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ORIGINAL ARTICLE

Association between serotonin transporter genotype, brain structure and adolescent-onset major depressive disorder: a longitudinal prospective study

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The extent to which brain structural abnormalities might serve as neurobiological endophenotypes that mediate the link between the variation in the promoter of the serotonin transporter gene (5-HTTLPR) and depression is currently unknown. We therefore investigated whether variation in hippocampus, amygdala, orbitofrontal cortex (OFC) and anterior cingulate cortex volumes at age 12 years mediated a putative association between 5-HTTLPR genotype and first onset of major depressive disorder (MDD) between age 13–19 years, in a longitudinal study of 174 adolescents (48% males). Increasing copies of S-alleles were found to predict smaller left hippocampal volume, which in turn was associated with increased risk of experiencing a first onset of MDD. Increasing copies of S-alleles also predicted both smaller left and right medial OFC volumes, although neither left nor right medial OFC volumes were prospectively associated with a first episode of MDD during adolescence. The findings therefore suggest that structural abnormalities in the left hippocampus may be present prior to the onset of depression during adolescence and may be partly responsible for an indirect association between 5-HTTLPR genotype and depressive illness. 5-HTTLPR genotype may also impact upon other regions of the brain, such as the OFC, but structural differences in these regions in early adolescence may not necessarily alter the risk for onset of depression during later adolescence.

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INTRODUCTION

Depressive disorders are common and debilitating, have a multifaceted etiology and often emerge during adolescence.[1,2] Recent efforts to understand the underlying biological basis of susceptibility to depression have focused on genetic risk factors.[3,4] However, comprehensive genome-wide association studies have had little success in identifying risk loci, with no replicated findings to date.[5] Increasingly, researchers are returning to more theoretically guided approaches based on biological systems implicated in depression. Such an approach can extend from candidate gene to whole-pathway analyses.[6,7] It is widely accepted that abnormal serotonergic function is implicated in the onset and course of depressive disorders.[8] The serotonin transporter gene (*SLC6A4,* synonyms: 5-HTT, SERT) controls transporter enzyme production and is a key regulator of serotonergic neurotransmission. Furthermore, the effects of genetic variation at this loci have been shown to interact with environmental stressors, such as child maltreatment,[9,10] however, this has not been consistently demonstrated,[11] suggesting a need for further refinement of research methodologies.

Detection of genetic risk could be enhanced by consideration of endophenotypes that occur at an intermediate stage in the causal pathway from a distal gene to the overt expression of disease.[12,13] Brain structure and brain function have been identified as particularly promising endophenotypes for depression, given the findings suggesting they are highly heritable[14,15] and the reported associations between the volume and activity of specific brain regions and the disorder.[16,17] In particular, variation in the volume of brain structures involved in emotional processing and stress responses, including the hippocampus, anterior cingulate cortex (ACC), orbitofrontal cortex (OFC) and amygdala, have been theorized to have a role in mood disorders.[18,19] Specifically, volume reductions in the hippocampi,[20–23] the ACC[24] and the OFC[23,25] have been consistently documented in patients with major depressive disorder (MDD). Smaller hippocampal and ACC volumes have also been linked to poorer clinical outcomes longitudinally.[26–28] Studies of the association between amygdala volume and depression have been somewhat more conflicting, with a recent meta-analysis indicating volume deficits in MDD patients compared to healthy controls,[29] although some earlier meta-analyses have indicated no structural difference between these groups.[23,30] These brain regions are also densely innervated by serotonergic neurons originating primarily in the dorsal and median raphe nuclei.[31] Emerging evidence from imaging genetics studies of mood disorders suggests that variations in serotonergic neurotransmission, due in part to 5-HTTLPR genotype, may be associated with variations in these brain structures, although current findings present a somewhat inconsistent picture.[18] Findings on the hippocampus have been equivocal, with the majority of studies failing to identify differences in hippocampal

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volumes associated with the 5-HTTLPR genotype in healthy individuals (for example, Eker *et al*.,[32] Taylor *et al*.,[33] Frodl *et al*.[34,35]). However, one study with a large sample has reported that individuals homozygous for the S-allele had significantly smaller left hippocampal volumes than those homozygous for the L-allele.[36] With regard to MDD, there have been reports of smaller,[32] larger[34–36] and equivalent volumes[33] in S-allele carriers compared to their L-allele homozygous counterparts.

