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Polymorphisms of the HTR1a Allele are linked to Frontal Brain Electrical Asymmetry

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Abstract

Polymorphic variations in genes related to serotonin synthesis, transport, recognition, or degradation may convey subtle changes in serotonin system architecture that may place an individual at risk for psychopathology when faced with life stressors. The relationship between three key serotonin alleles and frontal brain electrical asymmetry, a putative endophenotype of depression, was examined. Risk alleles were hypothesized to predict relatively greater right frontal brain activity regardless of current clinical state. A sample of 313 college-age individuals, spanning a range of depressive severity from no symptomatology to clinically meaningful levels, participated. Resting encephalographic (EEG) activity was recorded from 64 scalp sites on four occasions separated by at least 24 hours (two 8-min recording sessions occurring at each occasion). Alpha power asymmetry scores between homologous sites were calculated for each session and then averaged to form a trait metric of asymmetry for each pair. PCR based genotyping was conducted for the HTR1A, HTR2A, and HTTLPR genes. Variations in the HTR1A gene were related to trait EEG asymmetry, regardless of any history of depression. Compared to subjects with at least one non-risk allele, subjects with homozygous HTR1A risk alleles had significantly greater relative right frontal activity at sites F7/F8, F5/F6, & F1/F2. In conclusion, variation in HTR1A can influence trait level brain activity, which may ultimately be indicative of risk for psychopathology.

Keywords

Serotonin; EEG; Asymmetry; HTR1a; Endophenotype; Gene

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Introduction

Depression has a notoriously heterogeneous presentation, implying diverse disease contributions. Endophenotypes (measurable biological markers of disease conveyance (Gottesman & Gould, 2003; Iacono, 1998)) can aid in elucidating specific risk pathways by parsing the heterogeneity of symptom-based phenotypes into homogeneous clusters that share underlying neurophysiological mechanisms. The current study thus investigated the relationship between a putative endophenotype for depressive risk, frontal electroencephalographic (EEG) asymmetry, and three serotonergic candidate genes that have shown a previous association with depression.

Serotonergic dysregulation is implicated in the onset, course and recovery of depression (Arias, Catalan, Gasto, Gutierrez, & Fananas, 2005; Lesch, 2001), with studies showing an association between genetic polymorphisms in serotonin genes and individuals with depression or at risk for depression (e.g. (Brown & Hariri, 2006; Caspi et al., 2003; Ogilvie et al., 1996)), but not without exception (Frisch et al., 1999; Kato, 2007). Specific genes of interest in the present study were: 1) the Serotonin (5-HT) Transporter Linked Promoter Region (HTTLPR) gene polymorphism SLC6A4, located on chromosome 17, with the short polymorphism resulting in a decrease of serotonin transporter efficacy (in binding, reuptake, and mRNA concentrations), and was associated with increased depression in the presence of life stress in early studies (e.g., Caspi et al., 2003 but see Risch, Herrell, Lehner, Liang, Eaves, Hoh et al., 2009); 2) The C(-1019)G polymorphism of the 5HT Receptor-1A (HTR1A) gene, with the G allele showing a two-fold increase in depressed individuals, and the extensive distribution of these receptors in key brain areas associated with depression including prefrontal cortex pyramidal and inter-neurons, hippocampus, amygdala, hypothalamus and septum (Le Francois, Czesak, Steubl, & Albert, 2008); and, 3) The 5HT Receptor-2A (HTR2A), in which an increased frequency of the C allele polymorphism in exon 1 has been found in individuals with unipolar depression (Zhang et al., 1997). In addition, HTR2a receptors often function within limbic-cortical loops to moderate prefrontal cortex pyramidal neurons. Blockade of these HTR2a receptors (e.g. mirtazapine, trazodone, nefazodone, and perhaps atypical neuroleptics) in conjunction with the activation of 5-HT receptors 1A (Santarelli et al., 2003) have been proposed as mechanisms of action for 5-HT reuptake inhibitor antidepressants.

