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Association of Serotonin Transporter Gene Polymorphisms with Post-Stroke Depression

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Abstract

Context—Polymorphisms of the Serotonin Transporter (SERT) have been associated with mental illness. In people with chronic medical conditions, variants of the 5-HTTLPR and STin2 VNTR polymorphisms of SERT have been shown to confer a heightened vulnerability to comorbid depression.

Objective—To determine whether the 5-HTTLPR, STin2 VNTR, and rs25531 polymorphisms of SERT are associated with post-stroke depression (PSD) in stroke survivors.

Design—In this case-control study, stroke survivors were screened for depressive symptoms and assigned to either a group of depressed or a group of non-depressed participants.

Setting—Outpatient clinic.

Participants—75 stroke survivors with PSD and 75 non-depressed stroke survivors.

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Interventions—Blood or saliva samples were collected from each participant for DNA extraction and genotyping.

Main outcome measures—The 5-HTTLPR, STin2 VNTR, and rs25531 polymorphisms were genotyped for each subject.

Results—Subjects with the 5-HTTLPR s/s genotype had three-fold higher odds for PSD compared to l/l or l/×l genotype carriers (OR 3.1, 95%CI 1.2–8.3). Subjects with the STin2 9/12 or 12/12 genotype had four-fold higher odds for PSD compared to STin2 10/10 genotype carriers (OR 4.1 95%CI 1.2–13.6). An association of rs25531 with PSD could not be shown.

Conclusion—The 5-HTTLPR and STin2 VNTR, but not the rs25531, polymorphisms of SERT are associated with PSD in stroke survivors. This gives further evidence for a role of SERT polymorphisms in mediating resilience to biopsychosocial stress.

Introduction

Approximately 33% of patients suffer from major depression following stroke [1]. The etiology of PSD is thought to be multifactorial, involving psychosocial as well as biological mechanisms [2]. Premorbid history of depression and high levels of disability increase the risk for depression among stroke survivors, yet these factors are poor predictors of who will become depressed following stroke and who will not [3].

The goal of this study was to investigate the role of polymorphisms of the serotonin transporter (SERT) in the etiology of PSD. The SERT protein is localized on the presynaptic membrane of serotonergic neurons, where it controls the intensity and duration of serotonergic signaling through re-uptake of the neurotransmitter into the synapse [4]. Since SERT is the target of selective serotonin reuptake inhibitors, variations in the SERT gene have been widely studied as possible risk factors for psychiatric illness.

The SERT gene is located on chromosome 17q11.1-17q12 and organized into 14 exons spanning approximately 31kb [5;6]. Its most frequently studied variant, 5-HTTLPR, located in the promoter region, is subdivided into a short (s) and long (l) allele, based on the presence or absence of a 43bp insertion/deletion polymorphism [7;8]. The short (s) variant has been associated with depression in response to stressful life events [9;10] and with PSD in a small sample [11]. rs25531 is a single nucleotide polymorphism (SNP), present in either a common (A) or rare (G) variant, which is located immediately upstream of 5-HTTLPR in the SERT gene [8]. rs25531 was included in this study despite its low minor allele frequency because it is a functional polymorphism in which the rare allele (G) lowers SERT transcription. rs25531 and 5-HTTLPR have been shown to influence jointly SERT expression levels, resulting in high (l-A), intermediate (l-G), and low-expressing (s-G) genotypes [12]. A third SERT polymorphism, STin2 VNTR, is located in intron 2 and consists of a variable number (usually 9, 10, or 12) of nearly identical 17bp segments, with the 9-repeat allele (STin2.9) conferring increased odds of major depression and bipolar disorder in one study [13]. The 12-repeat allele (STin2.12) has been associated with bipolar affective disorder and schizophrenia [14–16]. Both the 9-repeat and the 12-repeat allele have been shown to enhance transcription of SERT compared to STin2.10 [17;18]. In people with chronic medical conditions, the 5-HTTLPR s/s and STin2 9/12 genotypes were more frequent among subjects with comorbid depression [19]. Our goal was to determine whether the odds for PSD are similarly heightened in carriers of alleles previously associated with mental illness: the 5-HTTLPR s allele, and the STin2.9 and STin2.12 alleles.

