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Converging translational evidence for the involvement of the serotonin 2A receptor gene in major depressive disorder

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ABSTRACT

An association between serotonin 2A receptor (5-HT2AR), encoded by HTR2A gene, and major depressive disorder (MDD) has been suggested. Here, we combined preclinical and ecological clinical approaches to explore the impact of impaired 5-HT2AR-mediated transmission on MDD or anxio-depressive-like phenotype in mice.

Htr2a knock-out mice ($Htr2a^{-/-}$) and wild-type mice were compared for the ability of chronic corticosterone to elicit some anxio-depressive-like phenotype in three behavioral paradigms (elevated plus maze, tail suspension test and splash test). Accordingly, two single nucleotide polymorphisms of the HTR2A gene (rs6314 ie His452Tyr and rs6313 ie *102C/T*), which specific allelic variants may decrease 5-HT2AR-mediated transmission (as in $Htr2a^{-/-}$mice), were studied in a sample of 485 Caucasian patients with MDD.

In response to chronic corticosterone exposure, $Htr2a^{-/-}$ mice displayed more pronounced anxiodepressive-like phenotype than wild-type mice, as shown by a significant higher "emotionality score" ($p < 0.01$). In patients, the *C* allele of rs6313 was more frequent in depressed patients ($p = 0.019$) and was also associated with a more severe major depressive episode ($p = 0.03$).

This translational and ecological study involving constitutive $Htr2a^{-/-}$ knock-out mice and related SNPs in depressed patients suggests that a lower neurotransmission at the 5-HT2AR may favor the susceptibility and severity of MDE. It also suggests that specific allelic variants of the rs6313 and rs6314 may reduce 5-HT2AR-mediated transmission.

1. Introduction

The serotonin 2A receptor ($5\text{-}HT_{2A}R$) is widely expressed throughout the central nervous system (Quesseveur et al., 2012) and the role of this receptor in the control of serotonergic tone has been examined. *In vivo* studies report that its acute activation attenuates the firing rate of 5-HT neurons (Boothman and Sharp, 2005; Boothman et al., 2003; Bortolozzi et al., 2003; Garratt et al., 1991; Martin-Ruiz et al., 2001; Quesseveur et al., 2013; Wright et al., 1990), reduces the extracellular 5-HT levels at the nerve terminals (Martin-Ruiz et al., 2001) and produces depressive-like behaviors (Diaz and Maroteaux, 2011). In a marked contrast, pharmacological or genetic inactivation of $5\text{-}HT_{2A}R$ with selective antagonists or antisense oligonucleotides respectively,

Abbreviations: 5-HT, serotonin; $5\text{-}HT_{2A}R$, serotonin 2A receptor; CGI, Clinical Global Impression; EPM, elevated plus maze; HAMD-17, 17-item Hamilton Depression Rating Scale; HW, Hardy–Weinberg equilibrium; MDD, major depressive disorder; MDE, major depressive episode; SNP, single nucleotide polymorphism; ST, splash test; TST, tail suspension test; UCMS, unpredictable chronic mild stress.

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elicits antidepressant-like activities in rodents (Pandey et al., 2010; Patel et al., 2004; Sibille et al., 1997; Zaniewska et al., 2010). Importantly, these preclinical studies were performed in naive rodents whereas data using a relevant animal model exhibiting hallmark characteristics of depression are lacking to determine the contribution of the 5-HT_{2A}R in pathological states. Animal models of depression are mainly based on stressful situations, such as unpredictable chronic mild stress (UCMS) (Farley et al., 2010; Surget et al., 2009). An alternative is to supply mice with exogenous corticosterone (David et al., 2009; Gourley and Taylor, 2009), a hormone found to be elevated in several animal models of depression and in patients with major depressive disorders (MDD) (Sterner and Kalynchuk, 2010). We have recently reported, in mice administered with corticosterone ("CORT model") some behavioral abnormalities reflecting major depression (David et al., 2009).