Frontal Functional Brain Asymmetry and Risk for Psychopathology

There is a large body of research suggesting the utility of frontal EEG asymmetry as an endophenotype for various psychopathologies. The asymmetry involves relatively greater left-versus right-frontal alpha-band (8-13Hz) power, implying decreased left-frontal activity, since alpha is seen during periods of cortical quiescence (Allen, Coan, & Nazarian, 2004; Allen, Urry, Hitt, & Coan, 2004). Individuals with a history of depression, independent of current clinical status, may be distinguished from never-depressed persons by frontal EEG asymmetry (Stewart, Bismark, et al., 2009; Allen, Iacono, Depue, & Arbisi, 1993; Gotlib, 1998; Henriques & Davidson, 1990, 1991; Reid, Duke, & Allen, 1998). This asymmetry also characterizes persons potentially at-risk for depression, including infants (Dawson, Frey, Panagiotides, Osterling, & Hessel, 1997; Jones, Field, Davalos, & Pickens, 1997) and adolescents (Tomarken, Dichter, Garber, & Simien, 2004) of depressed mothers, consistent with the notion that resting frontal EEG asymmetry may tap a diathesis for depression or other emotion-related psychopathology, particularly those associated with high levels of negative affect (for review see Coan & Allen, 2004). Research has established that high negative affect predisposes individuals to both mood and anxiety disorders, and accounts for the majority of comorbidity between them (Clark & Watson, 1991; Watson, O'Hara, & Stuart, 2008; Watson, Weber, Assenheimer, Clark, Strauss, & McCormick, 1995). Therefore, prefrontal brain asymmetry appears to be an important risk factor not only for MDD, but also for internalizing disorders

more generally, all of which are associated with a predisposition toward high levels of negative affect. In addition to depression, frontal EEG asymmetry has been found to characterize those with: anxious arousal/somatic anxiety (Mathersul, et al., 2008; Nitschke, Heller, Palmieri, & Miller, 1999; Stewart, Levin-Silton, Sass, Heller, & Miller, 2008); panic disorder (Wiedemann, Pauli, Dengler, Lutzenberger, Birbaumer, & Buchkremer, 1999); comorbid anxiety and depression (Bruder, Fong, Tenke, Leite, Towey, & Stewart, 1997); social phobia (Davidson, Marshall, Tomarken, & Henriques, 2000); premenstrual related dysphoria (Accortt & Allen, 2006; Accortt, Stewart, Towers, & Allen, 2009), seasonal affective disorder (Allen, et al., 1993), and a variety of childhood or adolescent internalizing psychopathology (Buss, et al., 2003, Fox, et al., 1995 Henderson, Marshall, Fox, & Rubin 2001, Schmidt & Fox, 1994).

Research has also suggested that prefrontal brain asymmetry is at least partly heritable. In a sample of 732 twins and their siblings, the heritability of prefrontal brain asymmetry was as high as 32% for men and 37% for women (Smit, et al., 2007) and approximately 30% in two smaller samples (Anokhin, Heath, & Myers, 2006; Coan, Allen, Malone, & Iacono, 2009). A more recent twin sample (n=951) looking at 9- 10 year old twins, estimates the heritability of frontal asymmetry at 11-28%. (Gao, Tuvblad, Raine, Lozano & Baker, 2009). While the reported heritability of frontal asymmetry in these studies is not large, it is in the range of that observed for depression. The heritability of depression has been estimated to be higher in women than men, and in the range of 22-24% for men (Bierut., L.J, Heath, A., Bucholz, K., Dinwiddie, S., Madden, P., Statham, D., Dunne, M. et al., 1999; McGue, M. & Christensen, K., 2003) and 37-39% for women (Kendler & Prescott, 1999; McGue & Christensen, 2003).

Serotonin and Frontal EEG Asymmetry

Bruder, Stewart, Tenke et al. (2001) reported global hemispheric differences in alpha band EEG in SSRI responders with a shift from relatively greater left alpha power (relatively greater right hemisphere activity) pre-treatment to no hemispheric differences post-treatment. Examining frontal asymmetry specifically, Allen, McKnight, Moreno, Demaree and Delgado (Allen, McKnight, Moreno, Demaree, & Delgado, 2009) examined rapid plasma tryptophan depletion (TD), a procedure that transiently lowers brain serotonin, finding that the magnitude of TD-induced change in frontal EEG asymmetry significantly predicted the development of depression during the ensuing six to twelve months.