Methods

Subjects

The case-control study presented here is a supplement to a larger parent study, the randomized clinical trial "Living Well with Stroke" which investigates the role of behavioral intervention in PSD. Subjects for the parent study were recruited from among 287 ischemic stroke survivors discharged from acute care hospitals in the Puget Sound region of Washington State who were within four months of an ischemic stroke and had signed informed consent to be screened for PSD. Upon intake, participants were screened for the presence of depressive symptoms with the 30-item Geriatric Depression Scale (GDS) and classified as suffering from PSD or not depending on whether they scored above (≥11) or below (<11) the depression cut-off level on the GDS. This cutoff point of GDS ≥ 11 has previously been validated with a sensitivity of 96% and specificity of 69% for major depression, using DSM IV criteria [20]. Screened patients with GDS ≥11 were invited to enroll in the parent study of a brief psychosocial intervention for depression. Patients with hemorrhagic stroke, receptive or global aphasia, reduced level of consciousness (Glasgow Coma Scale <15), inability to understand and follow directions, or psychosis were excluded from recruitment. Enrollment in the parent study began in November 2002 and ended in April 2007. Enrollment in this supplemental genetic study occurred between January and October 2006. Eligible stroke survivors who consented to be screened for the parent study were invited to co-enroll in the genetic study from January 2006. Simultaneously, all eligible subjects previously screened for the parent study were contacted and invited to enroll in the genetic study. Subjects consented separately for the supplemental genetic study and provided blood or saliva samples for analysis of SERT genetic polymorphisms. This process of inviting enrollment from newly and previously screened participants in the parent study continued until the predetermined group size of 75 subjects with depressive symptoms and 75 non-depressed individuals was reached (September 27, 2006). All study procedures were reviewed and approved by the University of Washington IRB committee.

Race and/or ethnicity were assessed in this study, because the distribution of genetic polymorphisms and their associations with medical illness or treatment response have been shown to differ among population groups. Study participants classified themselves as belonging to one or more of the race/ethnicity options defined by the investigators. Subject disability was assessed by the National Institutes of Health stroke scale (NIHSS) [21].

Sixty-two of the 75 subjects with GDS ≥ 11 in the genetic association study presented here chose to enter the behavioral intervention trial. In these subjects, a DSM-IV diagnosis of major depression (n=58) or a non-DSM-IV diagnosis of minor depression (n=4) was established by a structured diagnostic interview, the Depression Interview and Structured Hamilton (DISH) [22]. Among these 62 subjects the majority had a history of major depression by self-report (n=46, 74%), with the reported number of episodes ranging from one to eight with a median of two. The remaining 13 subjects with GDS ≥ 11 consented to genetic testing only, but declined to be part of the intervention trial. As the DISH and psychiatric history were obtained only as part of the intervention trial, a more detailed depression history was not obtained in these subjects, and neither in the control group (n=75).

Sample collection and genotyping

From participants who were mobile a sample of 10 ml EDTA-anticoagulated blood was collected at a local laboratory. Subjects for whom travel to a laboratory would have been a burden donated saliva samples instead. Saliva samples were obtained by asking participants to hold their saliva for 2 min and subsequently spit into sterile 50 ml polypropylene tubes. Samples were identified by subject number only. DNA isolation and genotyping was done by investigators blind to any subject information.

DNA was isolated from blood using buffy coat preparations in a modification of the procedure of Miller [23], using Puregene DNA Purification Kits (Gentra Systems, MN) and following the manufacturer's instructions. DNA was isolated from saliva using QIAamp DNA Blood Mini Kits (Qiagen, CA) and using the manufacturer's protocol for isolation of genomic DNA from saliva.

