THE HERITABILITY OF AND GENETIC CONTRIBUTIONS TO, FRONTAL ELECTROENCEPHALOGRAPHY

by

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Abstract:

The heritability of frontal EEG asymmetry, a potential endophenotype for depression, was investigated using a large set of adolescent and young adult twins. Additionally, the relationship between polymorphisms within three serotonin genes, two receptor genes and one transporter gene, and frontal EEG asymmetry was also investigated. Using Falconer's estimate, frontal EEG asymmetry was shown to be more heritable at lateral compared to medial cites across nearly all reference montages, and greater in males compared to females. Using structural equation modeling (SEM), and investigating both additive (ACE) and non-additive (ADE) models of genetic heritability, males displayed consistently greater additive genetic contributions to heritability, with greater lateral contributions than medial ones. For female twins pairs, the additive genetic model data provided a mixed picture, with more consistent heritability estimates observed at medial sites, but with larger estimates shown at lateral channels. For non-additive genetic models, male twin pairs demonstrated exclusive non-additive contributions to heritability across channels within AVG and CZ referenced data, with metrics in the CSD and LM montages more mixed between additive and non-additive contributions. However, consistent with Falconer's estimates, lateral channels were nearly always estimated to be more heritable than medial channels regardless of gender. These models demonstrate some combination of additive and non-additive contributions to the heritability of frontal EEG asymmetry, with the CSD and AVG montages showing greater lateral compared to medial heritability and CZ and LM montages showing mixed contributions with additive heritability at lateral channels and non-additive primarily at medial channels. The complex interaction of gender and reference montage on the heritability estimates highlight the subtle yet important roles of age, gender, and recording methodology when investigating proposed endophenotypes. However, no association was found

between the proposed polymorphisms in serotonin receptor 1a, 2a or serotonin transporter genes and frontal EEG asymmetry. Although the results support modest heritability of frontal EEG asymmetry, the proposed link to underlying serotonergic genetic markers remains an open question. Overall, these results indicate that frontal asymmetry may be a useful endophenotype for depressive risk with modest heritability, but is one that taps more environmental risk.

Introduction:

Depression is a serious mental health concern throughout the world. Recent research points to one-year point prevalence being about 9%, with rates higher in women (Kessler et al. 2005). Unfortunately, one of the hallmarks of depression is its heterogeneous presentation. A heterogeneous symptom profile implies heterogeneous disease contributions that are unlikely to result from any singular biological or psychosocial etiology, but that more likely are the product of complex interactions between a number of aspects related to risk. Research has demonstrated aspects of depressive risk in domains including genetic (Arias et al, 2005), psychophysiological (Allen et al, 2004; Drevets, 1998), psychosocial (Dimidjian et al., 2006; Ma and Teasdale, 2004) and their interaction (Stein et al 2007; Caspi et al 2003; Caspi et al 2002). With multiple pathways to disease presentation, it is likely that each domain conveys diatheses for disease development and that the summation and interaction of these diatheses with environmental conditions leads to illness development. Thus, it is of great importance to discover both the underlying diatheses but also the interactions of biological and psychosocial factors that place an individual at risk.

Endophenotypes can aid in elucidating etiology by parsing disease heterogeneity into more homogeneous phenotypes that should allow for a closer examination of the neurophysiological mechanisms that underlie causal disease pathways. The primary goal of this study was to investigate a putative endophenotype of depression, frontal EEG asymmetry, as a heritable disease marker and one associated with risk genotypes in serotonergic reception and transport. By examining the associations between genes, heritable patterns of frontal asymmetry (FA), and risk for depression, we assessed whether this marker had characteristics that make it

useful as an endophenotype, and whether previous findings relating it to altered serotonergic function can be replicated in a much larger sample.

Individuals with depression (or illness history) display different patterns of brain activity as measured by electroencephalographic (EEG) recordings. One particular pattern of interest is an asymmetrical pattern of activity in the Alpha band (8-13Hz) over the frontal cortex, which has been associated with depressive illness (Henriques & Davidson, 1990, 1991; Allen, Urry, Hitt, & Coan, 2004). This pattern of brain activity, characterized by relatively greater right than left frontal activity (as inferred from relatively less right than left frontal alpha power) has been suggested as a possible endophenotypic marker of depression, and one that may mediate the link between genetic risk factors and depression. Although various sources can influence neurochemical systems, genetics play a substantial role in determining both structural and functional capabilities of these systems. Previous research has shown a higher incidence of genetic polymorphisms in individuals with major depression or a history of depression (Brown and Hariri, 2006; Neumeister et al, 2004; Staley et al 1998), as well as serotonergic dysregulation in the onset, course and recovery of depression (Lesch 2001, Arias et al., 2005). The current study further investigated serotonergic genetic polymorphisms that may influence this putative marker of risk for depression.

This study utilized two approaches to examine whether frontal EEG asymmetry may prove useful as an endophenotype of risk for depression. First, a behavioral genetic approach was used to estimate the unique contributions of genetic, shared and non-shared environmental influences (ACE and ADE models) on frontal asymmetry. If an endophenotype is to be useful, it should be at least as heritable as and ideally more heritable than the phenotype. Previous work with asymmetry has found it to be modestly heritable (Smit et al., 2007; Gao et al., 2009), but

neither of these studies utilized an optimal EEG reference (Current Source Density; CSD) that previous work has found to be superior as a prospective endophenotype (Stewart et al., 2010), by virtue of distinguishing those with any lifetime history of MDD from never-depressed individuals. The present study assessed heritability for EEG alpha asymmetry derived from the conventional references and also CSD- transformed signals. Second, using a candidate gene approach, the study examined the relationships between frontal asymmetry and genes related to serotonin system functions, using a considerably larger sample then previous reported.

Frontal EEG Asymmetry as a Potential Endophenotype for MDD

Endophenotypes are subtle, stable, intermediate bio-behavioral manifestations of clinical symptoms or phenotypes with clear connections to underlying genes. Less deterministic than genes and more specific than clinical phenotypes, endophenotypes may prove extremely useful in classifying risk for disease development or recurrence. However, in order for the endophenotypic concept to apply, certain criteria be met, namely: the endophenotype is associated with the illness in the population of study; it is heritable; it is primarily state independent; within affected families, the endophenotype and illness tend to co-segregate; and that within affected families, rates of endophenotypic presentation in unaffected members is higher than in the general population (Gottesman & Gould, 2003). Despite their stringent criteria, endophenotypes offer several advantages over symptom-based phenotypes for the study of psychopathology. First, endophenotypes represent simpler clues to biological underpinnings of disease states, offering more objective measurements of risk. Second, endophenotypes are state-independent and can be evaluated in the presence or absence of an acute disease state, and even before disease onset. Third, phenotypes are heterogeneous and often subjectively rated adding substantial variability among individuals with the same diagnosis. Lastly, the brain is subject to

complex interactions in epigenetic, neurochemical, individual and neuronal circuits, that give rise to a phenotypic output that is more than the sum of its parts. This suggests that markers more closely related to biological processes would be more informative when investigating the underlying etiology of psychopathology. The current study investigated the heritability of frontal asymmetry (FA) and assessed its relationship to genes indicated as risk-relevant for depression.

One method by which to study the unique and interactive effects of biological and psychosocial factors is the twin study. On average, monozygotic (MZ) twins are twice as genetically similar as their dizygotic (DZ) counterparts, thus estimates of heritability for any particular trait are predicted to be twice the difference between the correlation in MZ twins and that same correlation in their DZ counterparts. By using this genetic relationship and modeling the environmental relationships (common and unique), allows for the separation of the contributions of genetic, shared and non-shared environmental influences to the heritability of that trait. Basic versions (not considering genetic dominance) of these models are commonly referred to as ACE models (see Figure 1), where **A** (additive genetic effects), **C** (common/shared environment) and **E** (unique/non-shared environment).