The Present Study

The present study thus examined the relationship of frontal EEG asymmetry to serotonin allele variations in a large sample of medication-free participants with the hypothesis that genotype risk status would be associated with greater relative right frontal activity. The primary aim of this study was to investigate how functional polymorphisms within specific genes in the serotonin system influence the putative marker of risk for depression, frontal EEG asymmetry.

Methods and Materials

Participants

Participants completed the Structured Clinical Interview for DSM disorders (SCID, (First, 1997)) and were excluded for any DSM-IV Axis I disorder other than Major Depressive Disorder (MDD) or Dysthymia. All participants were strongly right handed (Score > 35 of 39 on a handedness inventory (Chapman & Chapman, 1987)). Exclusionary criteria included history of head injury, loss of consciousness exceeding 10 minutes, concussion, epilepsy, electroconvulsive therapy and use of current psychotropic medications. Individuals with active or acute suicidal potential necessitating immediate treatment were also excluded. Interview screening and DNA sampling (via salivary collection or buccal cheek swabs) took place on a separate day prior to any EEG evaluations. A total of 323 participants from among 520 invited

for screenings were found eligible for participation; ten participants discontinued before beginning any EEG recording. These participants did not significantly differ from the remaining participants in age, gender or ethnicity. The final sample of 313 participants (32% male) aged 18-33 ($m=19.2$, $sd=2.0$) included: 74 experiencing current depressive symptomatology at the time of evaluation (52 Major MDD, 14 Dysthymia, 8 Double-Depression), 17 of whom were experiencing their first episode; 72 participants currently not depressed but with a history of depressive illness and 167 participants with no history of depressive illness. These participants are also reported in Stewart, Bismark, et al., 2009.

Gene frequencies as a function of Depression History status are presented in Table 1 for the genes examined in this study. The initial analyses were carried out using all participants with complete gene and EEG data. To assess whether varying rates of gene frequency between ethnicities (Cardon & Bell, 2001; Glatt, Tampilic, Christie, DeYoung, & Freimer, 2004) might indicate a need to assess an ethnically and racially homogeneous subsample, chi-square analyses were used to evaluate any ethnic stratification of allele frequency per gene. Given the racial/ethnic composition of the sample (72% Caucasian non-Hispanic, 10% Hispanic, 5% Black, 8% Asian, 3% American Indian, 1% Hawaiian or Pacific Islander, and 1% other/unknown), the chi-square examining all groups would result in extremely small cell sizes that can render chi-square inaccurate. Thus the chi-square analyses were conducted comparing Caucasian non-Hispanic to Caucasian-Hispanic samples, finding no evidence of significant stratification for HTTLPR ($\chi^2 = 1.5$, $p > .4$), HTR1a ($\chi^2 = 0.47$, $p > .7$), or HTR2a ($\chi^2 = 0.55$, $p > .7$). As an additional precaution, primary analyses were rerun using only Caucasian, non-Hispanic individuals, yielding a total n of 220 participants (42 MDD, 6 Dysthymic, 6 Double-Depression, 51 euthymic history +, and 115 history -) for these analyses. Race and ethnicity were self-reported by participants. For the purpose of analysis, participants with any history of depression (past or current, including Dysthymia) were classified as MDD History + ($n=105$) and participants with no history of depression were classified as MDD History - ($n=115$). Hx + and Hx- groups did not differ in terms of age or gender distribution. Gene frequencies as a function of Depressive History status for the Caucasian non-Hispanic sample are presented in Table 2 for the genes examined in this study.