Given the dysfunction of the serotonergic system in major depressive episodes (MDE) of MDD (Baldwin and Rudge, 1995), genetic association studies have focused on the genetic variants at the gene encoding for the 5-HT_{2A}R, *HTR2A* (see (Anguelova et al., 2003; Serretti et al., 2007) for review). The association between MDD and three single nucleotide polymorphisms (SNPs), G-to-A substitution at nucleotide −1438 (rs6311, *−1438G/A*), C-to-T substitution at nucleotide 102 (rs6313, *102C/T*) and C-to-T substitution at nucleotide 1354 (rs6314, His452Tyr, *1354C/T*) has been investigated, showing inconsistent results for the *C* allele of rs6313 (association: (Arias et al., 2001; Du et al., 2000; Zhang et al., 1997), no association: (Illi et al., 2009; Kishi et al., 2009; Minov et al., 2001; Tsai et al., 1999; Wang et al., 2009; Zhang et al., 2008)), for the *A* allele of rs6311 (association: (Christiansen et al., 2007; Enoch et al., 1999; Jansson et al., 2003; Kamata et al., 2011; Lee et al., 2006), opposite association: (Choi et al., 2004), no association: (Illi et al., 2009; Kishi et al., 2009; Ohara et al., 1998; Tencomnao et al., 2010)) and for rs6314 which has been poorly studied (no association: (Minov et al., 2001)). Moreover, the functional consequences of these SNPs on 5-HT_{2A}R function and/or *HTR2A* expression remain poorly studied (Serretti et al., 2007), especially for the *C* allele of rs6313, which could be submitted to methylation, a process known to prevent gene expression (Polesskaya et al., 2006) and for the *T* allele of rs6314 which could be associated with a decreased 5-HT_{2A}R-mediated intracellular signaling (Ozaki et al., 1997). The inconsistency of data regarding the functional consequences of these SNPs on 5-HT_{2A}R function and/or *HTR2A* expression warrants further investigations in pre-clinical and clinical studies.

This translational study aimed at assessing the functional role of 5-HT_{2A}R signaling in MDD. Indeed, in the present study, genetic evidence for a functional impact of an impairment of brain 5-HT_{2A}R-mediated transmission was studied both in mice with a constitutive genetic inactivation of the 5-HT_{2A}R exposed to corticosterone and in MDD patients considering two *HTR2A* SNPs supposed to alter the expression of the gene or the function of 5-HT_{2A}R.

2. Methods

2.1. Preclinical study

2.1.1. Animals

Adult male 5-HT_{2A}R mutants (*Htr2a*$^{-/-}$ mice) and wild-type mice (*Htr2a*$^{+/+}$ mice) (Weisstaub et al., 2006) were bred on a S129/Sv genetic background at the University of Paris-Sud (SCA Châtenay-Malabry, France). All *Htr2a*$^{-/-}$ and *Htr2a* $^{+/+}$ mice were 7–8 weeks old and weighed 23–35 g at the beginning of the experiments. They were maintained on a 12L:12D schedule, and were housed five per cage. Behavioral testing occurred during the light phase. All testing was conducted in compliance with the NIH laboratory animal care guidelines and with protocols approved by the Institutional Animal Care and Use Committee (Council directive # 87-848, October 19, 1987, Ministère de l'Agriculture et de la Forêt, Service Vétérinaire de la Santé et de la Protection Animale, permissions # 92-196 to Alain Gardier).

2.1.2. Drugs

The administration of corticosterone in the drinking water has been repeatedly used to induce a depressive-like phenotype in mice (David et al., 2009; Rainer et al., 2011). Corticosterone (Sigma-Aldrich, St. Quentin-Fallavier, France) was dissolved in hydroxy-propyl-beta-cyclodextrin (β-cyclo: 0.45%, St. Quentin-Fallavier, France) and delivered for 5 weeks in opaque bottles to protect it from the light. It was available *ad libitum* in the drinking water (David et al., 2009). Control mice received β-cyclo in the drinking water at the same final concentration (0.45%). A modified version of the CORT protocol was used here because the genetic background of the mice is different from that used in the initial characterization of the model (David et al., 2009) (129/Sv *for Htr2a*$^{-/-}$ and *Htr2a*$^{+/+}$ mice, and C57/Bl6 in the latter work).

2.1.3. Experimental procedures

Distinct groups of *Htr2a*$^{-/-}$and *Htr2a* $^{+/+}$ mice were used for behavioral analysis in presence or not of corticosterone. All animals were subjected to the same sequence of behavioral tests.

2.1.3.1. Elevated plus maze (EPM). The EPM was performed as previously described (David et al., 2009). Animals were placed into the central area facing one closed arm and allowed to explore the maze for 5 min. The testing took place in bright dimmed light conditions (800–900 lx). Automated scoring was done using ANY-maze (tm) behavioral video-tracking software from Stoelting Co (Bioseb, Vitrolles, France). The total entries and the time spent in the open arms were measured.

2.1.3.2. Tail suspension test (TST). The TST is a mouse behavioral test useful in the screening of potential antidepressant drugs, and in assessing other manipulations that are expected to affect depression related behaviors (Steru et al., 1985). Mice are suspended by their tails with tape. In such a position animals cannot escape or hold on to nearby surfaces. During the test, typically 6 min in duration, the resulting escape oriented behaviors are quantified using the Bioseb TST software (Bioseb, Vitrolles, France). A specific strain gauge linked to a computer quantifies properly the time of mobility and immobility. The latter parameter was scored and used in the present study as a measure of despair (Lucki, 1997).