The current research was designed to further assess the utility of FA as an endophenotype for psychopathological risk and builds upon this literature by investigating the heritability of frontal EEG asymmetry using a large, relatively young twin sample. More specifically, we employed twin designs and behavioral genetic (ACE/ADE) models, to model the unique additive genetic, shared and non-shared environmental contributions to frontal EEG asymmetry within the alpha frequency band range (8-13Hz). Using a subset of the sample for which genotyping is available, we attempted to replicate previous genetic findings demonstrating the relationship

between serotonergic genes coding for various aspects of the Serotonin system, and frontal EEG asymmetry (Bismark, Moreno, Stewart, Towers, Coan, Oas, et. al., 2010).

Frontal EEG Asymmetry & Emotion

EEG asymmetry is measured by quantifying the activity (power) of alpha frequency oscillations measured from the scalp. Alpha oscillations are inversely related to cortical activity, such that greater alpha power indicates lower activity (cf. Allen, Coan & Nazarian 2004). These hemispheric differences are *relative* differences in power, thus power comparisons will be made by the subtraction of natural-log transformed homologous sites across the scalp (ln[Right]-ln[Left]). This metric provides a quantification reflecting hemispheric differences in alpha activity with larger positive asymmetry scores indicating greater left-than-right cortical activity.

Frontal EEG asymmetry is conceptualized as a measure of motivational/behavioral style (Davidson, 1992; 1993) as well as an indicator of underlying affective processes (e.g., Coan, Allen & Harmon-Jones, 2001). In both conceptualizations, relatively greater right activity (relative to left) is associated with greater withdrawal motivated behavior, negatively valenced emotions and greater risk for depressive illness. Conversely, greater left activity (relative to right) is associated with greater approach motivated behavior and positively valenced emotions. If affective/motivational style(s) can be represented as relative differences in hemispheric asymmetry, then FA may be useful as a liability marker for depression. Research indicates that FA can distinguish individuals with current episode or a history of depression from never depressed individuals (Henriques & Davidson, 1991; Allen, Iacono, Depue, & Arbisi, 1993; Henriques & Davidson, 1990, Allen et al., 1993). Additionally, infants of depressed mothers display relative left frontal hypoactiivty compared to infants of non-depressed mothers (Dawson, Frey, Panagiotides, Yamada, Hessl, & Osterling, 1999).

Research indicates patterns of frontal activity are moderately stable, with 60% of the

variance attributable to a stable latent trait across four measurement occasions (Hagemann, Naumann, Thayer, & Bartussek, 2002) as well as intra-class correlations ranging from .50 to .75 across measurements spanning five months (Allen et al., 2004). High interclass correlations with Cronbach's alphas, ranging from .81 to .90, have also been reported for FA (Tomarken et al., 1992). This trait-like stability suggests the potential for FA heritability, supported by recent findings indicating heritability ranging from .11 to .28 (Gao et al. 2009) with higher estimates for single-site spectral alpha power ranging from .46 to .89 (Zietsch et al., 2007; Anokhin et al., 2006; Smit et al., 2005; and see Beijsterveldt et al., 2002 for review).

However, heritability estimates often have moderating factors such as age (Smit et al., 2007), gender (Smit et al., 2007; Coan et al., unpublished) and recording site (Anokhin et al., 2006), with many of the inconsistent or low estimates likely due to suboptimal reference montages (Averaged mastoids/earlobes [Gao et al., 2009; Smit et al., 2007; Anokhin et al., 2006; Coan et al., unpublished] or active reference (Cz) [Coan et al., unpublished]). These suboptimal reference montages provide less focal estimates of power, including at each site broad-source alpha from both local and distal sources distorting estimates of heritability. Alternatively, a current source density (CSD) montage greatly attenuates any broad-source activity and provides more localized measurements of alpha activity, allowing for focal estimates of heritability.[1] Regardless of montage used, studies to date provide solid rationale for a continued investigation of FA as a possible endophenotype for the development of psychopathology.

[1] It is worth noting that if asymmetric propagation of these distal sources is itself heritable, then these sources could have contributed to extant heritability estimates and thus CSD-referenced data would show lower heritability than other reference schemes. Such a finding would raise serious questions about the utility of frontal EEG asymmetry as an endophenotype, but would be important results nonetheless.

Serotonin Genetics and Frontal EEG Asymmetry

Should frontal EEG asymmetry prove to serve as an endophenotype for MDD, it would hold advantages for genetic association compared to association studies using MDD as the phenotype. Given frontal EEG asymmetry's links to emotion and depression, a promising focus of genetic association studies would be on serotonergic polymorphisms. Several studies suggest that EEG asymmetry may be, at least in part, a reflection of underlying serotonergic neural function (Bismark et al, 2010; Bruder et al, 2008, 2001). Moreover, Allen et al. (2009) found that rapid plasma tryptophan depletion (TD), a procedure that transiently lowers brain serotonin, influenced frontal EEG asymmetry and that the magnitude of TD-induced change in frontal EEG asymmetry significantly predicted the development of depression during the next ensuing six to twelve months. A recent study using non-psychiatric volunteers demonstrated that in response to acute administration of the SSRI citalopram (celexa), individuals with the SERT "s" allele showed decreases in left frontal, pre-central and middle temporal gyri BOLD signal than individuals homozygous for "l" genotype (Smith, Lotrich, Malhotra, Lee, Ma, Kramer, et al, 2004). In animal models, 5HT has been shown to induce rapid increases in excitatory postsynaptic potentials (EPSPs) in pyramidal cells, predominantly in the medial PFC and other serotonin enriched frontal regions (Marek & Aghajanian, 1998). Given the complexity of brain chemistry, it is unlikely that any singular neurotransmitter system is responsible for a large percentage of the variance in EEG patterns displayed during emotion or depression. However, these studies provide good rationale to expect changes in frontal EEG activity as a result of serotonergic modulation.

Specific Serotonin Polymorphisms of Interest

Previous studies have shown an association between genetic polymorphisms in serotonin genes and individuals with depression or at risk for depression (Brown and Hariri 2006; Caspi et al 2003; Ogilvie et al 1996), but not without exception (Frisch et al, 1999; Kato 2007 (review)). Of primary interest for the current study are three genes that have shown some degree of relationship to MDD in prior work.

Perturbations (e.g., decreased serotonergic neuronal size, number or arborization) in serotonin system architecture following early postnatal stressors have been demonstrated in animal models and can lead to long-lasting systemic changes that increase the predisposition to depressive phenotypes later in life (Albert & Lemond 2004). For those individuals with underlying genetic diathesis, or early life stress, genes may play a role in determining patterns of both motivational and behavioral style (as indexed by frontal asymmetry) that predispose them to develop depression when confronted with stressful life events.

SERT**.** The Serotonin Transporter (SERT) gene SLC6A4, located on chromosome 17, has been extensively studied in relationship to depression. Research indicates that polymorphisms within this gene can greatly affect transporter efficacy. The most studied of these polymorphisms is the 44base-pair insertion/deletion within the promoter region of the SERT gene. This insertion/deletion (in/del) represents the presence of (insertion or "long" (l) allele) or absence of (deletion or "short" (s) allele) of 44 base pairs that influence the amount of transporter protein expressed. A protein binding study using ligand [^{125}I]RTI-55, found 30-40% greater membrane presence of the transport protein for the homozygous long allele (l/l) compared to heterozygous individuals (l/s) or homozygous short (s/s) individuals (Lesch, Bengel, Heils, Sabol, Greenberg, Petri, et al.1996). Additionally, they found 1.4-1.7 times greater concentrations of expressed mRNA and 1.9-2.1 times greater reuptake for homozygous l/l carriers compared to heterozygous

l/s or homozygous s/s individuals (Lesch et. al. 1996). This research points to polymorphic differences in serotonin transport availability. Increases in extracellular serotonin can produce long-term changes in overall serotonin system architecture and function. These changes (like addiction models of neuronal adaptation in response to chronic drug administration) can lead to down regulation of 5HT1a and 5HT1b receptors in pre-synaptic raphe neurons and 5HT1a receptors in postsynaptic limbic target neurons. Although not fully understood, this could lead to alterations in emotional processing when this already less efficient serotonergic system is further taxed under stress (Marek, 2007). The serotonin transporter has been implicated in liability for depression (Caspi et al. 2003; Risch et al. 2009) and anxiety (Caspi et al, 2010; Stein et al., 2007), particularly in response to stressful life events. Research also indicates that 5HTT genotypes play a role in response time and efficacy of SSRI treatment of depression (Arias et al., 2003; Durham et al. 2004), further warranting an investigation of it's relationship with the potential endophenotype FA.