EEG Data Collection and Reduction

Resting EEG was recorded for eight minutes (four eyes open, four closed) during each of two resting baseline sessions on four separate occasions. EEG recordings were separated by at least 24 hours with all four sessions being completed within a 14-day period.¹ EEG data were collected using a 64-channel NeuroScan (Charlotte, NC) amplifier and electrode caps. Two ocular channels (vertical: superior and inferior orbit of the left eye; lateral: outer canthi) were collected for ocular artifact rejection. Electrode impedances were under 10K Ohms. Data were collected using 1000hz sampling rate, amplified 2816 times, and filtered with 200Hz low pass filter prior to digitization. EEG data were referenced online to an electrode site immediately posterior to CZ and subsequently re-referenced offline using a current source density (CSD), using algorithms from Kayser & Tenke, (2006) and based on the spherical spline approach summarized by Perrin, Pernier, Bertrand, & Echallier, (1989,1990). This approach mitigates the impact of broadly distributed sources thus revealing more focal frontal activity of interest for frontal EEG asymmetry (see also Stewart et al., 2009).

Data were visually inspected to remove epochs with movement and muscle artifacts, followed by a computer-based blink rejection algorithm (rejecting segments where vertical ocular activity exceeded ± 75 microvolts), and a separate computer-based algorithm to reject large

¹Note: Fourteen participants had at least one EEG session outside the 14-day period. Of these, nine completed all sessions within a 16-day window with the remaining five completing all sessions within 18-20 days after the initial session.

fast deviations in amplitude (e.g., DC shifts and spikes) that may have eluded human inspection. Data were segmented into one-minute EEG blocks and epoched into 117 two-second epochs per block, overlapping by 1.5 seconds. Power spectra were calculated via FFT (with a Hamming window with 10% taper applied). Power spectra from all artifact free epochs across the eight blocks were averaged to provide a summary spectrum for each resting baseline. Alpha power (8-13Hz) was then extracted for each resting baseline and electrode site. Asymmetry scores for each baseline were calculated by subtracting the natural log transformed scores (i.e., $\ln[\text{Right}] - \ln[\text{Left}]$) for each symmetrical left and right pair. Analysis focused on specific pairs (F7/8, F5/6, F3/4, F1/2) that correspond to regions commonly studied throughout the asymmetry literature pairs F7/8 & F3/4 (see review (Coan & Allen, 2004)) and neighboring pairs (F1/2 & F5/6).

DNA Collection & Analysis

All DNA samples were extracted from buccal cheek swabs or saliva samples using Oragene DNA Collection Kits (DNAGenotek, Ottawa, Ontario, Canada). Genotyping procedures were carried out in the Molecular Psychiatry Laboratory at the University of Arizona. Genomic DNA was amplified for all polymorphisms using standard PCR protocol. Amplification of each base pair region was carried out using sense and anti-sense primers specific for each analysis in a reaction volume of 25 μl consisting of 2.5 ng of DNA, .25 mM dNTP's, .25 μM sense and anti-sense primers, 1X QIAGEN PCR buffer and 1.5 U Taq DNA polymerase (QIAGEN). Thermocycling conditions consisted of an initial denaturation step of 95°C for 5 min, followed by 35 cycles of 94°C for 30 to 35 s, annealing temperatures specific to each genetic region of interest for 30 to 60 s, and 72°C for 60 s, with a final extension step of 72°C for 10 min. PCR product for both HTR1a and HTR2a were digested enzymatically at 36 °C for 14 and 2 hours respectively. Five μl of PCR product was added to 4 μl of Orange G (Sigma Life Biosciences) DNA loading buffer, loaded onto a 3% agarose gel and run in 0.5 $\times$ TAE buffer for 20 min at 72 V. Gels were pre-stained with GelStar® Nucleic Acid Gel Stain and images were captured via the Gel Doc XR System (BioRad, USA). HTTLPR sample analysis was visualized via microsatellite analysis with FAM labeling using the ABI 3730XL DNA Analyzer® (Applied Biosystems) and captured with Chromas Vs. 2.13 (Technelysium). Due to variables encountered during sample collection and/or genetic analysis, the number of usable genetic samples for each gene assayed varied slightly ((*HTR1a*: All ethnicities: 305; Caucasian non-Hispanic: 220; (*HTR2a*: all ethnicities: 264; Caucasian non-Hispanic: 193); (*HTTLPR*: all ethnicities: 308; Caucasian non-Hispanic: 220)).

