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Research Article

RESEARCH ARTICLE

Familial Risk for Major Depression is Associated with Lower Striatal 5-HT$_4$ Receptor Binding

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Abstract

Background: The 5-HT$_4$ receptor provides a novel potential target for antidepressant treatment. No studies exist to elucidate the 5-HT$_4$ receptor's *in vivo* distribution in the depressed state or in populations that may display trait markers for major depression disorder (MDD). The aim of this study was to determine whether familial risk for MDD is associated with cerebral 5-HT$_4$ receptor binding as measured with [^{11}C]SB207145 brain PET imaging. Familial risk is the most potent risk factor of MDD.

Methods: We studied 57 healthy individuals (mean age 36 yrs, range 20–86; 21 women), 26 of which had first-degree relatives treated for MDD.

Results: We found that having a family history of MDD was associated with lower striatal 5-HT$_4$ receptor binding ($p = 0.038$; in individuals below 40 years, $p = 0.013$). Further, we found evidence for a "risk-dose effect" on 5-HT$_4$ receptor binding, since the number of first-degree relatives with a history of MDD binding correlated negatively with 5-HT$_4$ receptor binding in both the striatum ($p = 0.001$) and limbic regions ($p = 0.012$).

Conclusions: Our data suggest that the 5-HT$_4$ receptor is involved in the neurobiological mechanism underlying familial risk for depression, and that lower striatal 5-HT$_4$ receptor binding is associated with increased risk for developing MDD. The finding is intriguing considering that the 5-HT$_4$ receptor has been suggested to be an effective target for antidepressant treatment.

Keywords: 5-HT$_4$ receptor, depression, MDD, PET, serotonin

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Introduction

The serotonin system is involved in regulation of mood and is the predominant target for antidepressant treatment, primarily with selective serotonin reuptake inhibitors (SSRIs). The serotonin 4 (5-HT_4) receptor is a G_s-coupled receptor and is believed to act by modulating other neurotransmitter systems. The heterogeneous cerebral distribution of the 5-HT_4 receptor is illustrated in Figure 1A. The 5-HT_4 receptor provides a new potential target for fast-acting antidepressant treatment (Vidal et al., 2013). Rodent experiments show that only 3 days of treatment with 5-HT_4 agonists elicits actions similar to those induced by 2–3 weeks of treatment with classical antidepressants, including desensitization of 5-HT_{1A} autoreceptors, increased tonus on hippocampal postsynaptic 5-HT_{1A} receptors, enhanced phosphorylation of the CREB protein, and neurogenesis in the hippocampus (Lucas et al., 2007). Recent rodent work confirms a fast-acting antidepressant and anxiolytic effect of 5-HT_4 receptor stimulation and also, notably, implicates 5-HT_4 receptor activation in the behavioral and neurogenic effects of SSRIs (Mendez-David et al., 2013). In the Flinder sensitive line rat model for depression, decreased levels of hippocampal 5-HT_4 receptor binding were reported (Licht et al., 2009), while regional changes in different directions were seen in two murine models of depression-related states, characterized by serotonin (5-HT) and hypothalamic-pituitary adrenal system changes of depression (Licht et al., 2010). Behaviorally, 5-HT_4 agonists reverse effects of chronic mild stress on sucrose intake and reduce the effects of olfactory bulbectomy on mice locomotor activity, thereby displaying an antidepressant potential (Lucas et al., 2007). Accordingly, 5-HT_4 receptor knock-out mice display a decreased reactivity to novelty seeking, which suggests a slight anxiety-like behavior (Compan et al., 2004).