There have been more consistent reports of smaller ACC structures in psychiatrically healthy S-allele carriers compared to L-homozygous individuals.[36–38] No apparent genotypic effects have been observed in individuals currently experiencing MDD; however, MDD patients homozygous for the L allele have been found to have reduced ACC volumes compared to psychiatrically healthy controls with the same genotype.[36]

Furthermore, there is some evidence suggesting decreased amygdala volumes (as well as reduced functional connectivity between the amydgala and the perigenual ACC) in S-allele carriers.[37,39] However, opposite[40] and null[38] findings have also been documented, albeit in smaller samples. Evidence of an impact of 5-HTTLPR on OFC volumes in humans is currently limited, with only one study to date showing S-allele-associated volume deficits in the left OFC, in psychiatrically healthy individuals.[38]

A key unresolved issue is the extent to which these brain structural abnormalities might serve as endophenotypes that mediate the putative link between 5-HTTLPR and depression. In general, a given variable may be regarded as a mediator to the extent that it accounts for the relationship between the predictor and the outcome. Because endophenotypes occur at an intermediate stage in the causal pathway from a distal gene to overt expression of disease, a mediation model is often assumed (for example, Waldman,[41] Munafò,[42] and Hyde *et al*.[43]) but has rarely been tested explicitly within the field of imaging genetics (see Nikolova *et al*.[44] for a notable exception). To our knowledge, there are no imaging genetic studies of this nature that have examined depression as an outcome. Studies so far have rather remained siloed, investigating either gene–brain structure *or* brain structure–depression relationships, and have not systematically tested mediation relationships within the same sample. There are also a limited number of longitudinal studies that have been able to examine whether neuroanatomic abnormalities are prospectively associated with later occurrence of the disorder (for example, Rao *et al*.[45]).

Thus, the purpose of the current study was to examine whether 5-HTTLPR genotypes predict variations in brain volumes in early adolescence, and whether these variations in turn prospectively predicted an onset of MDD in a 6-year follow-up period. We directly tested the hypotheses that (i) S-allele carriers would demonstrate reduced volumes of the hippocampus, ACC, amygdala and OFC, (ii) that smaller volumes of each of these structures would be prospectively associated with MDD onset, and, critically, (iii) that variation in brain structure would statistically mediate the association between 5-HTTLPR genotype and MDD onset.

MATERIALS AND METHODS

Participants and procedures

The current analyses are based on a subsample of 174 participants (71% of the total sample, 83 male) from the longitudinal Orygen Adolescent Development Study (ADS), conducted in Melbourne, Australia, who had provided a genetic sample during the course of their participation. The recruitment and screening of ADS participants has been reported previously.[46] These analyses draw on all four waves of ADS data collection: wave 1 (W1; *M* age 12.7 years, range 11.4–13.7 years) included a structural magnetic resonance scan and a diagnostic interview that assessed for current and lifetime mood disorders to exclude participants with a history of an episode of major depression. The diagnostic interview was repeated at waves 2, 3 and 4 (W2–W4), which were conducted ~2.5, 4 and 6 years after W1, respectively. The W2–W4 diagnostic interviews assessed for current MDD and any new episodes since the date of the last assessment.

Measures

MDD onset. MDD was measured at each of the four study waves by the Kiddie-Schedule for Affective Disorders and Schizophrenia for School-Age Children, Present and Lifetime version (K-SADS-PL), [47] a semistructured diagnostic interview that assesses current and lifetime symptoms and diagnoses of Axis I disorders in youths aged 6–18 years. Diagnostic interview data from each of the time points were used to construct a variable indicating whether participants had experienced their first occurrence of an episode of MDD between the W1 and W4 time points. Owing to attrition, this variable was able to be calculated for 138 of the 174 participants in the current study, and there were no differences between these participants and the 37 participants with missing data according to gender, $\chi^2(1) = 0.25$, $P > 0.05$, socio-economic status, $t[172] = -0.99$, $P > 0.05$, and W1 depression symptoms (as measured by the Centre for Epidemiological Symptoms – Depression scale), $t[160] = 0.77$, $P > 0.05$. A total of 36 participants had experienced their first onset of MDD between W1 and W4. Of these participants, 30 met criteria for one (or more) other lifetime psychiatric disorders compared to 34 of the 101 participants who did not experience an onset of MDD during adolescence (Supplementary Table 1).