2.1.3.3. Splash test (ST). The ST was performed as previously described (David et al., 2009). This test consisted of squirting 200 μl of a 10% sucrose solution on the mouse's snout. Because of its viscosity, the sucrose solution dirties the mouse fur and animals initiate grooming behavior. After applying sucrose solution, the time spent for grooming was recorded for a period of 5 min. A decrease in grooming, which is a particular feature of mice submitted to stress was used as an index of self-care and motivational behavior (Surget et al., 2008).

2.1.3.4. Emotionality z-score. Because the evaluation of behavioral parameters was performed with several tests in the same animal, a modified version of the z-score used by Guilloux et al. (2011) was calculated here in *Htr2a*$^{-/-}$and *Htr2a*$^{+/+}$ mice exposed to chronic corticosterone. This score evaluates consistency of behaviors across tests compared to random changes, and it provides an integrated and continuous measure of severity. Based on the depression definition (*i.e.* a syndrome as a collection of variable symptoms), z-score constitutes a relevant parameter to evaluate the severity of anxio-depressive-like states in mice taking into consideration different facets of the syndrome as human scales do. Notably, it has been validated in mice subjected to UCMS and corticosterone exposure. The higher the z-score, the higher the depression severity (Guilloux et al., 2011).

2.1.4. Statistical analysis

Statistical analyses were performed with StatView 5.0 software (SAS Institute Inc.). A one-way analysis of variance with treatment as main factor was applied to compare the emotionality score in *Htr2a*$^{+/+}$ administered with corticosterone or its vehicle. Treatment comparisons

were then analyzed using a post-hoc Bonferroni test. A student-*t* test was used to compare the effect of corticosterone between *Htr2a*$^{-/-}$ and *Htr2a*$^{+/+}$ mice.

2.2. Clinical study

2.2.1. Patients

485 Caucasian (*i.e.* with four Caucasian grand-parents) in or outpatients, aged 18–70 years, with a current MDE diagnosis (Diagnostic and Statistical Manual of Mental Disorders, fourth edition, text revised (DSM-IVTR)) based on the Mini International Neuropsychiatric Interview (MINI, (Sheehan et al., 1998)), were included in 3 university psychiatry departments in Paris, France. These patients required a new antidepressant treatment or a change of antidepressant treatment because of depression severity. A minimum depression score of 18 on the 17-item Hamilton Depression Rating Scale (HAMD-17, (Hamilton, 1960)) was also required to ensure that patients qualify for MDE. Patients with bipolar disorders (DSM-IVTR), psychotic disorders (DSM-IVTR), current substance abuse or dependence (DSM-IVTR), pregnancy, organic brain syndromes or unstable medical conditions were excluded. Patients provided written informed consent for study participation and for genetic analyses.

2.2.2. Study design

In this multicenter study, MDD patients with a current MDE were assessed for clinical characteristics and two *HTR2A* SNPs (rs6313 and rs6314) were genotyped. The study protocol was approved by the French ethics committee of Paris-Boulogne and the French national CCTIRS and CNIL.

2.2.3. Assessment instruments

The HAMD-17 and Clinic Global Impression Severity scale (CGI-S, (Guy, 1976)) were rated by trained clinicians to document the current MDE severity. In the whole sample, the HAMD-17 scores range from 18 to 40 and the mean score was 24.8 ± 5.0 (first quartile from 18 to 21, second quartile from 21 to 24, third quartile from 24 to 28, fourth quartile from 28 to 40). The CGI-S scores ranged from 3 to 7 and the mean score was 4.9 ± 0.75. The age at onset of MDD and number of previous MDEs were also recorded. Clinical assessments were performed blind to genotyping results.

2.2.4. DNA preparation and SNP genotyping

A sample of 5 mL of whole blood was collected. DNA from lymphocytes was extracted from 1 mL of blood sample using a Puregene Kit (Gentra systems, Minneapolis, USA) and cryopreserved at −20 °C.

Since the *HTR2A* genetic polymorphisms rs6311 and rs6313 are known to be in complete linkage disequilibrium (Myers et al., 2007; Spurlock et al., 1998), our analyses were performed on two *HTR2A* SNPs: rs6313 and rs6314. SNP genotyping was performed by a TaqMan SNP genotyping assay (assay ID: C_3042197_1 for rs6313 and C_11696920_20 for rs6314, Applied Biosystems) according to the manufacturer's instructions. SNP primers and probes were designed for genotyping using the Primer express software from Applied Biosystems (Forster City, CA, USA). Allelic discrimination was performed with the ABI prism 7900HT Sequence Detection System (Applied Biosystems, Courtaboeuf, France). Genotyping was performed blind to clinical data. For rs6313, patients were classified into two groups: *TT* group (patients with *TT* genotype) and *CC/CT* group (patients with *CC* and *CT* genotypes). *CC/CT* patients may have an impaired 5HTR2A expression, based on the study by Polesskaya et al. (2006) and correspond with the *Htr2a*$^{-/-}$ CORT mice.