5HTr1a. The 5HT1A receptor serves primarily as a regulator of serotonergic system activity, and 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). It has been hypothesized that 5HT1a genetic polymorphisms may affect the rate of pre- and post-synaptic receptor expression which, in turn, may affect the rate of negative feedback inhibition of the raphe leading to decreased serotonergic neurotransmission (Bismark et. al. 2010). Albert and Lemonde (2004) found that increased 5HT1a receptor concentrations in the raphe decreased serotonergic transmission to areas previously implicated in depression (such as PFC, hippocampal, and hypothalamic regions). A recent paper by Iritani and colleagues (2006), using

an animal model for mood disorders (using reserpine-treated rats), found a decreased concentration of 5HT1a receptors in the pyramidal cell layer of the hippocampus and the parahippocampal cortex, suggesting serotonergic receptor changes in animal models, which may be informative to human mood disorders. In human studies using positron emission tomography (PET), Drevets et al. (2007) found decreased binding potential of 42% in raphe neurons and 27% in mesiotemporal cortices of depressed subjects compared to controls. In addition, Lopez et al. (1998) reported decreased binding of 5HT1a postmortem and decreased physiological responses to 5HT1a agonists in vivo. This literature suggests effects of serotonergic receptor system dysfunction warrants evaluation the genetic contributions to this dysfunction and may prove to be one potential mechanism for FA.

5HTr2a. The serotonin 2a receptor gene was also of interest. Neuroscience research has indicted high concentrations of 5HT2a receptors in deep cortical layers, particularly in frontal and anterior cingulate cortices (Pazos et al., 1985; Fischette et al., 1987) and research has implicated thalamocortical circuitry in the alleviation of mood symptoms using 5HT2a antagonists (Marek et al, 2001). The FDA approved antidepressants mirtazapine, trazodone, and nefazodone, work at least partly by blocking 5HT2a. Interestingly, a common mechanism of action for atypical antipsychotics is the blockade of 5HT2a receptors. Osteroff and Nelson (1999) demonstrated that the addition of low-dose risperidone (a 5HT2a antagonist) to concurrent SSRI treatment significantly decreased remission time among depressed patients. Since then, most atypical antipsychotics have shown benefit as augmentation strategies for treatment individuals failing to improve with SSRIs or SNRIs alone. In addition, research suggests augmenting SSRIs with mirtazapine, increases the antidepressant efficacy of the SSRIs via selective antagonism of 5HT2a receptors in the pre frontal cortex (Celada, Puig, Amargós-

Bosch, Adell, & Artigas, 2004). With distribution of 5HT2a receptors in areas of frontal cortex linked with mood symptoms, and their potential antidepressant mechanisms, warrants the investigation of the 5HTr2A receptor genes' relationship with depression and FA.

Study Goals and Hypotheses:

This study focused on the investigation of the utility of frontal EEG asymmetry as an endophenotype for depression. This study also directly addresses methodological issues that may directly affect the interpretation of FA's utility as an endophenotype, particularly recording reference and gender differences. Lastly, this study provided a test with a large sample of whether the serotonergic neurotransmitter system related to the putative endophenotype of FA,. This last point illustrates the idea that biological difference may manifest as subtle intermediate risk diatheses that, in the face of life stress, may indirectly potentiate disease risk through cognitive and behavioral mechanisms.

For this study, it was hypothesized that FA will show modest heritability, with estimates near or above published estimates, with higher estimates of heritability for FA demonstrated for the CSD reference compared to the average (AVG), averaged linked-mastoid (LM), and CZ-channel reference montages. These hypotheses are predicted based on the greater specificity of the CSD reference at determining local EEG sources and sinks that minimizes distal alpha contributions, providing a more pure metric of FA. These estimates were calculated for both broad-sense (correlation-based) and narrow-sense (structural equation modeling, covariance-based) estimates of heritability.

The second study hypothesis involved the relationship between FA and allelic variations in specific serotonin genes. This study predicted that risk genotypes would be related to FA such that more negative (higher relative right frontal activity) co-segregate with single-nucleotide

variations previously associated with depression. Mixed linear models were used to test the relationship between FA and candidate genes across the frontal regions of interest.

Methods:

EEG data collection:

Subjects were instructed to sit quietly and maintain attention on the blank monitor in front of them, for 8 minutes while resting EEG data were recorded using a BioSemi Active Two system. The system uses an active reference scheme via a "referencing feedback loop" where one of the electrodes (common mode sensor; CMS) injects a small current into the subject to drive the "resting state" of the participant to the amplifier's zero-point. This voltage is monitored via another parietal-situated electrode that is passive (driven right leg; DRL). Data were referenced online using a CMS/DRL ratio. Data were recorded in DC mode from 61 scalp leads, 4 electrooculogram (EOG) recorded at the superior and inferior orbit of the left eye and outer canthi of each eye, and 2 ear-lobe electrodes with a sampling rate of 1048 Hz. Data were later down-sampled to 256 Hz and filtered with a .1hz high-pass Kaiser window prior to data processing. The data collection and initial preprocessing was performed on-site at the University of Minnesota. All subsequent data processing was performed at the University of Arizona.

Participants:

Exclusionary criteria for this study included history of head injury, left handedness, loss of consciousness exceeding 10min, concussion, epilepsy, electroconvulsive therapy and use of current psychotropic medications. The overall parent sample demographics were as follows: Caucasian (95%), Native American (1.41%), Asian (.26%), African American (.45%), Hispanic (1.54%), multiple ethnicities (.77%), and unreported (.26%).

Initially,1926 files were obtained for data processing. 1877 of those files were determined to have minimally usable data. The most common errors of the unusable files were blank EEG

records, no discernable EEG data within the file, or uncorrectable artifacts found in the file that were the likely result of recording errors or equipment malfunctions. The remaining 1877 files were subjected to data processing. During outliers analysis a further 365 individuals were removed due to an asymmetry score greater than three standard deviations from the mean at any channel pair used in the primary analysis, or for having their twin removed for outlier status. These non-outlier siblings were removed to provide complete data for more accurate modeling of the heritability analysis. The remaining 1512 individuals (756 pairs) were used for statistical analysis.

The final sample consisted of 480 MZ pairs, and 276 DZ pairs. MZ pairs consisted of 249 (52%) male pairs, of which 146 were 11years old at testing and the remaining 103 pairs were 17 years old. The female MZ pairs (n=231) consisted of 84 pairs of 11 year-old twins and 147 17-year-old pairs. DZ twin pairs consisted of 146 (53%) male twin pairs, of which 92 pairs were 11 years old and the remaining 54 were 17 years old at time of testing. The female DZ pairs (n=130) consisted of 54, 11 year-old pairs and 76, 17 year-old pairs.

Preliminary data reduction:

All EEG data files were visually inspected to remove movement and muscle artifacts and to document any channels where nonspecific artifacts consisted of greater than 15% of the total time for that file. All data reduction was then completed using EEGLAB, version 9.0.02b (Delorme & Makeig, 2004). Data were imported and stripped of all unessential channels (e.g. additional EMG channels included in original data collection), leaving 62 channels for all subsequent analysis. Bad channels were interpolated using a spherical spline approach (implemented in EEGLab),. Data were then segmented into two-second epochs overlapping by 1.5 seconds. This overlapping compensates for the minimal weight due to the taper that is

applied to the end of the epoch by the application of a Hamming window weighting function. Data were subjected to an automated blink rejection algorithm (rejecting segments where vertical ocular activity exceeded +/- 75mV), and a separate computer-based algorithm to reject large fast deviations in amplitude (e.g., DC shifts and spikes) that may have eluded human inspection. The range of epochs used in data processing ranged from 28-728 with an average of (s.d.) 232 (90). Separate reference montages were then computed for each file including re-references to CZ, Average whole-head power, linked mastoids, and finally using a current source density (CSD) approach using algorithms from Kayser and Tenke (2006) which are based on the spherical spline approach summarized by Perrin et al. (1989, 1990). Spherical spline current source density algorithms have demonstrated utility in the analysis of ERP and EEG data by adding specificity to ERP scalp topographies and minimizing volume-conducted activity in both types of signals (Kayser & Tenke, 2006; Tenke & Kayser, 2005). These reference-independent measures of CSD amplitude and power spectra are thought to more directly reflect underlying neuronal activity with less volume-conducted contributions. 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., 2010).