Neuroimaging

One-hundred and twenty-five participants of the current sample completed a structural magnetic resonance imaging (MRI) scan at W1, using a 3-Tesla GE scanner. Details regarding image acquisition, image preprocessing and tracing protocols for morphometric analysis can be found in Supplementary Information. Briefly, the guidelines for tracing the amygdala and hippocampus were adapted from those described by Velakoulis *et al*.[48,49] Watson *et al*.'s protocol[50] was used to separate the amygdala from the hippocampus (see Supplementary Figure 1). The boundaries of the OFC were based on a previously published method by Riffkin *et al*.[51] In accordance with Bartholomeusz *et al*.,[52] medial and lateral OFC regions were separated with the medial orbital sulcus[53] (see Supplementary Figure 2). The boundaries of the ACC were based on a previously published method,[54] which defines separate limbic and paralimbic regions according to individual differences in the morphology of the cingulate, paracingulate and superior rostral sulci (see Supplementary Figure 3).

Interrater and intrarater reliabilities were assessed by means of the intraclass correlation coefficient (absolute agreement) using 10 brain images from a separate MRI database established for this purpose. Intraclass correlation coefficient values were deemed acceptable for all ROIs (29 of the 36 ROIs were < 0.90 and none < 0.75), as shown in Supplementary Table 1. All brain structural measures were corrected for whole-brain size separately by gender by means of a covariance adjustment method[55] and converted from mm^3 to cm^3.

Genotyping

Saliva was collected from participants for genetic analysis using an ORAGENE saliva pot (www.dnagenotek.com). The methods used for PCR amplification and visualization by gel electrophoresis were as described by Edenberg and Reynolds.[56] The genotype distribution for 5-HTTLPR (LL: $n = 54$, SL: $n = 83$, SS: $n = 37$) was in Hardy–Weinberg equilibrium ($\chi^2(1, N = 174) = 0.24$, NS).

Statistical analysis

We used path analysis to test a multiple mediator model, with serotonin transporter genotype as an ordinal independent variable (IV), the left and right structures of a specific brain region of interest (corrected for whole brain volume) as continuous mediators, and MDD onset as the binary dependent variable (DV). Alterations in the normal asymmetry of brain regions, particularly limbic structures such as the hippocampus, have been implicated in depression, generally evidenced by greater reductions in the left, compared to the right, structure (for example, Mervaala *et al*.[57] and Bremner *et al*.[58]). Research, however, has tended to examine left and right structures separately, making it difficult to know whether asymmetrical changes have occurred, or whether there are bilateral changes that

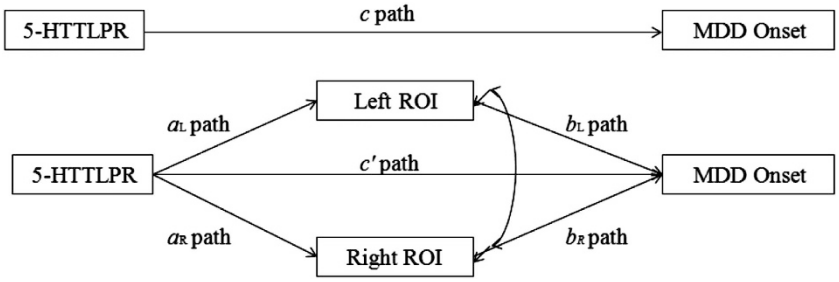

Figure 1. Hypothesized model outlining the tests for meditational effects. Path *a* (L or R) is the effect of 5-HTTLPR on the volume of a particular (left or right) brain region of interest (ROI), path *b* (L or R) is the effect of the volume of a particular (left or right) ROI on major depressive disorder (MDD) onset, path *c* is the total effect of 5-HTTLPR on MDD onset (that is, not controlling for left and right region of interest (ROI) volume), and path *c* is the direct effect of 5-HTTLPR on MDD onset (that is, controlling for left and right ROI volume).