We have recently found evidence in humans that the cerebral 5-HT_4 receptor is a biomarker for extracellular levels of serotonin (Haahr, Fisher, Jensen, et al., 2013), and that carriers of the short variant of the promoter serotonin transporter (SERT) gene have lower 5-HT_4 receptor binding (Fisher et al., 2012). Thus, it seems that the 5-HT_4 receptor is inversely regulated to the 5-HT tonus, albeit not responsive to acute changes in 5-HT (Licht et al., 2009; Marner et al., 2010). No *in vivo* studies of the cerebral 5-HT_4 receptor binding in depressed patients or at-risk populations have been published so far, but a postmortem study of 19 depressed suicide victims showed increased 5-HT_4 receptor density in the frontal cortex and caudate nucleus (Rosel et al., 2004).

Studying patients suffering from depression is intricate, and confounding effects of previous depressive episodes, co-morbidity with a current episode, and history of antidepressant treatment must be taken to account. An alternative approach is to study the serotonin system in relation to risk factors for depression. We have previously shown that personality risk factors associate positively with 5-HT_2 receptor binding (Frokjaer et al., 2008), and a familial risk for depression may enhance the effect (Frokjaer et al., 2010). A relevant family history is the most potent risk factor for depression (Kendler et al., 1999). The inheritance of depression is polygenetic (Levinson, 2006), and twin studies have suggested that the heritability is around 40% (Kendler et al., 2006b). People with a family history of depression are more prone to develop depressive symptoms (Klaassen et al., 1999), and show compromised emotional processing after dietary depletion of the serotonin precursor protein tryptophan, which decreases synaptic serotonin transiently in CNS (Feder et al., 2010).

Whether healthy individuals with a family history of depression have different cerebral 5-HT_4 receptor bindings compared to healthy individuals with no family history of depression may thus shed light on neurobiological mechanisms underlying risk for depression. The aim of this study was to investigate cerebral 5-HT_4 receptor binding, measured with brain PET imaging, in healthy people with varying degrees of familial predisposition to depression. We hypothesized that a family history of major depressive disorder (MDD) is associated with lower cerebral 5-HT_4 receptor binding based on the above-mentioned experimental studies.

Methods

Participants

Fifty-seven volunteers were included and gave a written informed consent for participation. The study was approved by the Copenhagen Region Ethics Committee ([KF]01-274821, [KF]01 2006-20 and H-D-2007-0067 with amendments). Demographic data are shown in Table 1. Exclusion criteria were significant medical history, drug or alcohol abuse, neurological or psychiatric disorders (also including depression and prior use of antipsychotics and antidepressants), pregnancy, or moderate-severe head trauma. All volunteers had a normal neurological examination, blood analyses within normal range, unremarkable brain magnetic resonance imaging (MRI) scans, and were screened for depressive symptoms using the MDI 10 questionaire (Bech et al., 2001; Olsen et al., 2003) on the day of the PET scan. The participants also completed the Danish version of the

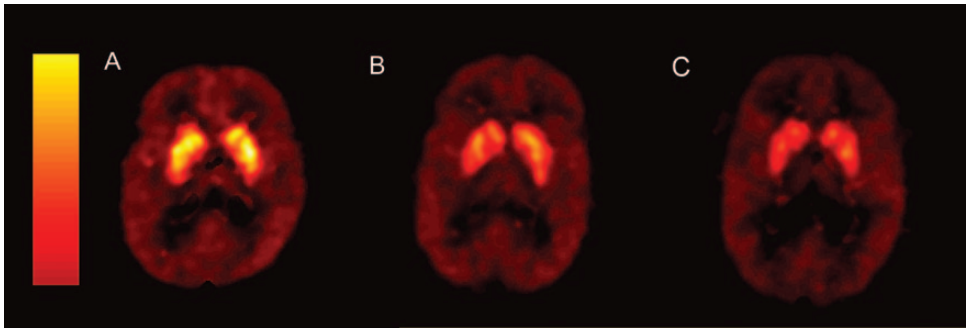

Figure 1. The distribution of [^{11}C]SB207145 binding potentials in 3 young (24–30 yrs) males scanned at the HRRT scanner. Binding levels are high in the striatum, intermediate in the temporal and limbic areas, and low in the neocortex.(A) The distribution in a subject without a family history of depression. (B) Lower binding potentials in a subject who reported to have one first-degree relative with MDD, and (C) even lower binding potentials in a subject who reported to have 2 first-degree relatives with MDD.

