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Chapter 2

Piccolo Genotype Modulates Neural Correlates of Emotion Processing but not Executive Functioning

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Abstract

Major depressive disorder (MDD) is characterized by affective symptoms and cognitive impairments, which have been associated with changes in limbic and prefrontal activity as well as with monoaminergic neurotransmission. A genome-wide association study implicated the polymorphism rs2522833 in the piccolo (*PCLO*) gene — involved in monoaminergic neurotransmission — as a risk factor for MDD. However, the role of the *PCLO* risk allele in emotion processing and executive function or its effect on their neural substrate has never been studied. We used functional magnetic resonance imaging (fMRI) to investigate *PCLO* risk allele carriers vs. non-carriers during an emotional face processing task and a visuospatial planning task in 159 current MDD patients and healthy controls. In *PCLO* risk allele carriers, we found increased activity in the left amygdala during processing of angry and sad faces compared with non-carriers, independent of psychopathological status. During processing of fearful faces, the *PCLO* risk allele was associated with increased amygdala activation in MDD patients only. During the visuospatial planning task, we found no genotype effect on performance or on BOLD signal in our predefined areas as a function of increasing task load. The *PCLO* risk allele was found to be specifically associated with altered emotion processing, but not with executive dysfunction. Moreover, the *PCLO* risk allele appears to modulate amygdala function during fearful facial processing in MDD and may constitute a possible link between genotype and susceptibility for depression via altered processing of fearful stimuli. The current results may therefore aid in better understanding underlying neurobiological mechanisms in MDD.

Keywords: Emotion processing – executive function – genome-wide association – major depressive disorder – neuroimaging genetics – *PCLO*.

Introduction

Major depressive disorder (MDD) is a common, multifactorial disorder and is one of the leading causes of years lost due to disability (World Health organization (WHO), 2005; World Health organization (WHO), 2009). Its core symptoms are affective, manifesting themselves in depressed mood accompanied by low self-esteem and loss of interest in normally enjoyable activities. Other MDD symptoms include cognitive impairments, such as concentration and planning problems (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition, Text Revision 2000). The presence and severity of both affective and cognitive symptoms can be evaluated by neuropsychological assessments (Weiner *et al.*, 2003), and may be reflected in altered regional brain activity as measured using fMRI (Demenescu *et al.*, 2011; Savitz & Drevets, 2009; van Tol *et al.*, 2011).

Twin studies have shown that up to 40% of MDD is genetically determined (Sullivan *et al.*, 2000). Much of the candidate gene literature has focused on genes from the monoaminergic neurotransmitter system, such as the serotonin transporter, monoamine oxidase A and tryptophan hydroxylase 2 (Dannlowski *et al.*, 2007a; Dannlowski *et al.*, 2007b; Heinz *et al.*, 2005; Lau *et al.*, 2009; Lee & Ham, 2008; Zill *et al.*, 2004). A recent study by our consortium demonstrated that candidate genetic association studies are not well replicated (Bosker *et al.*, 2011), which suggests that a hypothesis-free approach is more useful to identify possible genetic variants that contribute to MDD. Previously, we conducted a genome-wide association study (GWAS) in MDD using ~500 000 single-nucleotide polymorphisms (SNPs), showing one SNP (rs2522833) located at position 82453708 (hapmap genome build 37.1) in the piccolo gene (*PCLO*) in particular to be of interest in the genetic model for MDD (Sullivan *et al.*, 2009). This association was replicated in some studies (Bochdanovits *et al.*, 2009; Hek *et al.*, 2010; Sullivan *et al.*, 2009) but not in others (Shyn *et al.*, 2011; Wray *et al.*, 2012). The rs2522833 SNP changes the hydrophilic, uncharged amino acid serine to the charged amino acid alanine in the calcium-binding C2A domain of *PCLO* and may affect protein stability (Betts & Russell, 2007). The *PCLO* protein is localized at the cytomatrix of the presynaptic active zone and is important in monoaminergic neurotransmission in the brain (Südhof, 2008; Sullivan *et al.*, 2009). Recently, Schuhmacher *et al.* (Schuhmacher *et al.*, 2011) showed that the *PCLO* SNP rs2522833 is associated with antidepressant treatment response, supporting the involvement of *PCLO* in MDD. The mechanism through which this *PCLO* risk allele contributes to MDD is not known, but its primary role in monoaminergic neurotransmission suggests that it may affect activity of brain circuitry involved in emotional information processing and/or executive functioning in MDD (Robbins & Arnsten, 2009).

Abnormal emotion processing of, for example, facial stimuli is one of the intermediate phenotypes that can be considered closer to the neurobiological substrate of MDD than the clinical diagnosis itself (Savitz & Drevets, 2009). Identification of emotional facial expressions is reportedly impaired in MDD (Surguladze *et al.*, 2004), and functional imaging studies have shown aberrant activity of emotional face processing related areas, including the amygdala, striatum, insula, parahippocampal area and dorsolateral prefrontal cortex (PFC), using an emotional faces task in healthy controls (HC) (Fusar-Poli *et al.*, 2009). It is thought that serotonin has an important role in emotional face processing and dysregulation of the

serotonergic system is associated with altered amygdala activity as well as the occurrence of MDD (Daly *et al.*, 2010; Dannlowski *et al.*, 2007a; Ruhé *et al.*, 2011).