2.2.5. Data analysis

Genotype frequencies as well as deviation from the Hardy–Weinberg equilibrium were assessed using the Chi^2 test. Two sample t-tests were used to compare quantitative variables according to genotype groups. Chi^2 tests were applied to compare qualitative variables according to the genotype groups. A p-value of less than 0.05 was considered statistically significant. Statistical analyses were performed with StatView 5.0 software (SAS Institute Inc.).

3. Results

3.1. Pre-clinical study

In a first set of experiment, we determined the dose of corticosterone required to produce stress-related behavioral anomalies in *Htr2a*$^{+/+}$. The "emotionality score" was significantly higher in *Htr2a*$^{+/+}$ mice treated with corticosterone at the dose of 70 μg/kg than in *Htr2a*$^{+/+}$ mice administered with the vehicle whereas the dose of 35 μg/kg failed to elicit behavioral impairments (Supplementary Fig. 1). On the basis of these preliminary observations, we then compared the effects of corticosterone at the dose of 70 μg/kg between *Htr2a*$^{+/+}$ and *Htr2a*$^{-/-}$ mice (*Htr2a*$^{+/+}$*CORT* and *Htr2a*$^{-/-}$ CORT respectively). In the EPM, no differences between both genotypes were detected on the time spent in the anxiogenic areas such as the open arms ($t_{1,29} = 0.47$; Fig. 1A), the ratio of open/total arm entries ($t_{1,29} = 0.66$; Fig. 1B) and the total ambulatory distance ($t_{1,29} = 0.31$; Fig. 1C). We then explored mouse behavior in the TST. In this test, a higher immobility time was observed in *Htr2a*$^{-/-}$ CORT compared to *Htr2a*$^{+/+}$ CORT mice ($t_{1,29} = 0.02$; Fig. 1D). Finally, in the ST, a lower time of grooming was also detected in *Htr2a*$^{-/-}$ CORT compared to *Htr2a*$^{+/+}$ CORT mice ($t_{1,29} = 0.02$; Fig. 1E). The determination of the z-score revealed a higher emotionality score in *Htr2a*$^{-/-}$ CORT than in *Htr2a*$^{+/+}$ CORT mice ($t_{1,29} = 0.003$; Fig. 1F).

Importantly, when tested in basal conditions *i.e.* β-cyclo-containing drinking water (vehicle), none of these parameters were significantly influenced by the genetic inactivation of the 5-HT$_{2A}$R (Supplemental Table 1).

3.2. rs6313 HTR2A genetic polymorphism

The socio-demographic characteristics of the sample are shown in Table 1 according to *TT* and *CC/CT* groups. In this sample of depressed patients, 402 out of 485 (83%) patients carried the *CC* or the *CT* genotypes and 83 (17%) patients carried the *TT* genotype. Allelic frequencies were 0.56 for the *C* allele and 0.44 for the *T* allele. These measured genotype frequencies were significantly different from the expectations of Hardy–Weinberg (HW) equilibrium (Chi^2 = 5.05, p = 0.019), with an over-representation of *CC* and *CT* genotypes (389 expected carriers vs 402 measured) and an under-representation of *TT* genotype (96 expected carriers vs 83 measured). This result seems to be specific to the polymorphism studied, rather than related to the sample studied, as other SNPs studied in this sample show no deviation from HW equilibrium (data not shown). This argues for an over-representation of the rs6313 *C* allele in patients with MDD.

Moreover, whereas the 2 groups were not significantly different regarding socio-demographic characteristics and history of MDD, HAMD-17 scores were significantly different between the 2 groups (Table 1), with higher scores for *CT/CC* group than for *TT* group (p = 0.03, Table 1). These results also argue for an association between the *C* allele of the rs6313 polymorphism and higher MDD severity.