Power spectra were calculated via fast-Fourier Transform (FFT) (with a Hamming window) from all artifact free epochs. The power spectrum of interest is the Alpha band (8-13Hz). In addition to total Alpha frequency power, this frequency band was split into lower (8-10Hz) and upper (10-13 Hz) alpha bands and average power within each band will be taken as its index. Lastly, a total alpha asymmetry score was calculated by subtracting the natural log transformed scores for all symmetrical left and right locations, where left transformed values are always subtracted from its right homolog (i.e. ln[right]-ln[left]). Asymmetry scores were

calculated in in several ways with increasing regional specificity. Initially, averages of intra-hemisphere activity were calculated (e.g., Mean(F2,F4,F6,F8)), and those hemisphere averages were subjected to the same asymmetry calculated as described above. This provided a gross, hemisphere level measure of asymmetry for each twin. Medial and lateral averages were then calculated by averaging the two medial channels (e.g., F1, F3) and two lateral channels (e.g., F5, F7) for each hemisphere, then using those averages to calculate medial and lateral asymmetry scores. Lastly, the individual asymmetry scores were calculated to provide the greatest regional specificity for assessing heritability. Each of these metrics (hemisphere, medial, lateral and individual channels) was subjected to both Falconer's estimates and Structural Equation modeling to provide an increasingly focused investigation of the heritability and regional specificity for FA.

Statistical analysis:

All correlational and general linear modeling was performed in SPSS (version 20), IBM Corporation. Using these correlations, an initial broad sense heritability estimate was calculated using Falconer's formula estimates heritability (H_b^2) as $H_b^2 = 2(r_{mz} - r_{dz})$. As a heuristic, the logic of the classic twin study remains as follows: if the correlation between MZ twins is higher than that of DZ twins, then genetic factors are important for determining heritability. However, if the correlation between DZ twins is greater than half the correlation of the MZ twins, then common environment was an increasingly important factor in heritability. And if the correlation between MZ twins was less than one, then unique environmental factors are important in determining heritability.

However, correlations using same sex MZ twins will always be higher than their opposite sex DZ counterparts. In this case, gender differences are estimated to be heritable and can

confound results. Thus, only same sex pairs were used. Another factor in a heritability metric is the consideration for MZ twins reared together, which would make the relationship between genetic and common environmental factors more complicated to estimate, wherein twins reared apart, the r_{mz} is a more pure measure of additive genetic contributions. In addition to this, measures of non-additive genetic factors (i.e. genetic dominance or D) are indicated by r_{mz} that are greater than half of their DZ counterparts. However, when measuring twins reared together, these contrasting effects are confounded when estimating heritability only using ICCs. For example, if A is a true measure of additive genetic components, rDZ=.5rMZ. However, common environmental effects will inflate the rDZ above .5rMZ, but in contrast, dominant genetic effects will inflate the rMZ above twice that rDZ. These contrasting effects can partially cancel each other out with using estimates based solely on ICCs. The formulas below do not address the effects of dominance specifically but can indicate the type of model for use (i.e. ACE vs ADE) in more sophisticated structural equation modeling.

Having said that, it is possible to specifically estimate measures of additive genetic (A), non-additive genetic (D), shared environmental (C), and unique environmental (E) influences separately using only the MZ and DZ correlations. They are as follows:

$A = 2 (r_{mz} - r_{dz})$ or (σ^2_a)
$C = r_{mz} - A$ or (σ^2_c)
$E = 1 - r_{mz}$ or (σ^2_e)

Following Neale and Cardon (1992), the total variance would be $h_b^2 = A+C+E$ with a ratio of each individual component to the total providing the proportion of variance accounted for by that component. While this method provides a more detailed approach to measuring contributions to heritability by partitioning variance amongst the factors contributing to asymmetry; it still fails to take into account the covariance between A and C. Thus, more

sophisticated modeling is used to more accurately capture not only variance components contributing to asymmetry but also the covariance between those components that need to be statistically accounted for if we are to obtain an accurate heritability estimate.

To achieve this, more complex measures of heritability were modeled using structural equation modeling (SEM), performed within the open source statistical package R-Studio (version 0.97.551) using the OpenMX package (version 1.3.2-2301) (Boker, Neale, Maes, Wilde, Spiegel, Brick, et al., 2011, 2012). This method allows for the constraint of some relationships between twins (e.g. A, C/D) while allowing others to remain free (e.g. E) so that the modeled results more accurately represents the influences of the contributing factors on the heritability of frontal asymmetry. The structural equation model (see figure 1) contains both observed and latent variables, with the relationships between associated latent variables (A for twin 1 and twin 2) constrained to represent the hypothesized covariance between the factors. For example, identical twins share 100% of their genes, and twins reared together will share common environmental influences, thus in theory, the influences of these factors should be identical for each twin and their covariance should be 1. These model constraints will differ between models and are used to help explain the covariance between twins attributable to these latent factors. Initially, the model estimates an "ideal" covariance matrix based on these constraints, then compares the data-based covariance matrix to this "ideal model." The degree to which the data-based models align with the ideal model are represented by model fit statistics and the amount of variance explained by the latent variables are represented as unstandardized path coefficients. The standardized estimates are then calculated by taking a ratio of a given that path estimate to the total variance.

Results:

All analyses were run across each reference scheme, as it remains an empirical question

as to which reference montage provides the greatest measure of predictive validity for estimating the heritability of frontal asymmetry. While this may be an open question, generally any results that replicate across reference montages may be considered the most generalizable and less reflective of reference-specific method variance (cf., Campbell & Fiske, 1959). However, one goal of this study was to investigate this open question about the importance of methods for determining measures such as estimates of heritability. Thus, those results indicating method-specific internal consistency will be greatly informative to the developing body of literature. Additionally, evidence (Stewart et al., 2010) indicates possible sex differences in frontal asymmetry for men and women. Thus, all analyses were conducted separately for males and females.

Intra-class correlations/Falconer's Estimates:

Intra-class correlations (ICCs) within monozygotic (MZ) and dizygotic (DZ) twins were computed and input into Falconer's formula for estimating rough heritability of frontal EEG asymmetry (Tables 1 & 2). When intra-class correlations are estimated to be at or below zero, the correlation is assumed to be zero and this assumption was made in these analyses. Nonetheless, there were several aggregate correlations (Table 1) or individual channel correlations (Table 2) that were inestimable as either an inter-twin correlation was at or below zero or more commonly, that H^2 calculations were at or below zero. Occasionally, ICCs between DZ twins were higher than those of MZ twins. In these cases, the estimates of heritability produced by Falconer's formula are also less than zero and assumed to be zero. These instances provide more conservative heritability estimates and likely indicate complex interactions of genetic and environmental influences that may require more sophisticated statistical modeling to be clarified.

The ICCs and H^2s in table 2 indicate more consistency within references than between them, a result that seems to be moderated by gender. ICCs for MZ males were more consistent

than DZ males, which are likely the result of low (or in some cases) negative correlations between DZ twins. For females, the ICCs showed greater consistency across reference regardless of zygosity. According to Falconer's formula, heritability of frontal asymmetry is higher and more consistent for males compared to females, regardless of reference. Because the patterns of ICCs by zygosity did not indicate one type of model (ACE vs ADE) would be best suited for use in Structural Equation Modeling, both types of models were computed and compared for model fit.

Importantly, obtaining these diverse values all from the same data set indicates the importance of method variance, in addition to the trait variance of gender, in determining measures of heritability for frontal asymmetry. Methodologically, it should be noted that the CSD reference less often produced extreme outliers and thus allowed for the relative preservation of more data, potentially resulting in more stable heritability estimates.