Another intermediate phenotype for MDD may be executive dysfunction, including impaired working memory and planning/problem solving. Previous studies in MDD have shown abnormal task performance and increased activity in task-related brain areas (Fitzgerald, Sr., 2008; McClintock *et al.*, 2010; van Tol *et al.*, 2011) using the Tower of London visuo-spatial planning task (ToL) and the n-back working memory task (Goethals *et al.*, 2005; Matsuo *et al.*, 2006; Rose *et al.*, 2006; van den Heuvel *et al.*, 2003). Apart from the PFC, other areas involved in planning include the striatum, and a visuospatial processing system, including the precuneus and inferior parietal cortex (Fitzgerald, Sr., 2008; van den Heuvel *et al.*, 2003). The association of executive function with monoaminergic neurotransmission is thought to be bidirectional: PFC function is shaped by monoaminergic neurotransmitters, but ascending modulatory monoaminergic systems may also be affected by the PFC (Robbins & Arnsten, 2009). Studies suggests that the PFC is able to recruit monoamine systems in specific circumstances, for example, to optimize the coping strategy for stress (Amat *et al.*, 2005; Giorgi *et al.*, 2003).

Over the last few years, imaging genetics has shown to be a powerful tool in unravelling neurobiological pathways in various psychiatric disorders. However, to date, studies in MDD have been rare (Dannlowski *et al.*, 2007b; Friedel *et al.*, 2009; Lee *et al.*, 2009), and the role of the *PCLO* risk allele on emotional information processing and executive function has never been studied. Given that *PCLO* is associated with MDD, we hypothesized that the *PCLO* risk allele — relative to the non-risk allele — is associated with similar changes in activity in emotional processing and in executive function related areas as described for MDD. In addition, we aimed to investigate the relationship of *PCLO* with current psychopathology during emotional face processing and executive function.

Methods

Subjects

A sample of 159 subjects participating in the Netherlands Study for Depression and Anxiety (NESDA) was selected to take part in this genetic neuroimaging study. Details of the NESDA study are extensively described elsewhere (Penninx *et al.*, 2008). Genotype data were collected through the genetic association information network (GAIN) study for MDD, in which 1862 cases and 1857 controls were genotyped (Boomsma *et al.*, 2008). Magnetic Resonance Imaging (MRI) data were available from the MRI NESDA study, in which 301 subjects aged between 18 and 57 years participated (van Tol *et al.*, 2010). During a 1-h protocol, subjects underwent MR imaging while performing different tasks, including the ToL paradigm, the emotional faces paradigm and a verbal memory task, structural imaging and resting state imaging.

Exclusion criteria were: presence of MRI contraindications, DSM-IV axis I disorder other than MDD, Panic Disorder or social anxiety disorder (except generalized anxiety disorder) lifetime, or any DSM-IV disorder (for HC), dependence or recent abuse of alcohol and/or drugs, hypertension, major internal and/or neurological disorders and use of psychotropic medication (other than stable use of a selective serotonin reuptake inhibitor (SSRI) or incidental use of benzodiazepines). To assess depressive symptom characteristics and

severity scores, the Inventory of Depressive Symptomatology (Rush *et al.*, 1986) and the Montgomery–Åsberg Depression Rating Scale (Montgomery & Åsberg, 1979), were used. For the present report, we included only subjects that met the 6-month recent DSM-IV criteria for current MDD, or no lifetime DSM-IV diagnosis. Owing to missing genotype data, technical problems during scanning and/or insufficient task performance, our final sample for this study consisted of 126 subjects for the emotional face processing study and 159 subjects for the executive function study (see Supplementary Table S2.1 for an overview). All participants provided written informed consent and the study was approved by the Ethics Committees at the VU University Medical Center, Amsterdam, the Leiden University Medical Center and the University Medical Center Groningen.

Demographics

The 126 subjects in the emotional faces study included 91 *PCLO* risk allele carriers (*PCLO*+; 25 homozygous and 66 heterozygous for the ‘C’ risk allele) and 35 homozygous ‘A’ non-risk allele carriers (*PCLO*-). The ratio of *PCLO*+ and *PCLO*- in the executive function study did not significantly differ (110 *PCLO*+; 33 homozygous and 77 heterozygous for the ‘C’ risk allele, and 49 homozygous ‘A’ non-risk allele carriers; *PCLO*-) from the ratio in the emotional faces study ($\chi^2(1)=0.31$; $P>0.05$). For the analyses, we formed two groups: one group consisting of subjects carrying the risk allele, and one group consisting of subjects not carrying the risk allele. From this point forward, we will refer to these groups as *PCLO*+ and *PCLO*-, respectively. *PCLO* groups did not differ with regard to age, gender, education, MDD/HC ratio, depression severity or SSRI use/duration (see Table 2.1). Thirty-four individuals were using SSRIs, and 15 individuals were using non-psychotropic medication other than SSRIs. All subjects reported Western European ancestry.