Table 1. Means and standard deviations (s.d.) of regional brain volumes (before correction for whole brain volume) in cm^3

	Full sample (N = *125*)		*MDD onset status*				*Serotonin transporter genotype*					
	M	*s.d.*	*MDD onset* (n = *26*)		*No MDD onset* (n = *73*)		*SS* (n = *29*)		*SL* (n = *59*)		*LL* (n = *37*)	
			M	*s.d.*	M	*s.d.*	M	*s.d.*	M	*s.d.*	M	*s.d.*
Left hippocampus	2.77	0.33	2.70	0.35	2.77	0.33	2.65	0.25	2.80	0.35	2.81	0.35
Right hippocampus	2.95	0.34	2.94	0.35	2.91	0.33	2.88	0.28	2.96	0.36	2.99	0.35
Left amygdala	1.89	0.26	1.86	0.25	1.89	0.25	1.89	0.23	1.89	0.28	1.88	0.26
Right amygdala	1.83	0.28	1.75	0.24	1.85	0.29	1.85	0.27	1.85	0.26	1.80	0.31
Left medial OFC	7.55	1.80	7.13	1.47	7.62	2.00	6.96	1.78	7.58	1.60	7.94	2.05
Right medial OFC	7.19	1.71	6.80	1.27	7.27	1.87	6.62	1.92	7.16	1.54	7.66	1.70
Left lateral OFC	12.41	3.04	11.81	3.31	12.63	3.06	12.00	3.83	12.70	2.46	12.26	3.25
Right lateral OFC	13.33	2.75	13.00	2.63	13.50	2.92	13.11	3.07	13.57	2.33	13.11	3.16
Left limbic ACC	4.98	1.68	5.44	1.38	4.77	1.68	4.55	1.55	5.02	1.67	5.27	1.79
Right limbic ACC	5.77	1.91	5.51	1.99	5.99	1.87	5.98	1.79	5.60	2.01	5.88	1.88
Left paralimbic ACC	5.33	1.99	4.73	1.72	5.47	2.14	5.57	2.23	5.22	2.01	5.33	1.77
Right paralimbic ACC	4.79	1.80	4.79	1.55	4.67	1.89	4.67	2.02	4.91	1.82	4.67	1.63

Abbreviations: ACC, anterior cingulate cortex; MDD, major depressive disorder; OFC, orbitofrontal cortex.

happened to be significant for only one side. While an investigation of the presence of asymmetry was not a focus of the current study, we included left and right structures of a specific region of interest in the same path model to better understand the relative contribution of each structure to the risk for depression. Acceptable tolerance (>0.2) and variation inflation factor (<5) values indicated no significant multicolinearity between the left and right structures for any of the regions of interest. Separate mediation analyses were conducted for the hippocampus, the amygdala, the medial OFC, the lateral OFC, and the limbic and paralimbic ACC. Path models were estimated in *Mplus*[59] using weighted least squares with a mean- and variance-adjusted chi-square test statistic (WLSMV). Fit statistics are not reported as the models of interest were just identified.

A hypothesized model outlining the tests for mediational effects is presented in Figure 1. When both the relationship between the IV and the mediator (the *a* path) and the relationship between the mediator and the DV controlling for the IV (the *b* path) were significant, mediation was tested by assessing the significance of the cross product of the coefficients for these two paths (that is, the *ab* cross product). The product of coefficients method has been shown to yield more accurate results compared to other methods when the DV is binary,[60] and also allowed us to test for significant mediation in the absence of a direct effect of the IV on the DV.

The current analyses were based on 5000 bootstrapped samples and bias-corrected bootstrapped parameter estimates were used to test the significance level of the indirect effects, according to current recommendations for determining mediation.[61–64] If the 95% and 90% confidence intervals for these estimates of an indirect effect do not contain 0, it can be concluded that the indirect effect is statistically significant at the 0.05 and 0.10 level, respectively.[65] As both the left and riight structures of a specific brain region were included in the model, two specific indirect effects (a_Lb_L and a_Rb_R) were investigated. Given that the left and right volumes of a particular brain region would be expected to be related, their residuals were covaried in the model. Additional mediational analyses that included the covariates of adolescent gender, ethnicity, full-scale IQ and age at time of the MRI scan were conducted, but did not alter the pattern of results and hence are not reported.