For genotyping of 5-HTTLPR, 0.5μM oligonucleotide primers flanking the 5-HTTLPR (forward: 5'-ATGCCAGCACCTAACCCCTAATGT-3') and reverse: 5'-GGACCGCAAGGTGGGCGGGA-3') were used in 10μl PCR reactions containing 5μl HotStar Taq Master Mix (Qiagen, CA), 2.5μl Betaine (Sigma-Aldrich, MO) and 100ng genomic DNA from each subject. PCR reactions were run on an MJ Research PCT-200 DNA engine, using the following cycling parameters: 15-minute incubation at 95°C, followed by 33 cycles of 95°C for 1 min, 60°C for 1 min, and 72°C for 2 min, followed by a 10-minute final extension step of 72°C for 10 minutes. Results were size fractionated on a 3% agarose gel which allowed for easy distinction of the s allele, yielding a 376bp fragment, the l allele, resulting in a 419bp fragment, and the xl allele, resulting in an approximately 460bp fragment.

rs25531 was genotyped using an ABI7000 Gene Expression System. 100ng genomic DNA were amplified in the presence of gene-specific primers (forward: 5'-CCCTCGCGGCATCCC-3', reverse: 5'-ATGCTGGAAGGGCTGCA-3') and allele-specific fluorescent probes (VIC-CTGCACCCCCAGCAT-NFQ and FAM-CTGCACCCCCGGCAT-NFQ) obtained through Applied Biosystems Custom TaqMan SNP genotyping assay service, and following the manufacturer's instructions.

For genotyping of STin2 VNTR, 0.5μM oligonucleotide primers flanking the polymorphic site (forward: 5'-GTCAGTATCACAGGCTGCGAG-3' and reverse: 5'-TGTTCCTAGTCTTACGCCAGTG-3') were used in PCR reactions using the same reagents and cycling parameters as described above for 5-HTTLPR. Reaction products were size fractionated on a 5% non-denaturing polyacrylamide gel, allowing distinction of the 214bp STin2.7, 248bp STin2.9, 265bp STin2.10 and 299bp STin2.12 alleles.

Data analysis

Comparisons between SERT genotype groups and other categorical variables were made using the generalization of Fisher's exact test. Calculations for deviation from Hardy-Weinberg equilibrium were made using $\chi 2$ tests. Continuous variables such as age and NIHSS were compared between the groups of depressed and non-depressed subjects using two-sample t-tests or Mann-Whitney U tests when data were non-normally distributed. Odds ratios for depression by genotype were determined by logistic regression, controlling for age, gender, and NIHSS score.

Results

Although no deliberate attempt was made to match subjects with or without PSD in this study, both groups had similar distributions of gender and race or ethnicity. Likewise, there were no significant differences in stroke location by hemisphere or along the anterior-posterior brain axis between the two groups (Table 1). PSD subjects, however, were significantly younger than non-depressed individuals, with a mean age of 56.8 ± 12.5 years for depressed versus 62.6 ± 14.2 for non-depressed individuals (p=0.009). Also, the mean NIHSS score was significantly higher among subjects with depressive symptoms (5.6 ± 4.4) than non-depressed subjects (3.6 ± 3.0; p=0.002).

The distributions of SERT genotypes for the 5-HTTLPR, rs25531 and STin2 VNTR polymorphisms among all 150 stroke subjects were in Hardy-Weinberg equilibrium (5-

HTTLPR $\chi 2=5.1$, p=0.17; STin2 VNTR $\chi 2=1.6$, p=0.65; rs25531 $\chi 2=0.54$, p=0.46). Allele frequencies are shown in Table 2. The rare 5-HTTLPR xl allele was observed in only one non-depressed individual with the genotype l/xl. The STin2.9 allele occurred twice, in two PSD subjects with the genotype 9/12. In order to facilitate further analysis, we grouped the xl allele together with the l allele, and the STin2.9 allele with STin2.12. This grouping, along with the identification of potential risk alleles (Table 3) was based on prior genetic association studies on 5-HTTLPR or STin2 VNTR and mental illness as well as studies suggesting similar functional effects of the STin2.9 and STin2.12 alleles. Analysis results did not differ significantly depending on whether the rare allele carriers were included or not (alternate results excluding rare allele carriers not shown).