Genotyping

Genotyping for the genetic association information network study was performed by Perlegen and is described in detail elsewhere (Sullivan *et al.*, 2009). Observed genotypes in our sample did not deviate from Hardy–Weinberg equilibrium (emotional face processing paradigm): $\chi^2(2)=0.37$; $P>0.05$; CC: AC: AA=35:66:25; $P>0.05$; executive functioning paradigm: $\chi^2(2)=0.07$; CC: AC: AA=33:77:49; $P>0.05$; <http://www.oege.org/software/hardy-weinberg.html>).

Emotional information processing paradigm

An emotional faces task, based on the event-related fMRI paradigm used by Wolfensberger *et al.* (Wolfensberger *et al.*, 2008) was used in the present study and has been extensively described elsewhere (Demenescu *et al.*, 2011). Briefly, 24 stimuli —selected from the Karolinska Directed Emotional Faces System (Lundqvist *et al.*, 1998) — were chosen for each of five facial expressions (angry, fearful, sad, happy and neutral), comprising 12 female and 12 male faces.

Each particular face was not presented more than four times. The control condition (scrambled faces) was presented 80 times. While functional MRI data were collected, subjects

were asked to indicate each face's gender using a button box. During the presentation of scrambled faces, participants had to press left or right buttons in conformity with the instruction presented on the screen, that is, an arrow pointing to the left or to the right. The reaction time (RT) was recorded and used as performance measure.

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Executive function paradigm

An event-related parametric version of the ToL task was used to investigate the neural correlates of planning, an aspect of executive functioning (van den Heuvel *et al.*, 2003; van Tol *et al.*, 2011). Briefly, while functional MRI data were collected, subjects were asked to work out the minimum number of steps needed — ranging from one to five moves — to get from the start position to the goal position, which were both presented on a screen simultaneously. In the baseline condition, subjects were asked to count the number of yellow and blue beads. RT and proportion of correct answers were used as performance measures.

Magnetic Resonance Imaging data acquisition

The functional neuroimaging methods have been comprehensively reported elsewhere (Demenescu *et al.*, 2011; van Tol *et al.*, 2011). In summary, T2⁺-weighted echo-planar images sensitive to the blood oxygenation level-dependent (BOLD) effect were acquired using similar Philips 3T MR systems (repetition time (TR)=2300 ms, echo time (TE)=30.0 ms (UMCG: 28.0 ms), situated at different locations (Amsterdam, Leiden, and Groningen, the Netherlands). The echo-planar images' volumes were acquired at 35 slices (UMCG: 39 slices), interleaved acquisition, 3 mm thickness. The matrix sizes were 96 × 96 voxels (UMCG: 64 × 64 voxels). The in-plane resolution was 2.29 × 2.29 mm² (UMCG: 3 × 3 mm²). The images were acquired parallel to the anterior-posterior commissure plane. A T1-weighted anatomical MRI was also acquired for each subject (TR=9 ms, TE=3.5 ms, matrix size 256 × 256, voxel size 1 × 1 × 1 mm³).

Analysis

Performance

Faces paradigm: RT were analysed with SPSS (SPSS, Chicago, IL, USA) by means of a repeated-measures analysis of covariance (ANCOVA), using RT per valence as dependent factor, group (PCLO+, PCLO-) as between subject factor and age, scan location, and years of education as covariates. Significance for behavioural analyses was set at 5%, and *post-hoc* paired tests were Bonferroni-corrected for multiple comparisons. *Post-hoc* analyses were performed to test for interaction using a univariate general linear model.

ToL paradigm: performance data (accuracy and RT) were analysed with SPSS (SPSS). Performance was analysed by means of a repeated-measures ANCOVA, using the proportion correct scores and mean response times per trial type as dependent factors, group (PCLO+, PCLO-) as between subject factor, and age as a covariate. Significance for behavioural analyses was set at 5%, and *post-hoc* paired tests were Bonferroni-corrected for multiple comparisons.

Table 2.1: Demographic characteristics of total sample.