Table 1. Data of Groups

	No familial risk	Familial risk
Gender	13 F / 18 M	8 F / 18 M
Age (yrs)	42 (20–86)	32 (20–64)
BMI (kg/m^2)	26 (20–40)	25 (20–38)
5-HTTLPR status (ll/s-carrier)	8/21	9/17
Neuroticism (score)	74 (34–139)	66 (26–131)
Scanner (HRRT/Advance)	16 / 15	12 / 14
Mass dose SB207145 (μg)	2.3 (0.23–5.1)	2.7 (0.34–5.9)
Injected activity (MBq)	511 (222–611)	520 (226–617)

Demographic data, risk factors for depression, and scanner data for individuals with and without familial risk for depression. Data listed as numbers or means (range). No significant between-group differences was found.

240-item NEO Personal Inventory Revised self-report (NEO-PI-R) personality questionnaire, which evaluates the broad personality dimensions of neuroticism, extraversion, openness, agreeableness, and conscientiousness (Skovdahl-Hansen et al., 2004). Neuroticism is strongly related to lifetime prevalences of MDD, largely due to genetic factors (around 50%) that predispose to both neuroticism and MDD (Kendler et al., 1993, 2006a)

Fifty-one volunteers were recruited to the study by public advertisements or extracted from the civil registration system in Denmark. Six volunteers were included because they had a sibling or parent with MDD treated at an in- or out-patient hospital in Denmark; these volunteers also participated in a clinical trial study (Knorr et al., 2011). All volunteers were scanned in the period from 2006 to 2011, and some datasets have been included in previously-published studies regarding validation of the tracer (Marner et al., 2009; Madsen, Marner, et al., 2011) and studies of the 5-HT$_4$ receptor in healthy volunteers to determine the association between the receptor binding and gender and age (Madsen, Haahr, et al., 2011), 5-HTTLPR genotype (Fisher et al., 2012), body mass index (BMI) (Haahr et al., 2012), memory (Haahr, Fisher, Holst, et al., 2013), effects of SSRI (Marner et al., 2010; Haahr, Fisher, Jensen, et al., 2013), and in relation to patients suffering from Alzheimer's disease (Madsen, Neumann, et al., 2011). Relevant effects found in these studies were also evaluated in the statistical analysis of this study.

To identify participants with familial risk of depression, all participants were interviewed at the day of the PET scan using a Danish version of the Family History Assessment Module (FHAM) (Rice et al., 1995). This module is designed to assess major psychiatric disorders in relatives of the participant. For ethical reasons it was not possible to contact the affected relatives themselves. Instead, participants who reported to have one or more first-degree relatives with a major psychiatric disorder were subsequently interviewed by a trained physician with a structural interview regarding the symptoms and treatment of each affected relative. No participants had first-degree relatives diagnosed with schizophrenia or bipolar disorder.

In this enriched cohort, 26 out of 57 healthy volunteers (46%) reported to have one or more first-degree relatives diagnosed with depression according to the DSM-IV criteria, which were used as the diagnostic criteria in this study. A total of 34 affected relatives were identified: 97% had been treated for depression by a general physician or psychiatrist, 77% had been treated with antidepressants, 44% had been hospitalized, and 15% had attempted suicide. Consistent with the preponderance of women with depression, 68% of the affected relatives were women (14 mothers, 10 sisters, 8 fathers, 2 brothers, and 0 children).

Since short-allele carrier status of the 5-HTTLPR polymorphism in the promoter region of the SERT gene, SCL6A4, may modulate risk for developing depression (Caspi et al., 2003) and affect the 5-HT$_4$ receptor levels (Fisher et al., 2012), blood samples were drawn to determine the 5-HTTLPR status as previously described (Kalbitzer et al., 2010).