3.3. rs6314 HTR2A genetic polymorphism

In this sample, 407 out of 485 (84%) patients carried the *CC* (452His/His) genotype, 76 (15.6%) patients carried the heterozygous *CT* (452His/Tyr) genotype and 2 (0.4%) patients carried the *TT* (452 Tyr/Tyr) genotype. Allelic frequencies were 0.92 for the *HTR2A C* (452His) allele and 0.08 for the *HTR2A T* (452Tyr) allele, no significant deviation from the Hardy–Weinberg (HW) equilibrium being detected (Chi^2 = 0.61, p = 0.43). The *TT* variant was evidenced in two patients confirming

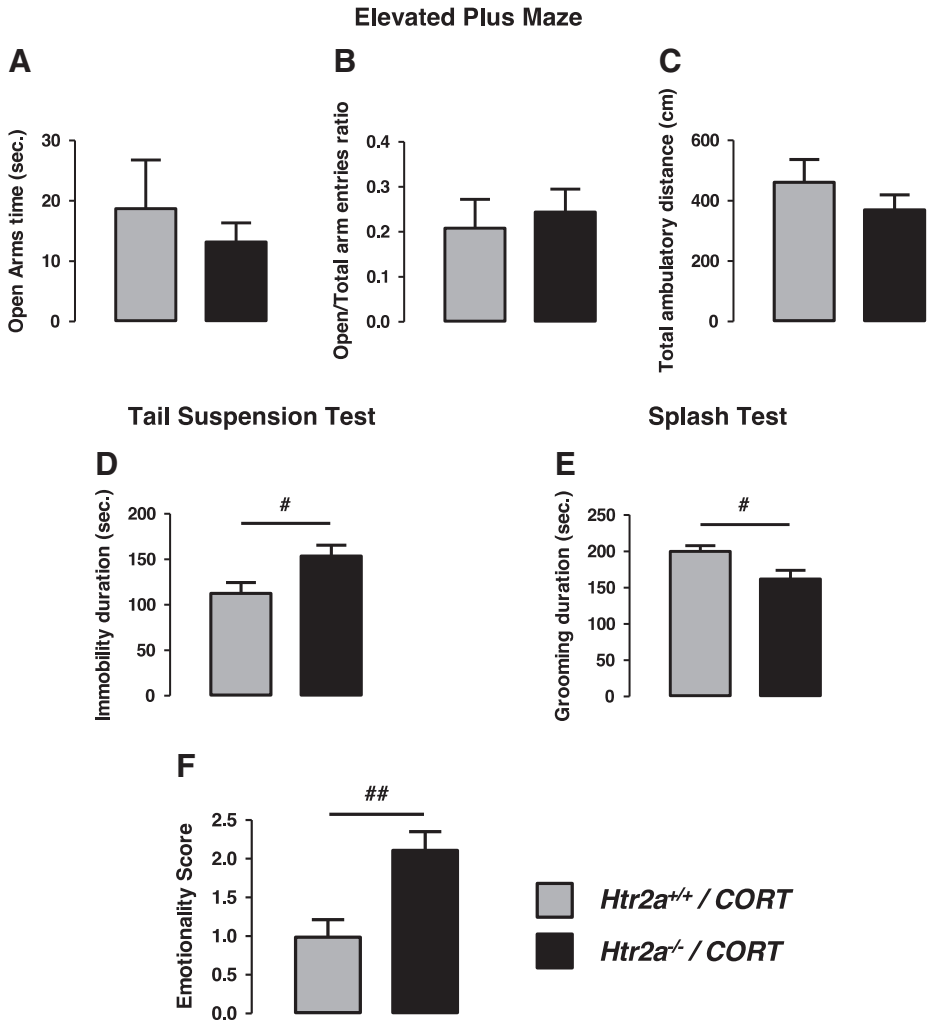

Fig. 1. Effect of the genetic 5-HT_{2A} receptor (5-HT_{2A} R) inactivation on behavioral responses related to anxiety and depression after corticosterone administration in mice. *Htr2a*$^{+/+}$ (n = 12) and *Htr2a*$^{-/-}$ (n = 18) mice were chronically exposed to corticosterone (70 μg/kg/day) in the drinking water for 5 weeks and then assessed in the elevated plus maze test (EPM), tail suspension test (TST) and splash test (ST). (A–C) Anxiety is measured in the EPM as means ± SEM of time spent in the open arms, the ratio of open/total arm entries and the total ambulatory distance (as an index of locomotor activity). (D) Despair is measured in the TST as means ± SEM of the immobility time in seconds. (E) Self-care is measured in the ST as means ± SEM of grooming after receiving a 10% sucrose solution of the coat. *p < 0.05, **p < 0.01 and ***p < 0.001: significantly different from *Htr2a*$^{+/+}$^{C}ORT mice (n = 5). #p < 0.05 and ##p < 0.01: significantly different from *Htr2a*$^{+/+}$^{C}ORT mice administered the vehicle. (F) Integrated emotionality z-scores in *Htr2a*$^{+/+}$^{C}ORT and *Htr2a*$^{-/-}$^{C}ORT mice exposed to chronic corticosterone administration. Data represent mean S.E.M. of "emotionality score". This score was calculated from mice behavioral performances in the TST, ST and EPM [$F(1,32) = 13.17$; $p = 0.0001$)]. *p < 0.05 and ***p < 0.001: significantly different from *Htr2a*$^{+/+}$^{m}ice. ##p < 0.01: significantly different from *Htr2a*$^{+/+}$^{C}ORT mice administered the vehicle.