These specific genetic polymorphisms were of interest. The serotonin transporter polymorphism (HTTLPR) is a 44 base-pair insertion or deletion (in/del) located in the SLC6A4 gene located on chromosome 11 with its primary function of encoding the transport protein necessary for the reuptake of synaptic serotonin into the presynaptic cell. There are two versions, the short (484 base-pairs) or long (528 base-pairs). Serotonin receptor 2a gene polymorphism (HTR2a) is the C/T single-nucleotide substitution at position 102 in exon 1 of the gene, which is located on chromosome 13q14-q21. The primary genetic function is the encoding of the serotonin receptor, type 2a. The serotonin receptor 1a gene polymorphism (HTR1a) is a cytosine to glycine single nucleotide substitution at position - 1019 from ATG start site within the gene, which is located on chromosome 5q11.2-q13. The gene's primary function is the encoding of a protein responsible for the regulation of HTR1a receptor transcription.

Results

There was no significant relationship between any serotonin gene frequency and depressive history that emerged within the full cohort (Table 1) or within the Caucasian non-Hispanic

cohort (Table 2), most likely due to the fact that this is a relatively young sample and many of the participants have not passed through a substantial portion of the period of risk for the development of depression. Dichotomous coding of risk status – homozygous risk vs heterozygous risk and homozygous non-risk – was based on a recent review by Le Francois et al. (2008). Individuals heterozygous for risk/non-risk genotypes have been found likely to display phenotypes more closely related to those homozygous non-risk individuals due to the compensatory action of the non-risk allele over the more inefficient risk allele.

Mixed Models analysis of genotype, MDD & frontal asymmetry

Mixed linear models analyses were used to evaluate the effect of MDD history (positive or Negative) and each genotype (High- vs low-risk) as well as their interaction on frontal asymmetry at each site. Models were repeated for each gene at each channel pair. Within each model, repeated factors included were both recording day (1-4) and session (1 or 2) along with the non-repeated factors of genotype and lifetime MDD status to evaluate genotype effects on frontal asymmetry but also to assess any meaningful interactions between state variance in frontal asymmetry and genotype. Effect sizes (Cohen's *d*) are presented for significant effects.

In terms of main effects of the three serotonin candidate genes, only the HTR1A gene consistently related to frontal EEG asymmetry in the full sample, which reached significance at regions F7/8 ($F(1,2361) = 4.2, p < .05, d = .04$); F5/6 ($F(1, 2341) = 6.0, p < .02, d = .05$); and F1/2 ($F(1, 2364) = 4.4, p < .04, d = .04$). In each case, 5HTR1A risk was associated with relatively greater right frontal activity. Region F3/4 was non-significant ($F(1,2356) = 2.3, p < .14, d = .03$) (See Figure 1). Effects for the other two genes were more limited. HTTLPR risk was significantly related to greater relative right frontal activity only at site F7/8 ($F(1, 2382) = 4.5, p < .04, d = .04$), with all other regions non-significant ($F_s < 0.7, p_s > .42$). HTR2a risk was significantly related to greater relative right frontal activity only at site F7/8 ($F(1, 2043) = 7.4, p < .01, d = .06$), all other regions were non-significant ($F_s < .70, p_s > .42$).

There were also main effects of lifetime MDD status (history positive vs negative) on frontal EEG asymmetry. Again for the full sample, significant lifetime MDD status effects on frontal asymmetry were found at sites F5/6 ($F(1,2341) = 6.4, p < .02, d = .06$), F3/4 ($F(1, 2354) = 12.9, p < .001, d = .08$) and F1/2 ($F(1,2364) = 8.4, p < .01, d = .06$). As shown in Figure 2, lifetime MDD was associated with relatively greater right frontal activity.

The interaction of lifetime MDD status and genetic risk in predicting frontal asymmetry was found in one site for each gene. There was a significant interaction of HTR1a genotype by lifetime MDD status at F7/8 ($F(1, 2362) = 4.6, p < .05, d = .02$). A pair-wise comparison indicated that individuals with a low-risk genotype and without a history of depressive illness showed greater relative left activity compared to those with either lifetime MDD or the risk genotype or both. Examining HTR2a and HTTLPR, significant interactions with lifetime MDD status were found only for F1/2 (HTR2a, $F(1,2048) = 4.1, p < .05, d = .055$; HTTLPR, $F(1,2389) = 7.5, p < .01, d = .045$). These effects at F1/2 in both instances reflected that although those individuals with a lifetime history of MDD had relatively greater right frontal activity than lifetime MDD- individuals, the effect was potentiated among those with the high risk genotype.