Characteristics	Tower of London planning task				Emotional face paradigm				
	Total	PCLO+	PCLO-	χ^2	Total	PCLO+	PCLO-	χ^2	p-value
number of subjects	159	110	49		126	90	36		
Gender(%female)	64.1	64.5	63.3	.024	61.9	61.1	63.8	.08	> .05
Age (mean years \pm SD)	38.17 (10.7)	37.9 (10.2)	38.8 (11.9)		38.1 (10.3)	38.0 (10.2)	38.4 (10.7)		> .05
education (mean years \pm SD)	12.5 (3.1)	12.8 (3.3)	11.9 (2.8)		12.6(3.1)	12.8(3.2)	12.1(2.8)		> .05
Scancenter (A/L/G)	42/62/55	28/46/36	14/16/19	1.21	24/54/48	19/40/31	5/14/17	1.99	> .05
Diagnosis (MDD/HC)	118/41	84/26	35/15	.86	96/30	69/21	27/9	.04	> .05
IDS (mean score \pm SD)	18.90 (13.5)	20.06 (13.2)	16.27 (14.0)		19.46 (13.9)	20.05 (13.6)	17.91 (14.8)		> .05
MADRS (mean score \pm SD)	11.89 (10.4)	13.01 (10.3)	9.39 (10.1)	0.042	12.78 (10.4)	13.53 (10.3)	10.80 (10.4)		> .05
Duration SSRI use (months \pm SD)	22.91 (37.6)	27.77 (38.8)	18.80 (36.5)		23 (36.9)	24.75 (37.7)	18.8 (36.5)		> .05
SSRI use (no/yes)	119/40	82/28	37/12		92/34	67/24	25/10		> .05

Abbreviations: HC, healthy controls; IDS, inventory of depressive symptomatology; MADRS, Montgomery–Åsberg Depression Rating Scale; MDD, major depressive disorder; PCLO, piccolo; SSRI, selective serotonin reuptake inhibitor.

Imaging data analysis

Image processing was performed using Statistical Parametric Mapping (SPM5; <http://www.fil.ion.ucl.ac.uk/spm/software/spm5>), software implemented in Matlab 7.5.0 (MathWorks, Natick, MA, USA). Details of preprocessing and first level single-subject analyses have been described elsewhere (Demenescu *et al.*, 2011; van Tol *et al.*, 2011). Briefly, following temporal and spatial preprocessing stimulus responses were modelled using delta functions convolved with a synthetic hemodynamic response function. For both paradigms, trial duration was also modelled (Friston *et al.*, 1998). In the faces task, four contrast images were generated for each subject: ‘angry>neutral’, ‘fearful>neutral’, ‘sad>neutral’ and ‘happy>neutral’. These contrasts ensured that visual input only differed in its emotional content and were entered in a second-level mixed effect analysis. For the ToL task, activation maps associated with increased task load were calculated per subject, and contrast images were likewise included in a second-level mixed-effect analysis.

The amygdala and ventral striatum were determined *a priori* as regions of interest (ROI) for the emotional faces task. Positive effects of task across whole-brain are reported at a threshold of $P < 0.05$ family-wise error corrected for multiple comparisons ($P_{FWE} < 0.05$). We conducted an ANCOVA for the faces task with genotype as an independent factor, to test whether the *PCLO* risk allele is associated with altered activity in the amygdala during processing of negative faces. Age and scan location were used as covariates. A threshold of $P < 0.05$ FWE corrected using the small volume correction ($P_{FWE_SVC} < 0.05$) procedure implemented in SPM5 for the amygdala was considered significant. For this small volume correction, we used the automated anatomical labelling atlas (Maldjian *et al.*, 2003), implemented in the Wake Forest University Pick Atlas toolbox. To test whether the *PCLO* risk allele is associated with altered increased activity in the amygdala and ventral striatum during processing of positive faces, we conducted an ANCOVA with genotype as an independent factor. Age and scan location were used as covariates. A threshold of $P_{FWE_SVC} < 0.05$ (using the automated anatomical labelling atlas, implemented in the Wake Forest University Pick Atlas toolbox) for amygdala and ventral striatum was considered significant.

For the executive function task, regions activated with increasing task load were determined *a priori* as ROIs according to the areas described in van den Heuvel *et al.* (van den Heuvel *et al.*, 2003) (including dorsolateral PFC). Main effects of increasing task load are reported at a threshold of $P < 0.05$ whole-brain FWE corrected for multiple comparisons. Next, an ANCOVA using genotype as independent factor was conducted, to test whether the *PCLO* risk allele is associated with altered brain activity associated with increasing task load. Age, gender, education and scan location were used as covariates. Owing to dorsolateral PFC size, a threshold of $P_{FWE_SVC} < 0.05$ (10 mm radius around the peak voxel that was found in the main effect of condition) was considered significant.

We conducted *post-hoc* analyses for both tasks to test whether interaction of genotype with current psychopathology occurred. A threshold of $P_{FWE_SVC} < 0.05$ (6 mm radius sphere for amygdala and striatum and 10 mm radius sphere for cortical areas around the peak voxel) in the ROIs was considered significant.

Results

Processing emotional faces

Task performance

We did not find significant genotype or genotype x valence effects on task performance (RT) during emotional face processing.

Additional genotype x diagnosis x valence effects on RT revealed interaction effects between PCLO genotype and MDD diagnosis for angry, fear and sad faces (RT_angry: $PCLO \times MDD$; $P=0.0595$; RT_fear: $PCLO \times MDD$; $P=0.0493$; RT_sad: $PCLO \times MDD$; $P=0.0395$; see Supplementary Table S2.3).