For the STin2 VNTR polymorphism, genotype frequencies differed significantly between both groups (Table 3), with the STin2 9/12 and 12/12 genotypes more common among patients suffering from PSD. The 5-HTTLPR s/s genotype was also more common among subjects with depressive symptoms, yet this effect did not reach statistical significance. The frequency of the rare rs25531 G-allele was similar in both groups. We next compared the odds ratios for depression between subjects carrying different numbers (0, 1, or 2) of potential risk alleles at each of the two polymorphic sites, 5-HTTLPR and STin2 VNTR (Table 3). For rs25531 no potential risk allele has been identified and genotypes were therefore compared without risk assignation. Since younger age and a higher NIHSS score were significantly associated with depressive symptoms in our study, odds ratios were adjusted for these two variables. In addition, odds ratios were adjusted for gender, since the prevalence of depression is higher among women. For both 5-HTTPLR and STin2 VNTR, carrying two potential risk alleles significantly raised the odds for PSD.

Genotypes at the three polymorphic sites, 5-HTTLPR, STin2 VNTR, and rs25531, were not independent from one another. As shown in Table 4, the 5-HTTLPR s allele occurred significantly more frequently in combination with the STin2.12 allele and the rs25531 A allele than together with STin2.10 or rs25531 G, indicating the presence of linkage disequilibrium between the three sites.

As a post-hoc analysis, we investigated a possible additive effect of 5-HTTLPR and STin2 VNTR. While being homozygous for a potential risk allele at either site significantly raised the odds for PSD, the odds were not much higher for individuals who were homozygous at both sites compared to individuals who were homozygous at only one of the two sites (Table 5).

Discussion

Our results show that the STin2 VNTR and 5-HTTLPR polymorphisms of SERT are associated with PSD in stroke survivors. Homozygous carriers of potential risk alleles for mental illness, the s-allele of 5-HTTLPR and the STin2.9 and STin2.12 alleles, had at least three-fold higher odds for depression compared to subjects with other genotypes. No association between rs25531 and depression was observed in our study. However, given the low minor allele frequency of rs25531, our study did not carry enough statistical power to detect anything but a strong association between PSD and rs25531. The limitations imposed by our sample size are also reflected in the fairly wide confidence interval on the odds ratios in table 3 and table 5.

The 5-HTTLPR s allele has previously been linked to depression moderated by life stress [9;10], suicidal behavior [24–26], neuroticism [27], and bipolar disorder [14;28], but not unipolar depression [14;28]. Moreover, the s allele has been associated with lower remission and response rates in depressed Caucasian patients treated with SSRIs [29–31]. Compared to the 5-HTTLPR

polymorphism, STin2 VNTR has been much less intensively investigated. The STin2.12 allele has been associated with schizophrenia [16] and bipolar disorder [15], but not unipolar depression [14], whereas the STin2.9 allele has been associated with depression and bipolar disorder in a single study [13]. However, a possible effect of STin2.9 was not confirmed in a subsequent meta-analysis of STin2 VNTR association studies [14].

To date, only very few studies have investigated of the role of SERT polymorphism in medical-psychiatric comorbidity. A single study comparing a 26 depressed and 25 non-depressed stroke survivors investigated only the 5-HTTLPR polymorphism and found the s allele to be more common in subjects with PSD [11]. In a cross-sectional population-based study Grabe et al [32] found that the s allele predisposed female carriers to higher levels of perceived mental and physical distress in the face of multiple chronic illnesses and unemployment. In a large study of cardiac patients, carriers of at least one s allele were more frequently depressed and had an increased risk for subsequent cardiac events [33]. In a study of 138 subjects with irritable bowel syndrome the STin2 VNTR 9/12, along with the 5-HTTLPR s/s genotype, were found to be more common in patients with a history of comorbid depression [19].