that it is a rare genotype (*cf.* HapMap results on dbSNP, NCBI). These 2 patients are particularly interesting since Ozaki et al. (1997) suggested that the *T* allele of rs6314 could be associated with a decreased 5-HT_{2A}R-mediated intracellular signaling. Thus, they were analyzed as single cases.

The first patient is a 58-year-old woman with a recurrent MDD, hospitalized for a MDE with melancholic features (DSM-IVTR). With a HAMD-17 score of 36, this patient pertains to the member of the upper quartile of our distribution. Her CGI-S score of 7, which is the maximal score corresponding to an 'extremely ill' patient.

The second patient is a 31-year-old man hospitalized for a current isolated MDE with melancholic features (DSM-IVTR), comprising marked depressed mood, guilt feelings, severe psychomotor retardation, marked weight loss (15 kg in 6 months), and recurrent suicidal ideation. His HAMD-17 score was 33, pertaining to the upper quartile of our distribution. Whereas he had no personal history of MDD, he had a significant familial history since one of his brothers had a MDD and his father committed suicide by drowning in a context of MDD.

Thus, the clinical presentation of these 2 patients argues for a possible association between the rare *TT* variant of rs6314 *HTR2A* polymorphism and severe MDEs.

4. Discussion

This study provides convergent preclinical and clinical genetic arguments in favor of an association between the impairment of brain 5-HT_{2A}R-mediated transmission and both susceptibility and severity of MDE in MDD. Indeed, *Htr2a*$^{-/-}$ mice are more prone than their wild-type littermates to develop anxio-depressive-like phenotype in different paradigms. And depressed patients with allelic variants suspected to decrease the expression/function of the 5-HT_{2A}R, *i.e.* the *C* allele of rs6313 and the rare *TT* variant of rs6314, have an increased severity of MDE.

In mice, the time of immobility in the TST was higher in *Htr2a*$^{-/-}$ CORT than in *Htr2*$^{+/+}$ CORT mice. Because previous works suggest that sustained exposure to corticosterone may reduce the locomotor activity in rodents, this parameter was analyzed. In agreement with recent

Table 1
Socio-demographic and MDD characteristics according to rs6313 TT and CT/CC genotypes (n = 485).

	Total number (n = 485)	TT (n = 83)	CT/CC (n = 402)	p
Socio-demographic characteristics				
Women (%)	344 (68%)	51 (61.5%)	278 (69%)	p = 0.21
Age <60 (nb (%))	414 (85%)	69 (83%)	345 (86%)	p = 0.64
Marital status				p = 0.60
Living alone (nb (%))	261 (54%)	42 (51%)	219 (54.5%)	
Married (nb (%))	224 (46%)	41 (49%)	183 (45.5%)	
Education level				p = 0.57
Low (nb (%))	46 (9.5%)	6 (7%)	40 (10%)	
Medium (nb (%))	222 (46%)	42 (51%)	180 (45%)	
High (nb (%))	216 (44.5%)	35 (42%)	181 (45%)	
Professional status				p = 0.27
Active (nb (%))	309 (64%)	51 (61.5%)	258 (64%)	
Pensioner (nb (%))	65 (13.5%)	14 (17%)	51 (13%)	
Unfit for work (nb (%))	22 (4.5%)	7 (8.5%)	15 (4%)	
Unemployed (nb (%))	39 (8%)	6 (7%)	33 (8%)	
Student (nb (%))	27 (5.5%)	2 (2.5%)	25 (6%)	
Other (nb (%))	22 (4.5%)	3 (3.5%)	19 (5%)	
MDD				
Age at onset (m(sd))	35.2 (14.9)	36.1 (14.6)	35.0 (15.0)	p = 0.54
Nb of previous MDEs (m(sd))	1.8 (2.0)	1.8 (2.1)	1.8 (2.0)	p = 0.93
Current MDE severity				
HAMD-17 (m(sd))	24.8 (5.0)	23.8 (4.5)	25.0 (5.1)	p = 0.03*
CGI (m(sd))	4.9 (0.75)	4.9 (0.7)	4.9 (0.7)	p = 0.91