MRI results

Main effect of task

Processing angry faces compared with neutral faces was associated with robustly increased activity of bilateral amygdala as well as bilateral putamen, ventrolateral PFC, superior and middle temporal lobe and inferior parietal cortex. Processing fearful faces compared with neutral faces was similarly associated with increased activity of bilateral amygdala as well as of bilateral parietal cortex, right temporal cortex, bilateral occipital cortex, bilateral amygdala, decreased activity of ventral striatum and right parahippocampal gyrus. Processing sad faces compared with neutral faces activated bilateral amygdala, striatum, insula, parahippocampal area and dorsolateral PFC.

For happy faces, we observed activation in bilateral amygdala and ventral striatum as well as in bilateral occipital cortex, inferior parietal cortex and posterior cingulate cortex.

PCLO genotype effect during processing of faces

We found significantly increased activity in the left amygdala in the PCLO+ group, relative to the PCLO- group, during processing of negative faces (angry vs. neutral: MNI [-21,0,-15], $Z=3.6$, $P_{FWE_SVC}=0.004$, (Figure 2.1); fear vs. neutral: MNI [-18,0,-18], $Z=3.24$, $P_{FWE_SVC}=0.014$, (Figure 2.2); and trend-wise for sad vs. neutral: MNI [-15,-3,-18], $Z=2.6$, $P_{FWE_SVC}=0.067$).

We did not observe significant effects of PCLO+ within our ROIs during processing of happy faces. However, we found subthreshold increased activity in the left amygdala during processing of happy faces compared with neutral faces (MNI [-24,3,-15]; $Z=2.47$; $P_{FWE_SVC}<0.09$, $P_{uncorrected}=0.007$). In addition, during processing of happy faces we found a near-significantly decreased activity for the PCLO+ group in the right ventral striatum (MNI [9,6,-9]; $Z=2.67$; $P_{FWE_SVC}\approx 0.05$ ($P=0.058$) $P_{uncorrected}=0.004$).

We found similar results as reported for negative vs. neutral faces, and plotting our data suggested that these effects were indeed driven by negative faces.

Interaction of PCLO genotype with current psychopathology during processing of faces

Post-hoc analyses showed a significant genotype by depression interaction ($F(1,119)=9.50$; $Z=2.8$) during processing of fearful faces compared with neutral faces:

PCLO+ demonstrated increased amygdala activity in the MDD group ($Z=3.02$; $p_{\text{FWE}} < 0.05$), but not in HC ($Z=1.1$; Figure 2.3). There was no interaction during processing of sad or angry faces or during processing of happy faces. Notably, we found no main effect of diagnosis on amygdala activation, which is similar to our previous study (Demenescu *et al.*, 2011).

2

Figure 2.1: Effect of piccolo (PCLO) genotype on emotion processing of angry vs. neutral faces (MNI [-21,0,-15]), $Z=3.6$, $\text{PFWE}=0.004$). Activation shown at $P < 0.005$, uncorrected.

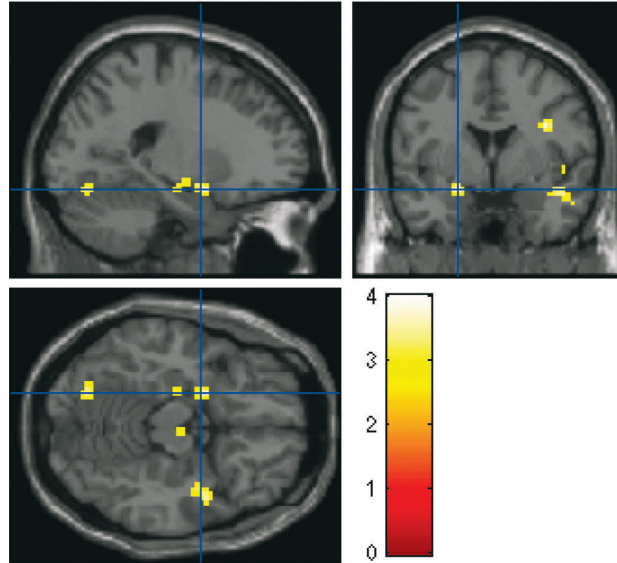


Figure 2.2: Effect of piccolo (PCLO) genotype on emotion processing of fearful vs. neutral faces (MNI coordinates [-18,0,-18]), $Z=3.25$, $\text{PFWE}=0.014$). Activation shown at $P < 0.005$, uncorrected.