Our results confirm previous studies that describe linkage disequilibrium (LD) between 5-HTTLPR, rs25531, and STin2 VNTR. For rs25531, the rare G allele has been shown to occur almost exclusively in the combination with the 5-HTTLPR l allele, a finding confirmed in our study [8]. Furthermore, we found evidence for association between the 5-HTTLPR s and the STin2.12 alleles. This agrees with prior population studies indicating LD between these two alleles in European populations [34;35]. As a result of LD, potential risk alleles at both polymorphic sites are more likely to occur in combination. Hence, disease associations ascribed to the 5-HTTLPR s allele in studies investigating this polymorphism alone might at least in part be due to an underlying 5-HTTLPR s – Stin2.12 association.

It is as yet unknown how SERT polymorphisms might influence the risk for mental illness. The s allele has been associated with lower transcriptional activity in cell culture and slower serotonin uptake in human platelets compared to the l allele [7;36]. Yet, several PET studies in adult human brain have failed to show a correlation between 5-HTTLPR genotype and SERT availability [37;38]. In contrast, laboratory studies of STin2 VNTR have identified the 9- and 12-repeat alleles as transcriptional enhancers [17;18], thus defying a simple explanation whereby a global increase or decrease of SERT expression through these polymorphism might influence the risk for depressive symptoms.

We used the GDS as a screening instrument for depressive symptoms consistent with PSD. While GDS sensitivity and specificity for major depression are fairly good [20], as a screening test it does not in itself make a DSM-IV diagnosis of major depression and only indicates the presence of depressive symptoms. Moreover, a GDS<11, while below the scale's cutoff for depression, is not synonymous with a complete absence of depressive symptoms. Hence, the term depressed as used in this paper indicates a group of subjects carrying either a DSM-IV diagnosis of a mood disorder due to stroke, with depressive features, or the more stringent DSM-IV diagnosis of a mood disorder due to stroke, with major depressive-like episode (both 293.83).

Our case-control study design did not call for PSD sufferers and not-depressed stroke survivors to be matched by demographic or clinical factors. Thus, it is relevant to note that subjects with or without depressive symptoms had similar distributions of stroke location, confirming a previous meta-analysis that did not show an association between lesion location and PSD [39]. Our findings that average NIHSS scores were higher among subjects with depressive symptoms confirms the conclusion reached in a prior systematic review of observational studies which showed a correlation between the incidence of PSD and stroke severity [40]. In addition, greater

levels of disability, such as caused by larger strokes, have been linked to an increased risk for PSD in a large prospective population-based study. The same study identified a history of depression before stroke as risk factor for PSD [3]. This is in keeping with the self-report of those study participants from whom we obtained an extensive psychiatric history (62 of the 75 depressed participants), as 74% of these subjects reported a prior history of major depression. The mean age in our subject group was young compared to the age of the average stroke patient [41;42]. This is interesting in light of results from the Framingham study, where symptoms of depression have been shown to significantly increase the risk of stroke in patients younger than 65 years [43]. Hence, our results are consistent with a possible effect where premorbid depression heightens the risk of both stroke and PSD in a younger patient group, thereby resulting in our observation that the PSD subjects were even younger than their non-depressed counterparts.

Our study is the first to characterize an association of both the 5-HTTLPR and STin2VNTR polymorphisms of SERT with PSD. While these polymorphisms, with the possible exception of STin2.9, do not appear to be associated with depression in the absence of psychosocial stress, our study shows them to raise significantly the odds for depressive symptoms in the context of medical illness. In the case of stroke, this can lead to a vicious circle, whereby some SERT genotypes lower resilience to psychosocial stress and thereby increase the risk for a depressive illness, which itself raises the risk for stroke at a younger age. Consequently, as stroke survivors, carriers of these genotypes appear to be at increased risk for PSD and further morbidity. This illustrates how genetic factors might participate in forming a vicious circle by increasing both medical and psychiatric morbidity in a biospychosocial framework.