TT and *CT/CC* groups are similar according to socio-demographic and clinical history. A significant difference is shown between these 2 groups according to the 17-item Hamilton Depression Rating Scale (HAMD-17) score (t = −2.14, df = 483, *p < 0.05), CGI: Clinical Global Impression, MDD: major depressive disorder, MDE: major depressive episode, m: mean value, sd: standard deviation, and df: degrees of freedom.

data obtained by our group in wild type mice (David et al., 2009), no difference in locomotor activity was detected between both genotypes in the open-field (data not shown). These results can therefore be interpreted as an exaggerated despair in *Htr2a*$^{-/-}$ CORT although this behavioral paradigm has been validated to unveil therapeutic activities rather than pathological states. The observation that the time of grooming in the ST was lower in *Htr2a*$^{-/-}$ CORT than in *Htr2*$^{+/+}$ CORT mice unveiled a decreased self-care behavior in mutants. Remarkably, in the EPM, no significant differences were detected between *Htr2a*$^{+/+}$ and *Htr2a*$^{-/-}$ mice exposed to corticosterone. To the best of our knowledge, such behavioral investigations in *Htr2a*$^{-/-}$ mice have never been performed in a mouse model of stress. In a separate study, we compared the behavioral responses of vehicle-treated *Htr2a*$^{+/+}$ and *Htr2a*$^{-/-}$ mice to rule out a putative effect of genotype in basal behavioral conditions. In this independent study (Supplemental Table 1), we did not find any basal modification of despair in *Htr2a*$^{-/-}$ mice in the forced swimming and tail suspension tests. Similar results have already been reported by Weisstaub et al. (2006) in *Htr2a*$^{-/-}$ mice with the same background. To better characterize the global impact of the genetic deletion of 5-HT$_{2A}$R on mice anxio-depressive-like phenotype, we employed the z-score method. This score, developed to integrate various behavioral disturbances as depression scales do in human, can be used as a relevant index of depression severity. In support of this assumption, it has been shown that wild-type mice subjected to UCMS or chronic corticosterone administration treatment display a higher z-score than their respective controls (Guilloux et al., 2011). In the present study, the severity anxio-depressive-like phenotype in response to corticosterone exposure was significantly higher in *Htr2a*$^{-/-}$ than in wild-type controls. This remarkable finding suggests that the inactivation of the 5-HT$_{2A}$R receptor subtype is an important process to potentiate the depressive-like effects of chronic corticosterone administration. Further experiments are required to definitely support this conclusion, notably by evaluating other depressive-like symptoms such as despair in the forced swimming test, anxiety in the novelty suppressed feeding and anhedonia in the sucrose consumption test. Nevertheless, in agreement with our hypothesis, preclinical studies reported that the chronic treatment with corticosterone desensitized the 5-HT$_{2A}$R receptors within the paraventricular nucleus (PVN) of the hypothalamus (Lee et al., 2009) whereas repeated stress decreased their density in the hippocampus (Dwivedi et al., 2005; Schiller et al., 2003). The mechanism by which glucocorticoids might have a repressive role on the 5-HT$_{2A}$R receptor subtype is presently unclear but recent investigations propose that glucocorticoids receptors may act directly as transcription factors at critical site of the *Htr2a* gene promoter (Falkenberg et al., 2011). It should be noted, however, that the apparent ability of glucocorticoids to alter the 5-HT$_{2A}$R receptor function and expression within the PVN or the hippocampus respectively contrasts with the results of several studies demonstrating that in the cerebral cortex, chronically elevated glucocorticoids increase serotonergic 5-HT$_{2A}$ receptor binding (Fernandes et al., 1997). Consequently, glucocorticoids modulate 5-HT$_{2A}$R receptor in a region-dependent manner. Therefore, the present study does not provide a definite insight into the precise mechanism by which the deleterious effects of corticosterone are enhanced in *Htr2a*$^{-/-}$ mice. Alternatively, the genetic inactivation of 5-HT$_{2A}$R receptor might have contributed to enhance plasmatic corticosterone concentrations. However, this assumption is not consistent with animal studies showing that the activation, rather than the inactivation, of 5-HT$_{2A}$R receptor elicits an increase in hypothalamo–pituitary–adrenocortical (HPA) axis activity (Hemrick-Luecke and Evans, 2002; Lee et al., 2009; Rittenhouse et al., 1994; Saphier et al., 1995; Van de Kar et al., 2001). Further studies investigating the reciprocal relationships between the HPA and the 5-HT$_{2A}$R receptor are clearly required to provide a better understanding of how their interaction relates to the development of depression.