Listwise deletion because of missing data would have resulted in only 98 cases remaining in the analysis due to non-participation in either the MRI at wave 1 or the psychiatric interview at waves 2, 3 or 4. Little's MCAR test[66] was non-significant, $\chi^2(163) = 179.54$, $P = 0.178$. We therefore used pairwise deletion (the default when using the WLSMV estimator in *Mplus*) to account for missing data. Pairwise deletion has been shown to be unbiased when data are missing completely at random.[67]

RESULTS

Table 1 presents mean brain volumes for each brain region considered in the current analyses before correction for whole brain volume.

For all analyses, the total effect of 5-HTTLPR on MDD onset (path *c*, that is, not controlling for ROI volumes) was non-significant (95% CI: −0.49 to 0.14, $\beta = -0.18$, s.e. = 0.16, $P > 0.05$). Each of the direct associations between 5-HTTLPR and MDD onset (path *c*′, that is, controlling for the relevant ROI volumes), 5-HTTLPR and the ROI volumes (path *a*), as well as between the ROI volumes and MDD onset (path *b*), can be seen in Table 2. In all path models, the direct effect of 5-HTTLPR on MDD onset (path *c*′) was non-significant.

Table 2. Path model of the effects of 5-HTTLPR genotype and brain ROIs on MDD onset

	b	*s.e.*	β	P
Hippocampus				
5-HTTLPR → MDD onset (path *c'*)	−0.21	0.17	−0.15	0.22
5-HTTLPR → left hippocampus (path *a*)	−0.08	0.03	−0.18	0.03
Left hippocampus → MDD onset (path *b*)	−1.79	0.79	−0.53	0.02
5-HTTLPR → right hippocampus (path *a*)	−0.05	0.04	−0.12	0.16
Right hippocampus → MDD onset (path *b*)	2.10	0.72	0.63	0.004
Amygdala				
5-HTTLPR → MDD onset (path *c'*)	−0.17	0.16	−0.12	0.30
5-HTTLPR → left amygdala (path *a*)	0.01	0.02	0.04	0.66
Left amygdala → MDD onset (path *b*)	1.44	1.08	0.31	0.18
5-HTTLPR → right amygdala (path *a*)	0.02	0.03	0.07	0.42
Right amygdala → MDD onset (path *b*)	−1.23	0.88	−0.29	0.16
Medial OFC				
5-HTTLPR → MDD onset (path *c'*)	−0.21	0.17	−0.15	0.23
5-HTTLPR → left medial OFC (path *a*)	−0.46	0.21	−0.21	0.03
Left medial OFC → MDD onset (path *b*)	−0.02	0.15	−0.04	0.88
5-HTTLPR → right medial OFC (path *a*)	−0.51	0.19	−0.25	0.006
Right medial OFC → MDD onset (path *b*)	−0.04	0.16	−0.05	0.83
Lateral OFC				
5-HTTLPR → MDD onset (path *c'*)	−0.19	0.16	−0.13	0.25
5-HTTLPR → left lateral OFC (path *a*)	−0.10	0.37	−0.03	0.79
Left lateral OFC → MDD onset (path *b*)	−0.06	0.11	−0.15	0.59
5-HTTLPR → right lateral OFC (path *a*)	−0.01	0.32	−0.003	0.98
Right lateral OFC → MDD onset (path *b*)	0.06	0.12	0.14	0.61
Limbic ACC				
5-HTTLPR → MDD onset (path *c'*)	−0.12	0.17	−0.08	0.49
5-HTTLPR → left limbic ACC (path *a*)	−0.39	0.20	−0.17	0.06
Left limbic ACC → MDD onset (path *b*)	0.16	0.08	0.27	0.04
5-HTTLPR → right limbic ACC (path *a*)	−0.33	0.22	0.01	0.87
Right limbic ACC → MDD onset (path *b*)	−0.02	0.08	−0.04	0.79
Paralimbic ACC				
5-HTTLPR → MDD onset (path *c'*)	−0.17	0.16	−0.12	0.29
5-HTTLPR → left paralimbic ACC (path *a*)	0.13	0.23	0.05	0.58
Left paralimbic ACC → MDD onset (path *b*)	−0.09	0.08	−0.16	0.25
5-HTTLPR → right paralimbic ACC (path *a*)	0.05	0.22	0.02	0.82
Right paralimbic ACC → MDD onset (path *b*)	0.09	0.08	0.15	0.28

Abbreviations: ACC, anterior cingulate cortex; MDD, major depressive disorder; ROI, region of interest.