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Table 1

Comparison of the depressed and non-depressed groups of subjects by demographic and clinical variables

For categorical variables (gender, race/ethnicity, stroke hemisphere and location) the numbers (n) as well as the percentages (%) of depressed (n=75) and non-depressed subjects (n=75) that met the demographic or clinical criteria listed are given. Participants who identified themselves as members of more than one race endorsed all races except Native Hawaiian/Pacific Islander. For age and NIHSS score, mean ± SD are given. Differences between the depressed and non-depressed groups were significant for age (two-sample t-test, p=0.009) and NIHSS (two-sample t-test, p=0.002) only, but not for any of the other, categorical variables (Fisher's exact test)

		Depressed n (%)	Non-Depressed n (%)
Gender	Male	43 (57%)	51 (68%)
	Female	32 (43%)	24 (32%)
Race / Ethnicity	White only	50 (67%)	58 (77%)
	African American only	7 (9%)	5 (7%)
	Asian only	4 (5%)	4 (5%)
	American Indian/Alaskan Native only	1 (1%)	0
	Native Hawaiian/Pacific Islander only	1 (1%)	0
	More than one race	12 (16%)	8 (11%)
	Hispanic ethnicity	2 (3%)	0
Stroke Hemisphere	Left	30 (40%)	33 (44%)
	Right	37 (49%)	37 (49%)
	Bilateral	8 (11%)	5 (7%)
Stroke Location	Anterior	38 (51%)	45 (60%)
	Posterior	22 (29%)	20 (27%)
	Anterior to posterior	15 (20%)	10 (13%)
Age		56.8 ± 12.5	62.6 ± 14.2
NIHSS Score		5.6 ± 4.4	3.6 ± 3.0

Table 2

Distribution of allele frequencies for 5-HTTLPR, STin2 VNTR, and rs25531

Shown are the allele frequencies (%) for depressed (n=75) and non-depressed subjects (n=75).

Polymorphism	Allele	Allele Frequency	
		Depressed	Non-Depressed
5-HTTLPR	s	49	38
	l	51	61
	xl	0	1
STin2 VNTR	STin2.9	1	0
	STin2.10	26	41
	STin2.12	73	59
rs25531	A	94	95
	G	6	5

Table 3

Influence of SERT genotype on depression risk

Genotypes were grouped together for analysis if they contained the same number of risk alleles, i.e. one subject carrying the l/xl genotype was counted with the l/l genotypes among the 5-HTTLPR 0 risk allele carriers, and two subjects carrying the STin2 VNTR 9/12 genotype were counted with the 12/12 genotypes among the STin2 VNTR 2 risk allele carriers. Shown are the numbers (n) as well as the percentages (%) of depressed (n=75) and non-depressed subjects (n=75) with any given genotype or genotype combination. Odds ratios were adjusted for age, gender, and NIHSS score. The number of risk alleles was significantly associated with depression for 5-HTTLPR and for STin2 VNTR, but not for rs25531.

Polymorphism	Risk Alleles	Number of Risk Alleles	Genotypes	Depressed n (%)	Non-Depressed n (%)	Adjusted Odds Ratio	95% Confidence Interval	p-values
5-HTTLPR	s	0	l/l or l/xl	24 (32%)	30 (40%)	1		
		1	s/l	28 (37%)	33 (44%)	1.2	0.5–2.7	
		2	s/s	23 (31%)	12 (16%)	3.1	1.2–8.3	
			Fisher's exact test:					0.11
			Logistic regression likelihood ratio test controlling for age, gender, NIHSS:					0.045
STin2 VNTR	9 and 12	0	10/10	7 (9%)	12 (16%)	1		
		1	10/12	25 (33%)	37 (49%)	1.6	0.5–5.2	
		2	9/12 or 12/12	43 (57%)	26 (35%)	4.1	1.2–13.6	
			Fisher's exact test:					0.02
			Logistic regression likelihood ratio test controlling for age, gender, NIHSS:					0.01
rs25531	neither		A/A	66 (88%)	67 (89%)	1		
			A/G	9 (12%)	8 (11%)	1.1	0.4–3.4	
			Fisher's exact test:					1.00
			Logistic regression likelihood ratio test controlling for age, gender, NIHSS:					0.83