Table 1: Intra-class correlation coefficients (ICCs) and estimates of heritability (H^2), based on Falconer's formula, of frontal asymmetry for MZ and DZ twins, by gender.

		Male			Female		
Reference	Channels	MZ	DZ	H^2	MZ	DZ	H^2
AVG	Aggregate	0.2		0.41	0.03	0.05	
	Lateral	0.2		0.4	0.08	0.02	0.13
	Medial	0.1		0.2		0.1	
CSD	Aggregate	0.12	0.1	0.02	0.08	0.12	
	Lateral	0.07	0.19		0.12	0.05	0.14
	Medial	0.13		0.25		0.21	
CZ	Aggregate	0.09		0.17	0.04	0.04	
	Lateral	0.1		0.21	0.05	0.01	0.1
	Medial	0.05		0.11	0.02	0.09	
LM	Aggregate	0.12	0.13		0.06		0.13
	Lateral	0.13	0.11	0.05	0.06		0.12
	Medial	0.09	0.1		0.04	0.05	

Aggregate refers to the average of all four frontal channels (F7/8, F5/6, F3/4, & F1/2). Lateral refers to the average of lateral channels (F5/6 & F7/8), and medial refers to the average of channels (F1/2 & F3/4).

Results from table 1 show ICCs and heritability estimates of FA by reference, gender, global hemisphere, and the average of the medial and lateral channels. The ICCs for the MZ and DZ twin pairs are inconsistent and may have significantly affect heritability estimates of traditional behavioral genetic estimates (i.e. rMZ=2rDZ). This is particularly evident in DZ males, where ICCs were frequently negative, regardless of reference. Heritability estimates for males were influenced by the predominately negative DZ ICCs while in females, they were influenced by the relatively higher ICCs for DZ compared to MZ twins, with the exception of the lateral pairs.

A very similar pattern of ICCs and heritability estimates arise when investigating the individual channel pairs compared to the hemisphere, medial and lateral averages (see Table 2). Again, for males, DZ ICCs were frequently negative, influencing heritability metrics for males across references. The heritability estimates were more consistent within each reference than across reference montages. In CZ, LM, and AVG lateral channels showed greater heritability, while in CSD, medial channels demonstrated greater heritability. In females, lateral channels demonstrated higher heritability estimates for AVG and CSD references, while CZ and LM references demonstrated greater medial heritability while maintaining weak lateral heritability estimates. The inconsistency of these results indicates a complicated picture and warrants investigation using more power statistical methods, such as structural equation modeling (SEM). Using SEM will allow for the parceling out of genetic and multiple environmental sources of variance on estimates of heritability to provide (potentially) more informative and stable estimates of FA.

Table 2: Intra-class correlation coefficients (ICCs) and estimates of heritability (H^2) of frontal asymmetry for MZ and DZ twins, by gender, for each frontal channel pair, and each reference scheme.

		Male			Female		
Reference	Channel Pair	MZ	DZ	H^2	MZ	DZ	H^2
AVG	F7/8	0.24		0.48	0.12	0.05	0.13
	F5/6	0.16		0.32	0.07		0.13
	F3/4	0.14		0.28	0.04	0.12	
	F1/2	0.10		0.20		0.11	
CSD	F7/8	0.12	0.08	0.09	0.19	0.03	0.33
	F5/6	0.03	0.23		0.04	0.05	
	F3/4	0.09		0.19	0.01	0.18	
	F1/2	0.13		0.25		0.10	
CZ	F7/8	0.11		0.21	0.10	0.09	0.02
	F5/6	0.09		0.17	0.01		0.01
	F3/4	0.09		0.17	0.11		0.22
	F1/2			---		0.12	
LM	F7/8	0.14	0.10	0.07	0.08	0.08	
	F5/6	0.12	0.04	0.16	0.03		0.06
	F3/4	0.06	0.07		0.07	0.03	0.09
	F1/2	0.11	0.11			0.05	

Channel F1/2 for the CZ reference was inestimable due to negative ICCs for both MZ and DZ twins.

Structural Equation Modeling (SEM):

In addition to Falconer's estimates, log likelihood latent variable modeling was used to compute path coefficients, variance components, and model fit statistics, to estimate the same heritability parameters as above. Since MZ ICCs were not consistently twice those of the DZ twins, both ACE and ADE models were run and evaluated to best establish which type of model best fit the data (See figure 1). All models were run using raw data as input for model fitting. As a reminder, within ACE models, three variance components are thought to influence the modeled variable: additive genetic components (A); common environmental influences (C); and unique environmental influences (E). In ADE models, both A and E components remain the same, but C is replaced by estimates of non-additive (i.e. Mendalian genetic dominance, D) genetic components.

Figure 1. Path diagrams for ACE and ADE models and the theoretical covariance weights between twin pairs for each latent variable.

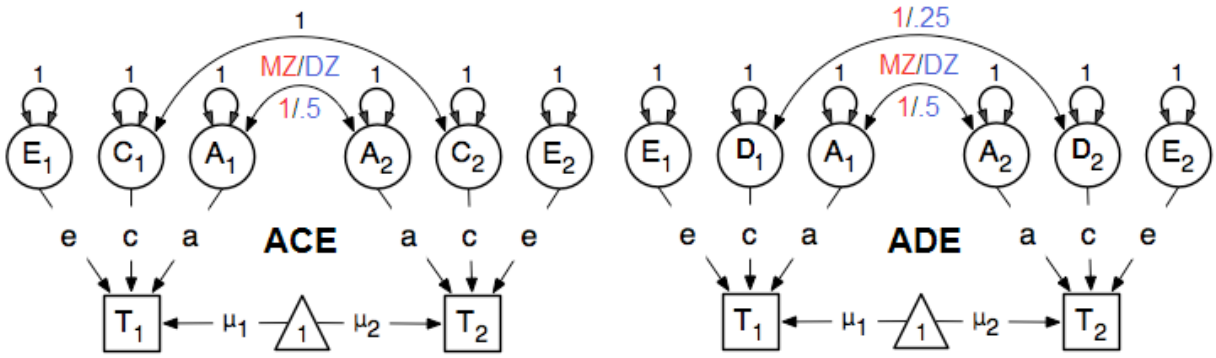

Figure reproduced with permission from Open Mx Genetic Epidemiology website: http://openmx.psyc.virginia.edu/ /1.2.0-1926/GeneticEpi_Path.html

All measures of model fit are based on model comparisons of the proposed model to the saturated model. Thus the fit statistics represent the current model compared to the "ideal model" based on the data. Thus, when measures of model fit (e.g. X^2 statistic) are statistically significant, the proposed model is judged to be a poor fit because the observed data are significantly different than the model specification. In addition to X^2, two other statistics are used to measure model fit, Akaike's Information Criterion (AIC) and the Root Mean Square Error of Approximation (RMSEA). The AIC statistic considers the number of unknown parameter estimates in its calculation, providing an additional estimate of model parsimony that is indicated with lower AIC values translating to relatively better model fit (Loehlin, 1998). The RMSEA is a population-based index, is relatively independent of sample size, and is also thought to be a measure of model parsimony. Perfect model fit as indicated by the RMSEA is zero, but values less than .05 are thought to indicate an "excellent" model, and RMSEA values less than .10 are thought to indicate "good" fit to the data (Loehlin, 1998, pp. 76-77). Overall, models fit the data reasonably well, and Appendix A presents tables detailing the model fit statistics for all models.

Standardized variance components were computed based on path diagrams using SEM (see figure 1) for each model type (ACE, ADE) and each reference montage (AVG, CSD, CZ,

LM), for each gender (Tables 1 & 2). These standardized variance components demonstrate how much variance is explained by the model for by each path, A, C, D, or E, and is interpreted as a measured of heritability. RMSEA values indicate model parsimony. Blank cells represent zero values, thus accounting for zero variance, and were removed for ease of interpretation.

Table 3: Standardized variance components (VARCOMPS) for each path (a^2, c^2, & e^2), and root mean square error of approximation (RMSEA) for each reference montage, for both males and females. Blank cells represent zero values, thus accounting for zero variance, and were removed for ease of interpretation.