Increasing copies of the S-allele predicted smaller left hippocampal volume (path a_L). Smaller left hippocampal volumes also predicted increased risk for MDD onset (path b_L). Bias-corrected 95% confidence intervals showed that smaller left hippocampal volume significantly mediated the relationship between S-allele copies and risk for MDD onset (indirect effect = 0.14, 95% CI = 0.009–0.42, s.e. = 0.10).

The association between S-allele copies and right hippocampal volume (path a_R) was not significant; however, larger right hippocampal volumes were predictive of increased risk for depression (path b_R).

Increasing copies of the S-allele of 5-HTTLPR predicted both smaller left and right medial OFC volumes (paths a_L and a_R); however, the associations between left medial OFC volume and MDD onset (path b_L) and between right medial OFC volume and MDD onset (path b_L) were non-significant; therefore mediation analyses were not conducted.

There was a trend ($P < 0.10$) towards increasing copies of the S-allele predicting smaller left limbic ACC volume, and a significant relationship ($P < 0.05$) between smaller left limbic ACC volume and decreased risk for MDD onset. Bias-corrected 90% confidence intervals indicated that left limbic ACC volume mediated the relationship between serotonin transporter genotype and risk for MDD onset (indirect effect = −0.06, 90% CI: −0.17 to −0.01, s.e. = 0.05), which is statistically significant at the 0.10 level. There were no significant findings relating to the right limbic ACC.

Given these results, further analyses were conducted on rostral, dorsal and ventral regions of the limbic ACC, which indicated that the finding obtained for the left limbic ACC was localized to the rostral region, such that a greater number of S-alleles was associated with smaller volumes of the left rostral limbic ACC, and that, in turn, smaller rostral limbic ACC volumes were associated with decreased risk for depression onset at trend level. The indirect pathway was also significant at trend level according to bias-corrected confidence intervals (indirect effect = −0.06, 90% CI: −0.17 to −0.003, s.e. = 0.05), suggesting possible mediation of the relationship between serotonin transporter genotype and risk for MDD onset by rostral limbic ACC volume. There were no significant findings relating to the right rostral limbic ACC. 5-HTTLPR did not predict left or right dorsal or ventral limbic ACC volumes, nor were these volumes related to risk for MDD onset. Mediation analyses for these regions were therefore not conducted.

5-HTTLPR did not predict left or right amygdala volume, left or right lateral OFC volumes, and left or right paralimbic ACC volume,

nor were these volumes related to risk for MDD onset. Mediation analyses were therefore not conducted for these ROIs.

Scatter plots of significant gene–ROI and ROI–MDD onset associations are provided in Supplementary Figures 4.

DISCUSSION

The aim of the current study was to investigate whether the volume of the hippocampus, ACC, amygdala and OFC mediated an association between variation in the serotonin transporter gene and a first onset of MDD in a large sample of adolescents using a longitudinal, prospective design. The findings are summarized in Figure 2. Our results support the role of left hippocampal volume deficits in early adolescence as salient mediators of the link between serotonin transporter genotype and increased risk for MDD onset in later adolescence. Specifically, we found that an increasing number of S-allele copies were associated with smaller left hippocampal volume, and smaller left hippocampal volume was in turn associated with increased risk of experiencing a first onset of MDD. Right hippocampal volume did not significantly mediate the pathway from 5-HTTLPR genotype to MDD onset, although larger right hippocampal volume did predict an increased risk of a depressive episode.

These results provide evidence that neurobiological factors may partly underlie the link between serotonin transporter genotype and depression. Furthermore, our finding that the S-allele predicted smaller left hippocampal volumes in early adolescence prior to illness onset is consistent with previous findings of a volume deficit in these structures in S-allele carriers.[32,33,36] Our finding that volume reductions in the hippocampus are associated with depression onset, but also predate its occurrence, also concords with suggestions that hippocampal volume deficits are one of the most consistently observed structural aberrations in depression,[19–23] and that this anomaly may represent a vulnerability factor that is present prior to emergence of mood disorder.[45,68]