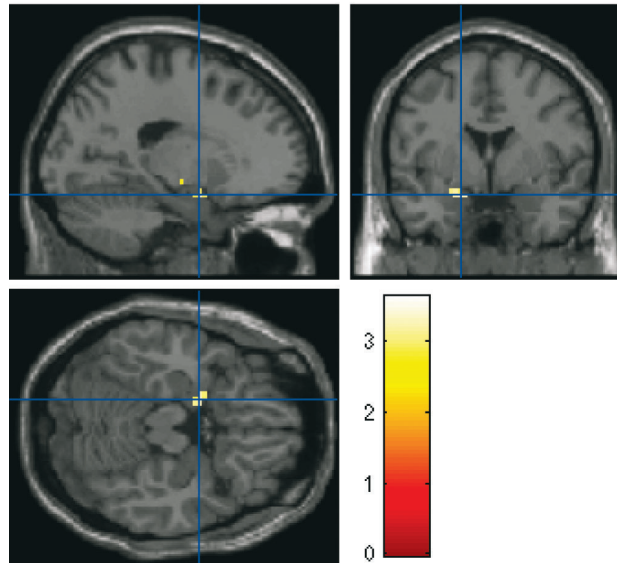
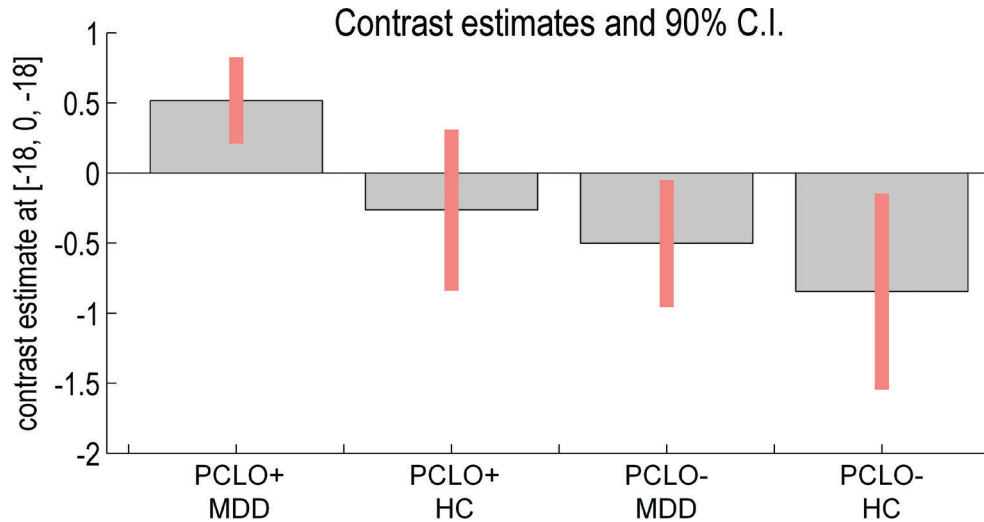


Figure 2.3: Parameter estimates and 90% confidence intervals showing direction of interaction effect of genotype and current psychopathology during processing of fearful faces.



2

Executive functioning

PCLO genotype effect on task performance

No effect of genotype was observed on task performance (mean RT: PCLO+: $8.30\text{ s} \pm 2.34$; PCLO-: $8.19\text{ s} \pm 2.11$; $P=0.76$; repeated measures ANOVA for RT per trial type: $P=0.094$; proportion of correct answers: PCLO+: 0.92 ± 0.08 ; PCLO-: 0.90 ± 0.11 ; $P=0.22$; repeated measures ANOVA for proportion of correct answer per trial type: $P=0.488$).

BOLD fMRI results

Main effect of task during executive functioning

In accordance with previous studies (van den Heuvel *et al.*, 2003; van Tol *et al.*, 2010), increasing task load during the ToL was associated with robust activity in the right dorsolateral PFC, right middle PFC, bilateral precuneus and bilateral inferior parietal lobe (Figure 2.4; Supplementary Table S2.2).

PCLO genotype effect during executive functioning

We found no significant effect of genotype in the predefined ROIs.

Interaction of PCLO genotype with current psychopathology during executive functioning

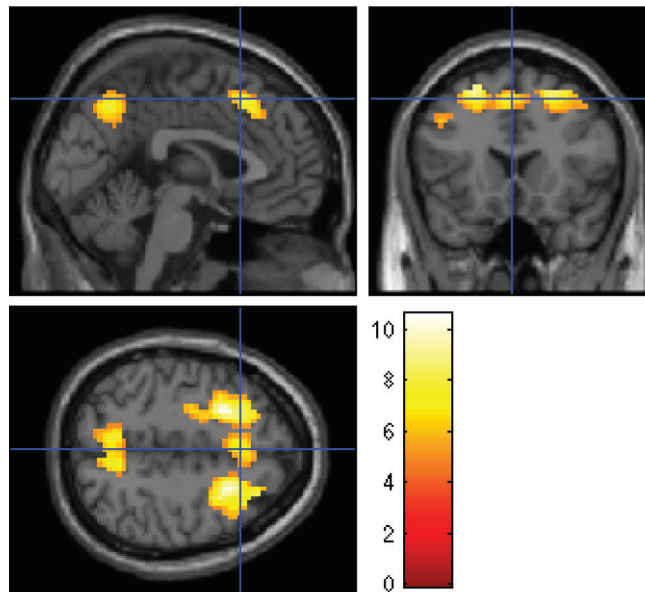
Explorative *post-hoc* analyses showed a genotype by diagnosis interaction effect in the left medial dorsolateral PFC (Brodmann's area 9, MNI $[-27,30,24]$, $Z=3.77$, $P_{\text{FWE}} < 0.05$). Increased activity in PCLO+ group compared with PCLO- was found in the MDD group but not in HC.