		Males				Females			
Reference	Channels	a^2	c^2	e^2	RMSEA	a^2	c^2	e^2	RMSEA
CSD	F7/8	0.043	0.064	0.893	0.06	0.172		0.828	0.00
	F5/6		0.094	0.906	0.00		0.042	0.958	0.00
	F3/4	0.068		0.932	0.03		0.067	0.933	0.00
	F1/2	0.085		0.915	0.05		0.029	0.971	0.00
AVG	F7/8	0.183		0.817	0.04	0.122		0.878	0.02
	F5/6	0.13		0.87	0.00	0.053		0.947	0.00
	F3/4	0.09		0.91	0.01		0.068	0.932	0.02
	F1/2	0.011		0.989	0.07		0.039	0.961	0.07
CZ	F7/8	0.071		0.929	0.00		0.057	0.943	0.10
	F5/6	0.073		0.927	0.00			1	0.00
	F3/4	0.024		0.976	0.02	0.098		0.902	0.05
	F1/2			1	0.02		0.032	0.968	0.05
LM	F7/8	0.09	0.052	0.858	0.00		0.076	0.924	0.00
	F5/6	0.116		0.884	0.00	0.005		0.995	0.02
	F3/4	0.002	0.061	0.937	0.00	0.06		0.94	0.05
	F1/2	0.027	0.09	0.882	0.05		0.01	0.99	0.05

Table 4: Standardized variance components (VARCOMPS) for each path (a^2, d^2, & e^2), and root mean square error of approximation (RMSEA) for each reference montage, for both males and females. Blank cells represent zero values, thus accounting for zero variance, and were removed for ease of interpretation.

		Males				Females			
Reference	Channels	a^2	d^2	e^2	RMSEA	a^2	d^2	e^2	RMSEA
CSD	F7/8	0.112		0.888	0.06		0.186	0.814	0.00
	F5/6	0.072		0.928	0.02	0.044		0.956	0.00
	F3/4		0.087	0.913	0.03	0.048		0.952	0.00
	F1/2		0.104	0.896	0.04	0.016		0.984	0.00
AVG	F7/8		0.226	0.774	0.02	0.035	0.095	0.87	0.02
	F5/6		0.15	0.85	0.00		0.058	0.942	0.00
	F3/4		0.12	0.88	0.00	0.067		0.933	0.03
	F1/2		0.013	0.987	0.07	0.025		0.975	0.07
CZ	F7/8		0.091	0.909	0.00	0.056		0.944	0.10
	F5/6		0.082	0.918	0.00			1	0.00
	F3/4		0.058	0.942	0.01		0.11	0.89	0.05
	F1/2			1	0.02	0.015		0.985	0.05
LM	F7/8	0.149		0.851	0.00	0.083		0.917	0.00
	F5/6	0.017	0.107	0.876	0.00		0.018	0.982	0.02
	F3/4	0.072		0.928	0.00	0.022	0.04	0.938	0.05
	F1/2	0.133		0.867	0.06			1	0.05

Table 5: Standardized variance components (VARCOMPS) and RMSEA for each path (a^2, c^2, & e^2) for each reference montage, 11 and 17 year old MALE twins. Blank cells represent zero values, thus accounting for zero variance, and were removed for ease of interpretation.

Males		11 y/o				17y/o			
Reference	Channels	a^2	c^2	e^2	RMSEA	a^2	c^2	e^2	RMSEA
CSD	F7/8	0.105	0.034	0.861			0.070	0.930	0.10
	F5/6		0.092	0.908	0.07		0.084	0.916	
	F3/4		0.009	0.991	0.03	0.142		0.858	0.09
	F1/2	0.021		0.979	0.04		0.114	0.886	0.09
AVG	F7/8	0.177		0.823	0.09	0.060		0.940	0.14
	F5/6	0.164		0.836		0.135		0.865	0.02
	F3/4	0.072		0.928	0.03			1	0.14
	F1/2		0.033	0.967	0.23			1	0.16
CZ	F7/8	0.063		0.937		0.075		0.925	
	F5/6		0.058	0.942		0.078		0.922	
	F3/4	0.015		0.985		0.004		0.996	0.11
	F1/2			1	0.03			1	
LM	F7/8		0.082	0.918	0.05	0.199		0.801	0.02
	F5/6	0.071		0.929	0.06	0.146		0.854	
	F3/4		0.077	0.923	0.03	0.028		0.972	
	F1/2	--	--	--	--	--	--	--	--

NOTE: Models for channel F1/2 for the LM reference was under identified making VARCOMPS and RMSEA values inestimable.

Table 6: Standardized variance components (VARCOMPS) and RMSEA for each path (a^2, c^2, & e^2) for ach reference montage, 11 and 17 year old FEMALE twins. Blank cells represent zero values, thus accounting for zero variance, and were removed for ease of interpretation.

Females		11 y/o				17y/o			
Reference	Channels	a^2	c^2	e^2	RMSEA	a^2	c^2	e^2	RMSEA
CSD	F7/8	0.186		0.814		0.164		0.836	0.06
	F5/6		0.024	0.976	0.11		0.047	0.953	
	F3/4		0.174	0.826			0.020	0.980	
	F1/2		0.016	0.984	0.07		0.022	0.978	
AVG	F7/8			1	0.12	0.137		0.863	0.13
	F5/6	0.124		0.876	0.26			1	
	F3/4	0.148	0.035	0.817	0.09			1	0.13
	F1/2			1	0.16			1	0.17
CZ	F7/8			1			0.146	0.854	0.07
	F5/6			1	0.04			1	
	F3/4		0.061	0.939	0.08	0.105		0.895	0.05
	F1/2		0.067	0.933	0.14			1	0.12
LM	F7/8	0.027	0.036	0.936			0.078	0.922	
	F5/6	0.090	0.022	0.887	0.06			1	
	F3/4	0.094		0.906	0.14	0.029	0.018	0.954	0.06
	F1/2	--	--	--	--	--	--	--	--

NOTE: Models for channel F1/2 for the LM reference was under identified making VARCOMPS and RMSEA values inestimable.

Table 7: Standardized variance components (VARCOMPS) and RMSEA for each path (a^2, d^2, & e^2) for each reference montage, 11 and 17 year old MALE twins. Blank cells represent zero values, thus accounting for zero variance, and were removed for ease of interpretation.

Males		11 y/o				17y/o			
Reference	Channels	a^2	d^2	e^2	RMSEA	a^2	d^2	e^2	RMSEA
CSD	F7/8	0.143		0.857		0.073		0.927	0.10
	F5/6	0.057		0.943	0.08	0.071		0.929	
	F3/4			1	0.03		0.203	0.797	0.08
	F1/2		0.065	0.935	0.04	0.123		0.877	0.09
AVG	F7/8		0.199	0.801	0.08		0.146	0.854	0.14
	F5/6	0.099	0.068	0.833			0.167	0.833	
	F3/4		0.102	0.898	0.02		0.049	0.951	0.14
	F1/2	0.031		0.969	0.23			1	0.16
CZ	F7/8		0.081	0.919			0.094	0.906	
	F5/6	0.064		0.936			0.100	0.900	
	F3/4		0.031	0.969			0.069	0.931	0.11
	F1/2			1	0.03		0.011	0.989	
LM	F7/8	0.085		0.915	0.05	0.075	0.130	0.794	0.01
	F5/6		0.080	0.920	0.06	0.129	0.019	0.852	
	F3/4	0.079		0.921	0.03		0.033	0.967	
	F1/2	--	--	--	--	--	--	--	--

NOTE: Models for channel F1/2 for the LM reference was under identified making VARCOMPS and RMSEA values inestimable.

Table 8: Standardized variance components (VARCOMPS) and RMSEA for each path (a^2, d^2, & e^2) for each reference montage, 11 and 17 year old FEMALE twins. Blank cells represent zero values, thus accounting for zero variance, and were removed for ease of interpretation.