MRI and Regions of Interest

MRI was conducted on a Siemens Magnetom Trio 3T MR scanner. T2-weighted sequences were acquired for brain-masking purposes. High-resolution 3D T1-weighted (matrix 256 x 256; 1 x 1 x 1 mm voxels) images were segmented into grey matter, white matter, and cerebrospinal fluid using Statistical Parametric Mapping (SPM5; Wellcome Department of Cognitive Neurology). A set of 17 brain regions was automatically delineated with the Pvelab software package (Svarer et al., 2005) on each volunteer's MRI in a user-independent fashion.

PET Imaging and Quantification of Non-Displaceable 5-HT$_4$ Receptor Binding

All PET scans were based on a 120-minute dynamic acquisition starting with a bolus injection of [^{11}C]SB207145 given over 20 seconds. Two different PET scanners were used over time, as the department added a scanner with a higher resolution. Twenty-nine volunteers had the PET scans performed with an 18-ring GE-Advance scanner (General Electric, Milwaukee, WI, USA) operating in 3D acquisition mode with an approximate in-plane resolution of 6 mm. After acquisition, attenuation- and decay-corrected recordings were reconstructed by filtered back projection using a 6 mm Hann filter. The remaining 28 volunteers completed the PET scans performed with a high-resolution research tomography (HRRT) Siemens PET scanner and the images were reconstructed with 3D-OSEM-PSF (Sureau et al., 2008) with a resolution of approximately 2 mm (Olesen et al., 2009).

The scan consisted of 38 time frames (6 x 5 seconds [s], 10 x 15 s, 4 x 30 s, 5 x 120 s, 5 x 300 s, and 8 x 600 s). Mean voxel movement between frames was assessed with AIR 5.2.5 (Woods et al., 1992), and, only when exceeding 3 mm, movement correction was applied as the rigid transformation of each frame to a selected single frame with sufficient structural information (frame 26: 15–20 min. post injection) using the scaled least squares cost-function in AIR.

For automatic co-registration of the PET scan to the MRI, the AIR algorithm was applied for GE-Advance scans while SPM5 was applied for HRRT scans and the quality of each co-registration was evaluated by visual inspection in three planes.

The regional *in vivo* outcome measure for 5-HT$_4$ receptor levels, the binding potential, BP_{ND}, was modeled with the simplified reference tissue model as validated previously (Marner et al., 2009). From the set of regions, volume-weighted means of BP_{ND} were calculated for three brain regions considered important for mood disorders: the striatum (high 5-HT$_4$ receptor binding, including caudate nucleus and putamen), the limbic regions (intermediate 5-HT$_4$ receptor binding, including hippocampus, amygdala, thalamus, and anterior and posterior cingulate gyrus), and the neocortex (low 5-HT$_4$ receptor binding, including parietal cortex, occipital cortex, lateral temporal cortex, insula,

and orbito-frontal and lateral-frontal cortex) as previously described (Madsen, Haahr, et al., 2011).

These regions were chosen since the striatum and the limbic regions previously have been shown to be involved in MDD (Price and Drevets, 2010), including findings of reduced grey-matter volumes, increased cerebral blood flow and metabolism, altered hemodynamic responses towards emotional stimuli, and reward-processing. The neocortex was included in our analysis as one large cortical region, as it has low 5-HT_4 receptor binding, albeit frontal regions also may be involved in MDD.

Statistics

As the primary investigation, a multiple–linear regression model was employed to study the association between having a family history of depression (binary) and the 5-HT_4 receptor bindings for each of the three selected brain regions. As expected, age, gender, and scanner type were significant covariates and were included in the regression model.

