus, in the future, it will be necessary to further examine the role of both parts of the brain and their underlying mechanisms to more clearly determine risk factors. We suggest a relationship between the risk variant in \(ODZ4\) and reward processing in the amygdala. Further analysis is needed to establish if there is another risk factor that influences the striatum or if there are environmental factors which contribute, in terms of a gene–environment interaction, to an imbalance between the amygdala and striatum. This might lead to more differentiated diagnoses and prospective therapies reliant on risk factors. It is important to note that our study examined adolescents, who might show differential activation compared to adults. Relative to adults, adolescents have been reported to recruit the ventral striatum and the amygdala to a lesser extent while anticipat-
ing gains, whereas no differences between adolescents and adults were found in the feedback phase (19). Concerning the face task for emotion processing, it has been demonstrated that, compared to adults, adolescents involve the amygdala more strongly in the response to fearful faces (20). We did not show genotype-dependent effects in the face task, but this does not contradict the previously mentioned finding as we only examined the differences between the two genotypes. A possible stronger activation in the amygdala compared to adults might still occur, as the results of the analysis of the main effects of the face task showed a robust BOLD response in the amygdala. Our results suggest that the relationship between the variation at ODZ4 (rs12576775) and bipolar disorder may involve amygdala-based reward processing, which is an important step in the necessary disentangling of the genotype–phenotype correlation.

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Disclosures

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References

Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Whole-brain analysis for the control condition contrast of the face task (concentric circles), T = 16.06, \( p_{FWE\text{corrected}} < 0.001 \) at 15, –97, 10. (A) Uncorrected; (B) family-wise error (FWE) corrected. Specific effects on the amygdala were \( T = 3.14 \) (\( p_{FWE\text{corrected}} = 0.126 \)) at 24, –4, –20, and on the striatum were \( T = 3.92 \) (\( p_{FWE\text{corrected}} = 0.064 \)) at –30, –1, 1.

**Figure S2.** Whole-brain analysis for the neutral condition contrast of the face task (neutral faces), \( T = 17.23 \), \( p_{FWE\text{corrected}} < 0.001 \) at 42, –49, –20. (A) Uncorrected; (B) family-wise error (FWE) corrected. Specific effects on the amygdala were \( T = 8.95 \) (\( p_{FWE\text{corrected}} < 0.001 \)) at 21, –4, –17, and on the striatum were \( T = 5.87 \) (\( p_{FWE\text{corrected}} < 0.001 \)) at –18, –7, –11.

**Figure S3.** Whole-brain analysis for the angry condition contrast of the face task (angry faces), \( T = 17.58 \), \( p_{FWE\text{corrected}} < 0.001 \) at 42, –52, –20. (A) Uncorrected; (B) family-wise error (FWE) corrected. Specific effects on the amygdala were \( T = 9.35 \) (\( p_{FWE\text{corrected}} < 0.001 \)) at 21, –4, –17, and on the striatum were \( T = 6.58 \) (\( p_{FWE\text{corrected}} < 0.001 \)) at –18, –7, –11.

**Figure S4.** Whole-brain analysis for the anticipation condition contrast of the monetary incentive delay task (sum of all anticipation conditions; i.e., large win, small win, no win, missed hits), \( T = 14.86 \), \( p_{FWE\text{corrected}} < 0.001 \) at 3, 11, 40. (A) Uncorrected; (B) family-wise error (FWE) corrected. Specific effects on the amygdala were \( T = 5.13 \) (\( p_{FWE\text{corrected}} < 0.001 \)) at 18, –7, –8, and on the striatum were \( T = 10.06 \) (\( p_{FWE\text{corrected}} < 0.001 \)) at –9, 8, –2.

**Figure S5.** Whole-brain analysis for the feedback condition contrast of the monetary incentive delay task (sum of all feedback conditions; i.e., large win, small win, no win, missed hits), \( T = 22.02 \), \( p_{FWE\text{corrected}} < 0.001 \) at 27, –79, –20. (A) Uncorrected; (B) family-wise error (FWE) corrected. Specific effects on the amygdala were \( T = 10.00 \) (\( p_{FWE\text{corrected}} < 0.001 \)) at –24, –1, –14 and on the striatum were \( T = 12.64 \) (\( p_{FWE\text{corrected}} < 0.001 \)) at –27, –13, 4.

**Figure S6.** Whole-brain analysis for the anticipation hit condition contrast of the monetary incentive delay task (anticipation of a large win and small win versus no win), \( T = 17.14 \), \( p_{FWE\text{corrected}} < 0.001 \) at 9, 8, 1. (A) Uncorrected; (B) family-wise error (FWE) corrected. Specific effects on the amygdala were \( T = 7.03 \) (\( p_{FWE\text{corrected}} < 0.001 \)) at –15, –1, –14, and on the striatum were \( T = 16.79 \) (\( p_{FWE\text{corrected}} < 0.001 \)) at –9, 11, –2.