In this sample of depressed patients, the over-representation of rs6313 *C* carriers suggests that this allele is associated with MDD. Indeed, this SNP fails to be in HW equilibrium. The classical causes of such a disequilibrium such as experimental mistake, the presence of non-Caucasian patients or consanguinity has been ruled out in this sample. This disequilibrium is probably evidenced because we examined a population of individuals with MDD (Wittke-Thompson et al., 2005) and suggests a selective pressure, which increases the *C* allele frequency in patients with MDD. However, we cannot rule out a genetic drift, due to a sampling effect in a relatively small sample of patients. Moreover,

even if the clinical relevance of the difference shown might be challenged in this sample of MDE patients, a higher severity of MDE observed in *CT*/*CC* patients as compared to *TT* patients further supports the association of 5-HT_{2A}R and MDD. According to Polesskaya et al. (2006), *CC*/*CT* patients may have an impaired 5HTR2A expression, which suggests that the severity of MDE in these patients might result from a decreased expression of the 5-HT_{2A}R. In this sample of depressed patients, 2 patients carrying the rare *TT* genotype (452Tyr/Tyr) of rs6314 had a severe melancholic MDE, one of them occurring in a context of familial history of depression, suggesting a high genetic contribution to the development of MDD. This might be related to the fact that the *TT* genotype has reduced ability to activate G proteins, downstream of 5-HT_{2A}R (Hazelwood and Sanders-Bush, 2004). And the difference evidenced in depression scores is coherent with the marked difference evidenced in *Htr2a*$^{-/-}$ mice. Interestingly, the association of 5-HT_{2A}R and MDD has been mainly reported in severe forms of MDD such as MDE with suicidal attempts (Du et al., 2000; Giegling et al., 2006; Li et al., 2006; Saiz et al., 2008; Vaquero-Lorenzo et al., 2008) or MDD with melancholic features (Akin et al., 2004). Moreover, our results are in line with those showing that the rs6313 *C* allele (Polesskaya and Sokolov, 2002) and the rs6314 *T* allele (Lee et al., 2009) are associated with lower 5-HT_{2A}R mRNA and protein levels. This observation together with our preclinical data in mutant mice supports the idea that a blunted 5-HT_{2A}R-mediated neurotransmission could favor MDD, even if negative results both *in vivo* (Bray et al., 2004) and *in vitro* (Parsons et al., 2004; Spurlock et al., 1998) have been published.

Our clinical results are also in line with those showing a greater 5-HT_{2A}R binding in post-mortem brain tissue (Arranz et al., 1994; Hrdina et al., 1993; Pandey et al., 2002; Shelton et al., 2009; Yates et al., 1990) or in platelets (Hrdina et al., 1995; Hrdina et al., 1997; Sheline et al., 1995) from individuals with MDD, and those evidencing that 5-HT_{2A}R mediated phosphoinositide synthesis was reduced in fibroblasts from patients with melancholic depression compared to controls (Akin et al., 2004).

This study has some limitations. First, the magnitude of the difference on the HAMD score, unless significant and coherent with animal data, is relatively low. Second, we cannot exclude that the severe symptoms in the two patients with the rare variant of rs6314 are a chance finding. Third, the medication status as well as non-drug treatment is uncontrolled in the clinical study, suggesting that differences between genotypes may reflect treatment differences. However, mice did not receive previous treatments, thus arguing against this possible confounding factor. Fourth, it could have been of great interest to assess whether this genotype affects mood/emotionality score in a non-depressed cohort.

This translational study has several strengths. First, the study design of the clinical study with clinical evaluation performed blind of the SNP results limits potential uncontrolled biases. Second, the relevance of this study is due to its ecological conditions. Indeed, an ecological mouse model of depression was used, in which “depressed” animals, *i.e.* animals displaying features of depressive disorders, instead of normal animals, were used based on the CORT model (David et al., 2009; Rainer et al., 2011; Sterner and Kalynchuk, 2010). And the data collected in depressed patients were obtained *in vivo*, instead of using post-mortem or platelet studies. Third, the clinical study has a reasonable sample size, which permits to be confident about the reproducibility of our results. Finally, this translational study involving both constitutive *Htr2a* knock-out mice and related SNPs in depressed patients is particularly relevant since the impact of genetic mutation in both species is present throughout life. This approach allows us to provide converging results suggesting the association between the impairment of 5-HT_{2A}R mediated neurotransmission with higher susceptibility to depression in mice and with higher severity of depression in patients. Further studies are needed in order to replicate our clinical results in an independent cohort, and to assess whether this genotype affects mood/emotionality score in a non-depressed cohort.

5. Conclusion

Thus, this translational and ecological study in mice and depressed patients suggests that a lower neurotransmission at the 5-HT_{2A}R may favor the susceptibility to MDD and the severity of MDD.

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