We also examined the effect of the number of affected relatives with a history of depression to investigate a possible "risk-dose effect" of family history of depression on 5-HT_4 receptor binding. Also, the interaction of being female and having a female relative with a history of depression was investigated to see if the heritable effect could be sex-specific (Kendler et al., 2006b).

All statistical tests were two-sided, and p values were considered statistically significant when less than 0.05.

On one hand, the assessment of familial risk for depression may be biased by the age of the volunteers: e.g., being older increases the likelihood of a higher number of MDD-diagnosed first-degree relatives. On the other hand, remaining mentally healthy in spite of a family history may also result from being protected against depression. Also, the availability of efficient antidepressant treatment and more attention to the diagnosis in society over time may have an impact. Therefore we *post hoc* estimated the model in the subset of the cohort <40 years (n = 39).

Results

A family history of depression was associated with a significant decrease in striatal 5-HT_4 receptor binding (p = 0.038, -0.20 BP_{ND}, 95% CI: [-0.39; -0.012] BP_{ND}), whereas 5-HT_4 receptor binding in the limbic regions (p = 0.20) and in the neocortex (p = 0.87) did not differ significantly between groups (Table 2). The results were even more significant when only including individuals below 40 years in the model (n = 39; striatum p = 0.013; limbic regions p = 0.16; neocortex p = 0.99).

When considering the "risk-dose" of first-degree relatives with a history of depression, a significant negative correlation was observed with 5-HT_4 receptor binding in both the striatum (p = 0.001, -0.22 BP_{ND}/relative, 95% CI: [-0.352; -0.097] BP_{ND}, Figure 2) and limbic regions (p = 0.012, -0.043 BP_{ND}/relative, 95% CI: [-0.076; -0.010] BP_{ND}), but no correlation was observed in the neocortex (p = 0.20, 0.017 BP_{ND}/relative, 95% CI: [-0.044; 0.009] BP_{ND}). The results are illustrated with examples of the distribution in different subjects in Figure 1. A leave-one-out sensitivity analysis showed that the estimated association was not strongly driven by any single observation in the data.

Consistent with a previous study in a subset of this cohort (Madsen, Haahr, et al., 2011), a decline is found with aging in all regions (Table 2). Female gender status was associated with reduced limbic 5-HT_4 receptor binding in the previous study; however, in this larger study the association was significant in all regions (Table 2). As expected (Nilsson et al., 2010; Svarer et al., 2010), the HRRT PET scanner generated higher 5-HT_4 receptor BP_{ND} than the GE scanner (Table 2). Inclusion of 5-HTTLPR genotype status, neuroticism score, and BMI in the model (all predictors were statistically insignificant in all three regions) resulted in very similar p values and parameter estimates of the

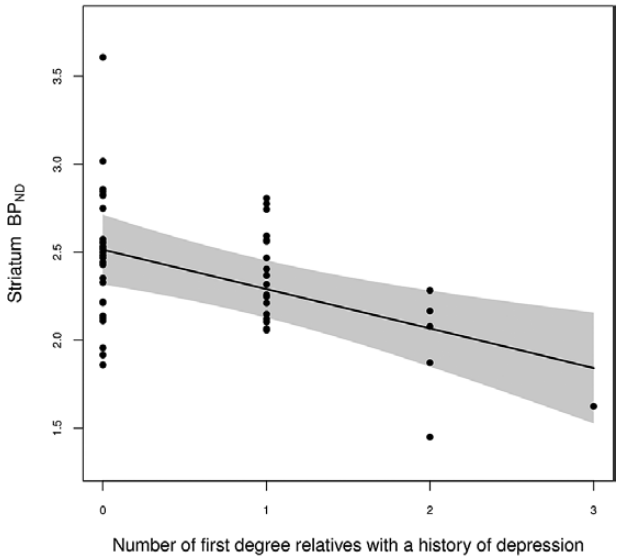

Figure 2. The estimated linear association between 5-HT_4 receptor binding in the striatum (corrected for age, gender, and scanner type) and the number of first-degree relatives treated for major depression (p = 0.001), with pointwise 95% confidence limits and partial residuals (reference: male, mean age 36 years; GE Advance). A leave-one-out analysis showed that the estimated association was not strongly driven by any single observation in the data (p values in the range 0.0003–0.005).