Table 4

Associations of genotypes between 5-HTTLPR, STin2 VNTR, and rs25531

All observed genotype combinations between pairs of polymorphic sites are tabulated separately for depressed (n=75) and non-depressed (n=75) subjects. Shown are number (n) and column percent (%) of genotype carriers as well as p-values of Fisher's exact test for association between genotypes within each of the two subject groups. A: 5-HTTLPR and STin2 VNTR, B: rs25531 and 5-HTTLPR, C: rs25531 and STin2 VNTR.

A

Depressed					**Non-Depressed**				
5-HTTLPR	STin2 VNTR				5-HTTLPR	STin2 VNTR			
	10/10	10/12	12/12	9/12		10/10	10/12	12/12	9/12
s/s	0	7 (28%)	16 (39%)	0	s/s	1 (8%)	2 (55%)	9 (35%)	0
s/l	1 (14%)	7 (28%)	20 (49%)	0	s/l	2 (17%)	21 (57%)	10 (39%)	0
l/l	6 (86%)	11 (44%)	5 (12%)	2 (100%)	l/l	9 (75%)	13 (35%)	7 (27%)	0
l/xl	0	0	0	0	l/xl	0	1 (3%)	0	0
p-value	0.000				p-value	0.004			

B

Depressed					Non-Depressed				
rs25531	5-HTTLPR				rs25531	5-HTTLPR			
	s/s	s/l	l/l	l/xl		s/s	s/l	l/l	l/xl
A/A	23 (100%)	26 (93%)	17 (71%)	0	A/A	12 (100%)	31 (94%)	24 (83%)	0
A/G	0	2 (7%)	7 (29%)	0	A/G	0	2 (6%)	5 (17%)	1 (100%)
p-value	0.005				p-value	0.046			

C

Depressed					Non-Depressed				
rs25531	STin2 VNTR				rs25531	STin2 VNTR			
	10/10	10/12	12/12	9/12		10/10	10/12	12/12	9/12
A/A	6 (86%)	20 (80%)	39 (95%)	1 (50%)	A/A	12 (100%)	32 (87%)	23 (89%)	0
A/G	1 (14%)	5 (20%)	2 (5%)	1 (50%)	A/G	0	5 (14%)	3 (12%)	0
p-value	0.06				p-value	0.53			

A	Non-Depressed		
Depressed			

Table 5

Combined effects of 5-HTTLPR and STin2 VNTR

Genotypes were grouped together in three risk categories based on the number of sites that were homozygous for a risk allele. Only genotype combinations that were observed in our sample are listed. Shown are the numbers (n) as well as the percentages (%) of depressed (n=75) and non-depressed subjects (n=75) for each group. Odds ratios were adjusted for age, gender, and NIHSS score. Depression risk significantly increased with the number of homozygous sites.

Number of Homozygous Sites	Observed Genotypes	Depressed n (%)	Non-Depressed n (%)	Adjusted Odds Ratio	95% Confidence Interval	p-values
	5-HTTLPR s/l + STin2 10/10					
	5-HTTLPR s/l + STin2 10/12					
0	5-HTTLPR l/l + STin2 10/10	25 (33%)	46 (61%)	1		
	5-HTTLPR l/l + STin2 10/12					
	5-HTTLPR l/xl + STin2 10/12					
	5-HTTLPR s/s + STin2 10/10					
	5-HTTLPR s/s + STin2 10/12					
1	5-HTTLPR s/l + STin2 12/12	34 (45%)	20 (27%)	3.9	1.7–8.8	
	5-HTTLPR l/l + STin2 12/12					
	5-HTTLPR l/l + STin2 9/12					
2	5-HTTLPR s/s + STin2 12/12	16 (21%)	9 (12%)	4.2	1.4–12.4	
	Fisher’s exact test					0.003
	Logistic regression likelihood ratio test controlling for age, gender, NIHSS:					0.000