The hippocampal region has been found to have moderate concentrations of the serotonin transporter.[69] An *in vivo* positron emission tomography study has revealed a strong leftward asymmetry in serotonin transporter distribution in the hippocampus,[70] suggesting greater expression of the serotonin transporter gene in the left hippocampal structure. Higher concentrations of serotonin transporters in the left compared to the right hemisphere may explain why serotonin transporter genotype was predictive of left hippocampal volume only in the current study. The hippocampus is known to be involved in the regulation of the stress response, specifically in the inhibition of the hypothalamic–pituitary–adrenal (HPA) axis.[71–73] Smaller hippocampal volumes associated with S-carrier status may affect negative feedback inhibition of the HPA axis, which could result in HPA hyperactivity. Alternatively, the S-allele may be associated with greater stress responsivity in the form of higher basal cortisol or a greater cortisol response,[74] which may have neurotoxic, atrophying effects on the hippocampus,[75] in turn increasing the risk for depression.

The finding that left and right volumes have opposite effects on the onset of MDD may initially seem inconsistent with previous studies that have found bilateral reductions in hippocampal volume that were predictive of depression. As far as we are aware, however, our study is unique in having considered the relative contribution of the left and right hippocampi to depression (that is, controlling for hippocampal volume in one hemisphere while assessing the effect of the volume in the other hemisphere). This renders it difficult to directly compare our findings with those of previous studies, which have focused on absolute volume in each hemisphere. It may still be worth noting that a number of these studies documented substantially greater left hippocampal volume reductions compared to the right in depression,[57,58] including child- or adolescent-onset depression,[76,77] raising the possibility that the presence of asymmetry in this region may have a role in the disorder. The implication of the finding of a difference in the directionality of the relationship between the left and right hippocampal volume with depression onset is unclear but is intriguing given suggestions that asymmetries in the limbic system, including the hippocampus, are associated with hemisphere asymmetries,[78] and there are suggestions that the right hemisphere may be more dominant in processing of negative emotions while the left hemisphere may be more dominant in processing of positive emotions.[79,80] It is not implausible that changes to asymmetry may have consequences for emotional processing that alters the risk for depression.

Possession of a greater number of S-allele copies also predicted both smaller left and right medial OFC volumes, although neither medial nor lateral OFC volumes (whether on the left or on the right) were prospectively associated with a MDD during adolescence. The finding that serotonin transporter genotype was associated with variation in medial but not lateral OFC volumes is consistent with the fact that the medial region of the OFC shows strong connections to limbic structures involved in emotion processing and reward, such as the amygdala, dorsolateral prefrontal cortex and ACC.[81,82] One factor that may be relevant to the lack of a prospective relationship between OFC volume and onset of depression is the time at which OFC volumes were measured. The OFC, which is thought to have an important role in inhibitory control and reward-based decision-making,[83] undergoes significant remodelling throughout adolescence and early

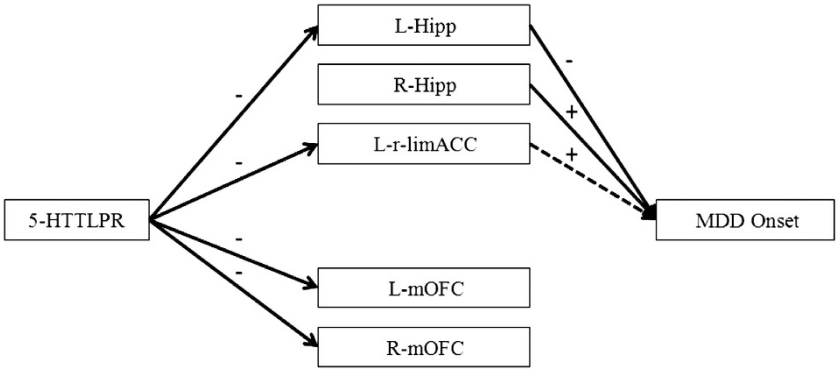

Figure 2. Summary of significant findings. A greater S-allele load was found to predict smaller left hippocampal volume, smaller left rostral limbic anterior cingulate cortex (ACC) volume, and smaller left and right medial orbitofrontal cortex (OFC) volumes. Smaller left but larger right hippocampal volumes predicted an increased probability of major depressive disorder (MDD) onset. There was a trend for smaller left rostral ACC volume to be associated with a decreased probability of MDD onset.

adulthood,[84] and it has been suggested that abnormalities in the maturation in this region may contribute to the etiology of depression.[85] Given that the OFC has not yet fully developed at 11–13 years old, it is possible that differences in OFC volume across adolescence may be more predictive of depression at a later age.