Confounding factors

As is known from other studies, SSRI use may alter regional brain function in psychiatric diseases (Linden, 2006). We conducted *post-hoc* analyses for both the emotional face paradigm and the planning paradigm to test for possible effects of SSRI use. During processing of the various emotional faces (angry vs. neutral, fear vs. neutral, sad vs. neutral and happy vs. neutral), we found similar results after excluding subjects using SSRIs from the main analyses. Notably, we replicated the effect of PCLO+ for the left amygdala.

Also, *post-hoc* analyses of imaging data of our executive functioning paradigm revealed similar results after excluding subjects using SSRIs as in the main analyses (data not shown).

Figure 2.4: Positive effect of increasing task load on activation during planning. Areas involved in the effect of increasing task load include the dorsolateral prefrontal cortex (PFC), middle PFC, precuneus and inferior parietal lobe. Family-wise error corrected at $P < 0.05$. Colors indicate Z-scores.



Discussion

We report, for the first time and in a large group of subjects, an effect of the *PCLO* risk allele for MDD on amygdala activity during processing of angry, fearful and (trend-wise for) sad facial expression, but not during happy facial processing. During angry and sad facial processing, *PCLO* risk allele carriers demonstrated increased amygdala activation, independent of psychopathological status. During the processing of fearful faces, only MDD patients with the *PCLO* risk allele showed increased amygdala activation, whereas no effect of genotype was observed in HC. At a behavioural level, MDD patients with the *PCLO* risk allele performed near-significantly faster than MDD patients with the non-risk allele, whereas in

HC, no significant differences between PCLO genotype were observed during perception of negative emotions. We found no main effect of genotype on executive function, or on its neural substrate, although explorative analyses indicated MDD-specific effects of PCLO risk allele carriers in the medial prefrontal area.

Dysregulation of the amygdala during emotional information processing has previously been associated with MDD (Drevets, 2003; Fu *et al.*, 2004; Leppänen, 2006; Sheline *et al.*, 2001). However, imaging findings have been inconsistent, as several studies failed to demonstrate altered amygdala activity during emotional information processing of faces (Almeida *et al.*, 2010; Demenescu *et al.*, 2011; Gotlib *et al.*, 2005; Lawrence *et al.*, 2004; Lee *et al.*, 2008; Norbury *et al.*, 2010). We suggest that genetic heterogeneity may explain, at least in part, the inconsistencies seen in the literature. Increased amygdala responsiveness during negative face processing and decreased ventral striatum activity in response to positive stimuli have also been reported in MDD studies. Our findings provide further evidence for PCLO being involved in the pathogenetic mechanisms underlying MDD. We found a significant interaction between genotype and current psychopathology, demonstrating increased activity in the amygdala in MDD patients relative to HC during processing of fearful faces. Involvement of the amygdala in the neural response to fearful faces, but not to angry or sad faces has been suggested by some studies (Adolphs *et al.*, 1994; Morris *et al.*, 1996; Thomas *et al.*, 2011), but not by others (Fitzgerald *et al.*, 2006; Sander *et al.*, 2003).

Our study showed lateralization effects in the amygdala in the PCLO risk allele group, which may reflect a bias towards a more specific, sustained emotional reaction in the left amygdala, rather than the general emotional reaction in which the right amygdala is reportedly involved (Baas *et al.*, 2004). It has also been proposed that lateralization may be associated with the temporal dynamics of the amygdala in response to emotional stimuli. Whereas the left amygdala is presumably involved in stimulus evaluation, the right amygdala has been implicated in the rapid detection of emotional stimuli (Sergerie *et al.*, 2008). Following this line of argument, we suggest that the PCLO variant may modulate evaluation of emotional faces, rather than their rapid detection.

In MDD, treatment with SSRIs may reduce negative bias in perception and memory (Alves-Neto *et al.*, 2010; Del-Ben *et al.*, 2008; Harmer *et al.*, 2002). In addition, it is thought that changes in serotonergic neurotransmission can modulate identification of emotional faces, particularly fearful faces (Alves-Neto *et al.*, 2010; Del-Ben *et al.*, 2008). Recently, the PCLO risk allele was found to enhance the biological response to SSRIs among MDD patients (Schuhmacher *et al.*, 2011). However, the mechanism of PCLO modulation on serotonin transmission is unclear and may not involve the serotonin transporter, also in view of the inconsistent findings associated with other serotonergic polymorphisms (Holmes *et al.*, 2010; Lewis *et al.*, 2011).