Table 2. Multiple Linear Regression Model Analysis

	5-HT_4 Receptor Binding		
	Estimate ± SE	p value	R^2
Neocortex			0.86
constant = 0.468			
Age [/year]	-0.0019 ± 0.0006	0.002	
Sex [female]	-0.062 ± 0.021	0.004	
Scanner [HRRT]	0.259 ± 0.021	<0.0001	
Family history of MDD	-0.0031 ± 0.019	0.87	
Limbic regions			0.71
constant = 0.689			
Age [/year]	-0.0017 ± 0.0008	0.028	
Sex [female]	-0.089 ± 0.027	0.002	
Scanner [HRRT]	0.184 ± 0.027	<0.0001	
Family history of MDD	-0.031 ± 0.024	0.20	
Striatum			0.81
constant = 2.859			
Age [/year]	-0.012 ± 0.003	0.0003	
Sex [female]	-0.233 ± 0.105	0.031	
Scanner [HRRT]	1.069 ± 0.105	<0.0001	
Family history of MDD	-0.203 ± 0.095	0.038	

Outcome of multiple–linear regression model analysis used to determine the effect of a family history of depression on 5-HT_4 receptor binding. Age, gender, and scanner type were significant co-variates in all regions and are included in the model.

associations between our primary predictor—familial history of MDD—and the regional 5-HT_4 receptor binding.

Discussion

Consistent with our hypothesis, we found that familial risk of MDD was associated with lower striatal 5-HT_4 receptor binding ($p = 0.038$), but no significant effect was found in the neocortex and limbic regions. The effect was even more significant in participants below 40 years (striatum $p = 0.013$) in spite of the reduced sample. Analysis using the same statistical model showed that the association to striatal 5-HT_4 receptor binding was even more pronounced when considering the number of affected relatives ($p = 0.001$) and an association in the same direction was observed in the limbic region ($p = 0.01$), indicating that there may be a "risk-dose effect" of the heritability of depression on 5-HT_4 receptor binding. The association towards the striatum and the limbic regions is in concordance with previous findings of involvement of these regions in MDD (Price and Drevets, 2010). Anhedonia is a common symptom in MDD and reward responsiveness (hedonic capacity) may be heritable (Bogdan and Pizzagalli, 2009). The striatum, particularly its ventral part, and the limbic regions are key structures in the reward system, and patients suffering from MDD have a reduced striatal activation response to rewards (Pizzagalli et al., 2009; Stoy et al., 2012). Reversal learning is also associated with striatal responses and has a negative bias in MDD (Robinson et al., 2011), and the 5-HT_4 receptor has been suggested to be involved in cognitive function (King et al., 2008; Haahr, Fisher, Holst, et al., 2013). The involvement of the limbic and striatal regions in MDD has also been demonstrated by induction of mood changes from deep brain stimulation (including symptoms of hypomania, dysphoria, and anhedonia), and experimental investigations are currently being conducted in treatment-resistant MDD (Cusin and Dougherty, 2012).