Females		11 y/o				17y/o			
Reference	Channels	a^2	d^2	e^2	RMSEA	a^2	d^2	e^2	RMSEA
CSD	F7/8		0.196	0.804			0.178	0.822	0.06
	F5/6	0.025		0.975	0.10	0.049		0.951	
	F3/4	0.147		0.853		0.010		0.990	
	F1/2	0.001		0.999	0.09	0.007		0.993	
AVG	F7/8			1	0.12		0.163	0.837	0.13
	F5/6		0.171	0.829	0.26			1	
	F3/4	0.188		0.812	0.09			1	0.13
	F1/2			1	0.16			1	0.17
CZ	F7/8			1		0.162		0.838	0.07
	F5/6			1	0.04			1	
	F3/4	0.050		0.950	0.08		0.127	0.873	0.05
	F1/2	0.064		0.936	0.15			1	0.12
LM	F7/8	0.068		0.932		0.083		0.917	
	F5/6	0.114		0.886	0.06			1	
	F3/4		0.106	0.894	0.13	0.048		0.952	0.06
	F1/2	--	--	--	--	--	--	--	--

NOTE: Models for channel F1/2 for the LM reference was under identified making VARCOMPS and RMSEA values inestimable.

Addressing hypothesis 1, results indicate that frontal EEG asymmetry is indeed heritable, but to a smaller extent than previously reported. The discrepant results could be due to a number of methodological factors (reference montage and model type), and demographic factors (gender and age), or their interaction. Regardless of gender or reference montage, FA at lateral channels was modeled as more heritable than at medial channels, and there was no significant difference in model fit between ACE and ADE models. However, gender, reference montage, and model type proved to be important factors in determining estimates of heritability. Regardless of these factors, the vast majority of variance was accounted for unique environmental influences, ranging from 77-100% in males and from 81-100% in females. Overall, genetic factors accounted for 0-22.6% of the variance in males and 0-18.6% for females.

Within ACE models, lateral channels accounted for greater proportions of variance than medial channel pairs. This is consistent with the model fit parameters and Falconer's broad sense heritability estimates. Males demonstrated greater estimates of heritability of FA across channels and reference with a heritability range between 0-18.3%, compared to a range heritability in females of 0-17.2%. Within each reference some proportion of the variance was accounted for by additive genetic factors, unlike females whose variance was accounted for by larger common environmental factors compared to additive genetic factors. In males, within the CSD and LM montages, there existed estimates of A, C, and E. Whereas, for AVG and CZ montages for males, and all montages for females, proportions of variance were estimated as A or C, but not both. This suggests that gender plays a significant role in estimation of heritability for frontal asymmetry, with males showing greater additive genetic contributions with females showing a mixture of additive genetic and common environmental contributions (Table 1).

ADE model results display patterns that indicate the heritability of FA in males is attributed to primarily non-additive genetic factors, while females heritability estimates are a combination of additive and non-additive factors, depending on the reference montage. Like their ACE model counterparts, genetic estimates accounted for more variance at lateral channels compared to medial ones, across genders and references. This is again consistent with ADE model fit parameters and Falconer estimates of broad sense heritability. Males also demonstrated greater estimates of heritability of FA, across channels and reference, compared to females.

For males, estimates of heritability related to genetic factors ranged from 0-22.6%, with a similar range in females of 0-18.6%. For males, the AVG reference displayed the highest and most consistent estimates of heritability, attributing all genetic variance to non-additive factors with a range of 1.3-22.6%. The CZ reference also displayed exclusively non-additive genetic

variance but had the lowest overall heritability, ranging from 0-9.1%. The LM reference in males displayed a range of heritability from 7.2-14.9% with nearly all variance being accounted for by additive genetic factors except for channel F5/6, where the 12.4% variance was a combination of additive and non-additive genetic influences. Lastly, for CSD-transformed data in males, the range of heritability was 7.2-11.2% and was split such that lateral channels greater variance attributed to additive genetic factors, while medial channels were explained by non-additive genetic factors (Table 2, middle column). For females genetic estimates for heritability were more mixed, with each reference montage consisting of some combination of additive and non-additive variance. The CSD montage displayed the highest overall heritability estimates, with F7/8 accounting for 18.6% of the variance attributable to non-additive genetic factors (D), and all other channels displaying additive variance between 1.6-4.8% explained. The AVG reference displayed a range of explained variance between 2.5-13% with most of the explained variance for the lateral channels attributed to no-additive factors and the variance for the medial channels attributed to additive genetic factors (A). Both CZ and LM references were mixed, each having one channel with zero variance attributable to genetic factors (CZ: F5/6 & LM: F1/2), with an overall range of modeled variance between 0-11%. These references displayed the poorest model fit and these estimates should be interpreted with caution (Table 12, right column).

In addition to reference and gender as moderators of heritability, age was also investigated. While not specifically hypothesized, research has indicated that age may have an effect on estimates of heritability, with traits maintaining or increasing in heritability over time (McGue & Christensen 2013; Bergen, Gardner & Kendler. 2007; Edwards, Sihvola, Korhonen, Pulkkinen, Moilanen, Kaprio, Rose, & Dick. 2011). Models for each age group were duplicated with estimates at each age group showing some consistencies as well as

inconsistencies with models of pooled age. The age moderated models showed far less consistency and poorer fit, across age, reference and gender, than their pooled-age counterparts. Additionally, LM models for F1/2 were inestimable due to model unreliability and underidentification, likely due to the small ns. Thus, the results from the age-moderated results should be interpreted with caution. (See appendix A tables A9-A16). Overall, lateral channels remained more heritable than medial channels, particularly for males. In addition, non-additive genetic models were more consistent across age group and gender (Tables 13-16).

Males demonstrated an age by reference interaction, such that for additive models, CSD and AVG montages showed decreasing heritability with age, while heritability increased in age in CZ and LM references. However, in non-additive models (ADE) there was a mixture of heritability patterns in CSD and AVG reference montages, while in CZ and LM montages, heritability generally increased as a function of age. For males, 11 y/o twins revealed estimates of heritability from 0-17.7%, while the 17y/o's showed similar ranges 0-19.9% with the AVG reference showing the greatest consistency. For non-additive models, younger males' estimates ranged from 0-19.9%, and older males from 0-20.5%, again with the AVG reference demonstrating the greatest cross-channel consistency.

Within females, results were more mixed with estimates increasing and decreasing across ages within all references and regardless of additive vs non-additive models. Female ACE models revealed variable heritability estimates with younger female estimates ranging from 0-18.6% and older females between 0-16.4%. However, 11yo females were shown to have no genetic heritability for frontal asymmetry for the CZ reference, with all variance accounted for by non-shared environmental factors. ACE models for older females indicate only one channel (at varying locations) for each reference demonstrating genetic heritability. For ADE models,

heritability ranged from 0-19.6% in the younger females to 0-17.8% in the older females, with the CSD montage revealing the largest and most consistent estimates. (Tables 5 & 6).

Genetic Analysis:

Mixed linear modeling, using a random intercept at the level of twin pairs, was used to evaluate the association between measured and imputed genetic variations in serotonin transporter, serotonin receptor 1a and 2a genes with the aggregated hemisphere, medial, and lateral metrics of frontal asymmetry as well as the individual channel pairs of F7/8, F5/6, F3/4 and F1/2 for each reference montage. It should be noted that although the *genes* of interest themselves (5htr1a, 5htr2a, and SERT) were sampled in the genetic analysis, only one of the proposed SNPs of those genes (rs6313), previously linked to depressive risk was specifically sampled in the parent genetic analysis. The genome wide association study (GWAS) procedure did not include these specific loci in the genetic sampling battery and the proposed markers were not imputed based on the sampled data. Thus, most of the proposed hypotheses linking specific genetic polymorphisms (rs25531, rs25532, rs6295) to FA, were not able to be completed. The SNP that was proposed, and included in the parent genetic sampling (rs2695), was not significantly related to FA. The genetic data of the parent study primarily included many SNPs that were not originally hypothesized as risk SNPs for the current study, and of those that were collected, few were linked to risk for mental illness. Thus all genetic analysis relating these genetic markers to FA were exploratory.