We found no effect of the PCLO risk allele on performance or BOLD signal during an executive paradigm, the ToL planning task. However, *post-hoc*, we found an interaction between genotype and current psychopathology during performance of the ToL in the left middle dorsolateral PFC, which was also found to be associated with depression severity (van Tol *et al.*, 2011). However, these results should be interpreted with care, as these findings were based on explorative analyses. Another potential limitation of our study is that our

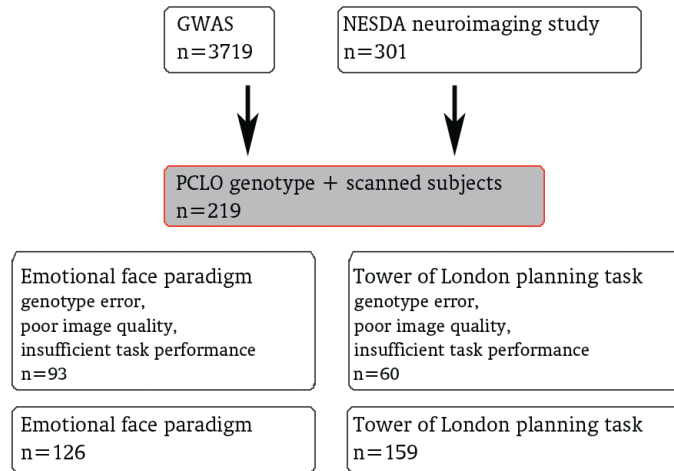
results cannot be readily generalized outside the field of planning and problem solving, and emotional faces processing field.

Taken together, our findings during emotional face processing and planning indicate that *PCLO* is associated with emotion processing rather than executive function. Although our findings suggested selectivity of *PCLO* for serotonergic neurotransmission relative to other monoamines, such a hypothesis is in need of empirical testing. Therefore, future fMRI studies should seek to employ combined cognitive-emotional paradigms coupled with pharmacological manipulation to further investigate the role of *PCLO* in emotional and cognitive processing.

In conclusion, the *PCLO* risk allele is associated with increased activity in the left amygdala during emotional information processing of negative faces, but not with executive function or its neural substrate. We suggest that our data support the role of the *PCLO* risk allele as a risk factor for MDD through altered amygdala activity and, possibly, altered serotonergic neurotransmission in brain areas related to emotional information processing.

Supplemental material

Supplementary table S2.1: Flowchart of participants used in this study



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Supplementary table S2.2: Positive effect of increasing task load during the Tower of London task

Region	side	BA	MNI coordinates			Z Value
			x	y	z	
Superior Frontal	L	6	-24	12	54	>8
	R	6	24	12	54	>8
	R	8	27	30	51	7.5
Medial Frontal	L	8	-6	24	45	7.7
Middle Frontal	L	10	-33	51	15	7.7
	L	6	-24	-12	54	6.7
	R	9	36	33	39	>8
	R	10	33	60	9	7.1
Inferior Frontal	L		-39	45	6	5.7
Inferior Parietal	L	40	-54	-48	39	6.2
	L	40	-45	-51	48	5.6
	R	40	48	-57	36	6.8
	R	40	51	-48	42	6.6
Precuneus	L	7	-6	-63	48	7.5
	L	39	-36	-66	36	5.3
	R	7	3	-66	45	7.3
Angular Gyrus	R	39	45	-69	33	6.6

FWE corrected at $P < .05$, clustersize minimal 10 voxels; side = hemisphere; L = left; R = right; BA = Brodmann Area.

Supplementary table S2.3: Performance during emotional faces paradigm

Response times fearful faces (mean +/- SD in ms)			
	PCLO+	PCLO-	<i>p</i> -value
MDD	753.4 (121.4)	826.8 (203.9)	0.0493
HC	804.3 (119.4)	747.8 (121.4)	

Response times angry faces (mean +/- SD in ms)			
	PCLO+	PCLO-	<i>p</i> -value
MDD	723.7 (112.8)	769.4 (132.3)	0.0595
HC	781.9 (128.6)	722.7 (118.2)	

Response times sad faces (mean +/- SD in ms)			
	PCLO+	PCLO-	<i>p</i> -value
MDD	752.3 (112.8)	812.6 (146.8)	0.0395
HC	791.3 (115.6)	734.6 (134.0)	

Response times happy faces (mean +/- SD in ms)			
	PCLO+	PCLO-	<i>p</i> -value
MDD	764.4 (126.4)	734.5 (183.1)	0.7536
HC	778.6 (121.8)	768.7 (111.6)	

Response times neutral faces (mean +/- SD in ms)			
	PCLO+	PCLO-	<i>p</i> -value
MDD	755.9 (122.6)	759.9 (199.2)	0.5913
HC	823.3 (148.5)	791.3 (120.5)	