There was also evidence that an increasing number of S-allele copies predicted smaller left (but not right) rostral limbic ACC volume, a finding that accords with the results of previous investigations of this particular gene–brain linkage.[37,38] Somewhat surprisingly, there was a trend for smaller left (but not right) rostral limbic ACC volume to be associated with decreased risk of depression onset during adolescence (or, alternatively, that larger left rostral limbic ACC volumes were associated with increased risk for depression onset), and the mediating pathway from the 5-HTTLPR genotype to the left rostral limbic ACC volume to depression onset was also significant at the trend level. The presence of an association between larger rostral limbic ACC volume and depression onset in the current study is somewhat inconsistent with past research, which has generally suggested that volume deficits are associated with depression.[24] It is important to note, however, that evidence supporting the presence of smaller ACC volumes prior to illness onset comes exclusively from a few studies that have examined brain structure in high-risk samples, which are defined by the presence of a family history of depressive disorder (for example, Boes *et al*.).[86]

The lack of evidence supporting amygdala volume as an intermediate phenotype between serotonin transporter gene and depression onset is perhaps somewhat unsurprising, given the heterogeneous findings regarding the association between 5-HTTLPR and amygdala structure,[37–40] and between amygdala structure and depression.[23,30] These null findings may reflect a need to take additional mediating or moderating factors, such as psychosocial risks (for example, stressful life events, trauma, family environment and peer relationships), into account. Our research group has previously found that amygdala volume and parenting interact to predict depressive symptoms.[87] The structure of the amygdala is thought to be highly plastic to environmental changes and behavioral manipulations,[88–90] and there is also indication that alterations in amygdala volume may occur during the course of depression,[19,30,91] raising the possibility that structural differences in this region could represent the epiphenomena of, or consequential change associated with, the disorder rather than a premorbid vulnerability factor.

A number of study limitations must be acknowledged. First, examining brain structure in an adolescent sample at only one time point renders it impossible to determine whether these findings reflect stable differences present prior to illness onset or abnormal developmental changes that emerge during early adolescence. Second, the current investigation also did not take into account the contribution of environmental factors, such as stressful life events, trauma, parenting and peer relationships to these associations. Hippocampal volume has been found to be affected by environments that are regarded as often having an etiological role in the development of depression, including early life adversity, such as abuse or neglect,[92,93] as well as more normative caregiving experiences.[94] Both increased depression risk [95] and hippocampus diminishments [96,97] have been documented in S-carriers who have experienced severe childhood adversity. Future studies may wish to consider how potential mediating paths such as those documented here might be moderated by these relevant developmental risk or protective factors. A third point for consideration is the higher rates of other lifetime psychiatric conditions in the group of participants who experienced an onset of MDD compared with participants who did not. Although comorbidity with depression is extremely common (for example, Merikangas *et al*.,[2] and Rohde *et al*.[98]) it limits our ability to attribute the observed relationships to depression specifically as opposed to the presence of psychopathology more generally. Finally, it should be noted that, although these results would not survive Bonferroni adjustment, the magnitude of the difference in left hippocampus volume between individuals who experienced an onset of depression and those who did not is comparable to that found by a meta-analysis examining hippocampal atrophy in first episode depression patients.[22] Given the large effect sizes required to survive the loss of power associated with such a conservative test as the Bonferroni adjusted significance test[99,100] and that the effects of individual genes on the risk for psychiatric disorder tend to be small,[101] we would contend that uncorrected results retain valuable information that would otherwise potentially be lost to Type 2 error.

In summary, despite much supposition about the extent to which brain structures involved in the stress response and emotion regulation might serve as intermediate phenotypes in the pathway from the serotonin transporter gene to depression, for example, Savitz and Drevets,[17] and Schaninger *et al*.[18]), these indirect relationships had not been formally assessed prior to the present study. Our results provide evidence that during early adolescence structural abnormalities in the left hippocampus and, potentially, the left rostral limbic ACC may exist prior to onset of depression and may be partly responsible for the link between 5-HTTLPR genotype and depressive illness.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on the Translational Psychiatry website (http://www.nature.com/tp)