Our finding of lower striatal and limbic 5-HT_4 receptor binding in relation to the heritability of depression may reflect that decreased receptor availability is a trait marker of MDD. This is consistent with observations in a genetic rat model of depression (Licht et al., 2009) and a slight hyperanxiety-like behavior of the 5-HT4 receptor knock-out mice in studies of activity in the open field (Compan et al., 2004). Our finding of lower 5-HT_4 receptor binding in mentally-healthy individuals at familial risk for developing depression could be interpreted as reflecting higher chronic endogenous serotonin levels, since the 5-HT_4 receptor is inversely regulated to the 5-HT tonus (Haahr, Fisher, Jensen, et al., 2013). *In vivo* studies have reported associations between depression and altered SERT and elevated 5-HT_{1A} binding (Meyer, 2007; Miller et al., 2009), and between behavioral phenotypes related to risk for depression and increased 5-HT_{2A} binding (Frokjaer et al., 2008). We cannot determine whether the lower striatal 5-HT_4 receptor binding found in our study represents a protective or compensatory mechanism for the included participants to remain mentally healthy, as part of being MDD resilient. It could be that those subjects at highest risk additionally down-regulate limbic 5-HT_4 receptors to remain mentally healthy, maybe by modulation of the 5-HT tonus. The answer to this question can only be obtained through longitudinal follow-up studies, which would also reveal whether the low 5-HT_4 receptor binding is predictive of development of depression later in life, or by examining unmedicated patients remitted from a depressed state.

The heritability of MDD is higher in women than in men and some genetic risk factors for MDD are sex-specific in their effect (Kendler et al., 2006b). A *post hoc* test showed no interaction between being a female and having a female relative with a history of depression ($p = 0.51$ in striatum, $p = 0.84$ in limbic regions, $p = 0.74$ in neocortex), even though females have lower limbic 5-HT_4 receptor binding (Madsen, Haahr, et al., 2011). Thus, based on our data it seems that the effect of familial risk for depression on 5-HT_4 receptor binding is not sex-specific.

Some potential limitations of our study should be considered. The number of first-degree relatives could bias our investigation, since some have more siblings and children than others. As being older increases the likelihood of a higher number of MDD-diagnosed first-degree relatives, age could be an important confounder. However, the participants were quite young, and none reported to have children who had suffered from depression. On the other hand, being elderly and having stayed healthy despite a family history of MDD may index protective factors. Yet, when we considered only individuals younger than 40 years old we continued to see a significant association between familial risk and striatal 5-HT_4 receptor binding.

One could speculate whether participants with an affected relative had an overrepresentation of other neuropsychiatric disorders, which could potentially contribute to decreases in 5-HT_4 receptor binding. However, absence of neuropsychiatric disorders was thoroughly assessed. In further support, participants were screened for depressive symptoms on the day of the PET scan.

Despite our inability to interview the affected relatives of the participants themselves, participants were able to give detailed information regarding their relatives. For example, they reported that 97% of the affected relatives had been treated for depression by a general physician or psychiatrist and 77% with antidepressant drugs, which we find underpins the validity of the MDD diagnosis. We experienced that the characteristics of the affected relatives were more difficult to clarify for elderly participants who reported a parent suffering from depression. This might explain why the association between familial risk and striatal 5-HT_4 binding appeared weaker when including participants above 40 years of age. However, MDD is a heterogeneous disorder and 5-HT_4 receptor binding could be more strongly related to a more homogenous phenotype of MDD (Bogdan et al., 2013), as, for example, in patients with predominant symptoms of anhedonia, anxiety, or suicidal behavior. However, we were not able to reliably characterize the affected relatives in such detail based on the interviews of the participants.

Conclusion

The finding of lower 5-HT_4 receptor binding in healthy individuals with familial risk for MDD suggests that the 5-HT_4 receptor is involved in the neurobiological mechanism underlying familial risk for depression. Our current finding is intriguing considering that the 5-HT_4 receptor may be an effective target for antidepressant treatment (Lucas et al., 2007; Vidal et al., 2013). Future studies are needed to elucidate whether 5-HT_4 receptor binding is changed in the depressed state of MDD, and clinical trials are needed to determine the effects of 5-HT_4 agonists on depressive symptoms and cognitive performances in MDD.

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Statement of Interest

Dr Knorr has been a consultant for Astrazeneca. Otherwise, the authors declare no conflicts of interest and no non-financial form of support has been given to the study.

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