The main mixed model analysis was repeated on the Caucasian, non-Hispanic sample as a conservative approach to ensure that the HTR1a risk genotype effects were not due in part to racial or ethnic admixture. Results were virtually identical to those obtained with the full sample. Risk genotype was significantly related to frontal asymmetry at sites F7/8 ($F(1, 1712) = 7.1, p < .01, d = .07$), F5/6 ($F(1, 1712) = 10.4, p < .01, d = .09$), and F1/2 ($F(1,1712) = 7.7, p < .01, d = .07$) with a trend at F3/4 ($F(1, 1712) = 3.2, p < .08, d = .05$).

Discussion

Results indicate that both lifetime MDD and HTR1a risk genotype are independently associated resting frontal brain activity. Individuals with a history of MDD showed greater relative right frontal EEG activity than those without a history of depression. Results also indicate that individuals homozygous for the G allele of the HTR1A C(-1019)G polymorphism, display patterns of frontal brain activity previous literature suggests are correlated with withdrawal-related motivational and behavioral styles, negative affective states, and a history of depressive illness. Although a significant interaction of lifetime MDD status and HTR1a risk genotype was limited to a single region, these findings would suggest that at least for the most lateral regions, frontal asymmetry is sensitive to genetic risk but only among those individuals not already affected by MDD; while at other regions, frontal asymmetry is sensitive to genetic risk regardless of MDD status.

Although the link from receptor genotype to surface EEG remains unclear, these findings are consistent with imaging evidence indicating lateralized HTR1a binding potential (BP) in favor of the right hemisphere compared to left (Fink et al., 2009). Using an animal model of depression, Shively, Friedman, Gage, Bounds, Brown-Proctor, Blair, et al (Shively et al., 2006) reported that monkeys engaged in friendly interaction, grooming and locomotion (approach related behaviors) showed increased HTR1a binding potential in the left cingulate while those engaged in simply attending to their surroundings (withdrawal related behaviors) showed an increased binding potential in the right cingulate cortex. A lateralized increase in binding potential at HTR1a receptors indicates a lateralized serotonergic response to environmental conditions that may contribute to patterns of motivational and behavioral tendencies, and potentially to lateralized potentials measured at scalp sensors.

Proposed mechanism of the conveyed functionalism of HTR1a gene polymorphism C(-1019)G

The HTR1a receptor is expressed both as a presynaptic autoreceptor on raphe neurons, and as a major postsynaptic receptor in regions involved in mood, emotion and stress response such as hippocampal, cortical, and hypothalamic regions (Le Francois et al., 2008). Within the HTR1a gene, the Cytosine to Glycine (C-G) SNP occurs at -1019 nucleotides from the beginning of the 5' region. This polymorphism is located within the gene promoter region and encodes for a protein that binds to the transcription factor nuclear auto-regulatory factor DEAF-1/suppressin (NUDR). The function of NUDR is to inhibit HTR1a transcription; this inhibition is impaired in the G-allele. . In concert with the current findings, Albert and Lemond (Albert & Lemonde, 2004) found that increased HTR1a receptor concentration in the raphe decreased serotonergic transmission to areas implicated in depression (such as PFC, hippocampus and hypothalamus). One proposed mechanism is that this failed transcription inhibition leads to increased receptor expression both pre- and post synaptically, enhancing negative feedback inhibition of raphe neurons, ultimately leading to decreased serotonergic neurotransmission. Recent imaging research indicates lateralization of HTR1a receptor binding potential in superior and middle frontal gyri as well as the triangular part of inferior frontal cortex (Fink et al., 2009). This increased binding potential indicates greater receptor expression and implies enhanced serotonergic negative feedback inhibition specific to right frontal regions.

Limitations and Conclusions

Although this sample is relatively large by EEG standards, candidate gene findings are best treated as preliminary until cross-validated in additional samples. Nonetheless, the consistency of the effects across prefrontal regions that have been found important in other studies of EEG

and MDD, and the fact that findings follow from a theoretical rationale predicting this pattern of results, lend confidence that replications will prove successful.

Evaluating gene-psychopathology relationships may be profitably explored by looking not at symptom-based diagnostic entities, but instead on underlying endophenotypes that link genetic risk to overt symptomatology. The current dataset demonstrates the utility of linking serotonergic genetic polymorphisms with the putative endophenotype of frontal EEG asymmetry, as frontal asymmetry was related both to genetic risk and lifetime history of MDD. Although HTR1a risk alleles were not related to lifetime MDD in this young adult sample, these individuals had not passed through even half the period of risk for MDD; the genotype-MDD relationship may not yet be apparent as some lifetime MDD- participants may go on to develop MDD later in life. The HTR1a-asymmetry link suggests that frontal brain asymmetry may indeed serve as an endophenotype of risk for MDD, although other findings in the literature suggest that it may serve more broadly as an endophenotype of risk for a variety of emotional disorders and internalizing psychopathology. Future work might profitably explore whether the combination frontal brain asymmetry, alone or in combination with genetic risk, can predict future phenotypic depression in unaffected individuals.

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Figure 1.

Asymmetry scores by site by HTR1a risk genotype for the full sample of 305 individuals with complete genetic data.. Individuals with genotype (G/G) show significantly more relative right activity than individuals with genotype (C/C) or C/G) across regions F7/8, F5/6 and F1/2. Symbols indicate significantly greater relative right frontal activity for G/G compared to the C/C and C/G genotypes at a given region. (*= $p < .05$ comparing risk to low-risk genotypes for a give scalp region).

Figure 2.

Asymmetry scores by site by MDD history (+/-) for all the full sample of 305 individuals with complete 5HTR1A data. Individuals with lifetime depressive history (+) show significantly more relative right activity than individuals without a history of depressive illness at regions F5/6, F3/4 and F1/2. Symbols indicate significantly greater relative right frontal activity for MDD (+) compared to MDD (-) at a given region. (*= $p < .05$, comparing MDD Hx (+) to MDD Hx (-) for a given scalp region).

Table 1Genotype by Depressive History for *the full sample*

	HTR1a			
	CC	CG	GG	
Hx–	48	82	34	$\chi^2 = 0.65, ns$
Hx+	46	63	32	
Total	94	145	66	

	HTR2a			
	GG	GC	CC	
Hx–	31	80	27	$\chi^2 = 0.41, ns$
Hx+	24	69	33	
Total	55	149	60	

	HTTLPR			
	LL	LS	SS	
Hx–	55	84	27	$\chi^2 = 0.67, ns$
Hx+	47	77	18	
Total	102	161	45	

Note: There were no significant differences in allele distributions as a function of depressive history. Numbers of subjects vary somewhat across alleles (HTR1a=305, HTR2a=264, HTTLPR=308) due to problems in sample collection and/or genetic analysis. For each gene, when dichotomizing for risk vs. non-risk, the leftmost and center columns were combined to represent non-risk while the rightmost column was the genotype most associated with risk. For example: HTR1a Non-risk genotype: CC/CG vs risk Genotype: GG.

Table 2Genotype by Depressive History for the *Caucasian non-Hispanic sample*

	HTR1a			
	CC	CG	GG	
Hx-	32	55	28	$\chi^2 = 0.61, ns$
Hx+	35	48	22	
Total	67	103	50	
	HTR2a			
	GG	GC	CC	
Hx-	20	58	21	$\chi^2 = 0.59, ns$
Hx+	19	50	25	
Total	39	108	46	
	HTTLPR			
	LL	LS	SS	
Hx-	42	58	15	$\chi^2 = 0.87, ns$
Hx+	35	55	15	
Total	77	113	30	

Note: There were no significant differences in allele distributions as a function of depressive history. Numbers of subjects vary somewhat across alleles (HTR1a=220, HTR2a=193, HTTLPR=220) due to problems in sample collection and/or genetic analysis.