Addressing hypothesis II, there were no significant main effect between any serotonin genetic variations and any metric of frontal asymmetry, for any reference montage, after correcting for skew in allele distribution within each gene. All $Fs<2.69$, $ps >.05$. In addition, there were no significant gene x sex, gene x zygosity, or gene x zygosity x sex interactions predicting frontal asymmetry after correcting for skew in allele distribution within each gene

loci. This was likely due to uneven allele distributions within the loci investigated, such that one allele would usually be associated with only a few subjects while another would be heavily represented within the sample.

Overall, the results for heritability of FA suggest that previous estimates of heritability may have been upper bounds, and that there are important moderating factors that influence these estimates. The current study of the heritability of frontal EEG asymmetry, the largest sample to date, indicates that gender and age can have a substantial influence on estimates of heritability, in addition to the method variance associated with reference montage used in data processing. Non-additive models (ADE) and genetic results further indicate a complex and likely epigenetic relationship, between genetic risk markers and FA, that likely influence measures of heritability.

Discussion:

This study focused on a continued investigation of the utility of frontal EEG asymmetry as an endophenotype for depression, with particular emphasis on its heritability and serotonergic genetic influences. More specifically, this study was designed to replicate estimates of the heritability of FA and examine methodological factors that may be directly relevant to the interpretation of FA's utility as an endophenotype, particularly recording reference, gender and age moderation. This study also was designed to replicate prior research linking FA to genes involved in serotonergic reception and transport. The overarching goal of this study was to provide a more definitive test of the argument that FA, an established risk factor for depression, is heritable and associated with specific depression-related genetic variation.

This study is the largest behavioral genetic investigation of the heritability of FA to date. It illustrates the utility of FA as an endophenotype but also some of the challenges surrounding the endophenotype approach, namely methodological, age, gender, and genetic challenges that

plague these studies. Of particular methodological interest, is the fact that all the heritability results are derived from the same dataset and differ only by the reference used to process the data. Thus, differences in estimates of broad sense heritability determined by Falconer's formula and the ACE/ADE modeling computed in SEM are (at least theoretically) attributable directly to the varying methodological impacts of varying reference montages. Previous research indicates that choice of electrode reference may play a significant role in study outcome (Stewart et al. 2010; Hagemann 2004). The CSD reference is thought to provide the most focal link to activity generated by the frontal cortex, while minimizing nonfrontal sources whose activity may be reflected in other reference montages such as CZ, LM, or AVG (Hagemann 2001). However, to date, no study of heritability of frontal EEG asymmetry has investigated the differing effects of EEG reference montage on heritability estimates. The current study thus suggests that previous estimates may be potentially inflated by the inclusion of alpha activity from non-frontal generators (Anohkin et al, 2006; Smit et al. 2007). Although the current study's estimates of heritability were low to moderate, they were within the range of other published estimates (Gao et al, 2009).

While the estimates of heritability for the current study were modest, asymmetries between contralateral power spectra may be informative as to how biological diatheses can be affected by environmental changes to predict a risk for disease development over time. Given the lack of specific genetic associations and the high proportions of variance attributed to environmental influences in the current study, FA may still have utility as a risk marker for depression (Stewart et al. 2010), but one that is primarily non-genetic in nature. The variance component estimated in the current study indicate a minority of risk tapped by FA is genetic, but more importantly that FA is primarily influenced by environmental effects. This implies a

modest underlying genetic diathesis that can be aggravated by early environmental influences to impact FA in a trait-like fashion, given its stability estimates in the literature (Hagemann et. al 2002).

The developmental literature holds some clues about the development of FA as a potential risk factor with both genetic and environmental components. FA studies investigating infants of depressed mothers revealed that the infants showed relative left frontal hypoactivity when interacting with their mothers as well as familiar, non-depressed adults (Dawson et al. 1999). Another study found that infants of depressed mothers exhibited relatively less left frontal activity that was related to decreased levels of affection and physical interaction with their mothers during mother-infant play (Dawson, Frey, Self, Panagiotides, Hessl, Yamada, & Rinaldi, 1999). While infants of symptomatic depressed mothers show relative less frontal activity during infant-maternal playtime, they also demonstrate less distress and less relative right frontal activity during maternal separation, compared to asymptomatic mothers (Dawson, Klinger, Panagiotides, Hill, & Spieker, 1992). These studies highlight the trait-like nature of FA and the potentially observable emotional and behavioral sequelae evident at an early age. In a sample of three year-olds, children with chronically depressed mothers exhibited overall lower frontal brain activity and increased behavioral problems compared to children of never depressed mothers or those with remitted depression. Interestingly, the child's brain activity and levels of contextual risk (e.g. stress and marital discord) mediated the relationship between maternal depression and child behavioral problems (Dawson, Ashman, Panagiotides, Hessl, Self, Yamada, & Embry, 2003). Taken together, these results indicate the subtle heritability of FA, but more importantly its sensitivity to environmental factors (i.e. parental discord, emotional challenge, and perceived caretaker) in predicting emotional and behavioral responses. These early environmental

influences could invoke epigenetic mechanisms that continue to establish trait-like diatheses of FA through the brain maturation process, and further sensitize the individual to the development of depression under future life stress.

The current study sought to further investigate FA as a potential risk marker as well as its moderating factors. The results indicate that, on average, frontal-lateral channels demonstrated higher heritability estimates than medial channels, but these results were moderated by, gender, age, reference montage, and the type of genetic influence modeled (ACE vs ADE models). These moderating factors indicate useful methodological considerations for future studies. When considering strictly additive genetic influence, males' frontal asymmetry displayed varying degrees of heritability from zero to modest estimates (0-18.3%) with similar heritability ranges in females (0-17.2%). However, the inclusion of non-additive genetic factors increased heritability estimates in both genders (males (0-22.6%); females (0-18.6%)) but more substantially in males. Interestingly, the inclusion of non-additive genetic factors to either model did not remove all variance from any channel displaying additive factors (that weren't already zero), but rather specified the type of genetic variance accounting for the heritability with genetic variance being shared between additive and non-additive factors. Comparing male ACE to ADE models, genetic estimates increased overall in ADE models but non-additive estimates account for more variance than their non-additive counterparts. Females display an interesting pattern when comparing ACE and ADE models such that when non-additive genetic effects are modeled in addition to the additive effects, overall genetic effects increase slightly but with a far larger proportion of genetic variance attributable to the additive influences, specifically increasing the number of channels displaying genetic heritability while keeping any channel's genetic variance proportion to the entire model relatively stable.

Despite the low to modest genetic contributions, the majority of the variance in any model, for both genders, for all references, was attributed to unique environmental influences. This is in line with previous findings of other individuals of roughly the same age range (Gao et al. 2009). Additionally, given that none of the variants of the genes tested were individually related to frontal asymmetry, it may be that several genes contribute small amounts of risk and only through their combination (i.e. risk haplotype) does the relationship with FA reach statistical significance. This remains an open question in the current dataset. Alternatively, the current findings may indicate more complex epigenetic relationships that moderate, and are moderated by, unique environmental contributions (Caspi et al 2010; Byers, Levy, Kasi, Bruce, & Allore. 2009). However, not all studies find that common environment affects aspects of genetic heritability (Smit et al, 2005, 2007), although the Smit et al. (2005) study investigated EEG power and Smit et al. 2007 was investigating asymmetry. Estimates of heritability of EEG power are generally higher than those for asymmetry, particularly the alpha band (Zietsch, et al. 2007). However frontal asymmetries may be useful as potential risk markers by providing clues about underlying lateralization differences in information processing. Subtle inter-hemispheric differences in EEG power, as demonstrated by asymmetries, and the heritability of those asymmetries, may prove to be informative of Carver and White's conceptualization of Gray's behavioral activation/inhibition system theory and can be a source for future research. If the ultimate goal is to utilize FA to predict behavioral and motivational tendencies in the face of life stress, then changes in FA could prove to be a useful neuro-bio-behavioral risk marker for depression.

Previous research has suggested that the heritability of FA changes over time, displaying higher heritability in young adult twins, but not in middle age twins (Smit et al. 2007). A recent

study of the heritability of depressive symptoms, whose samples consisted of twin cohorts in age nearly identical to the current sample, indicated stability of heritability for depressive symptoms across adolescence with estimates between 40-50% across age groups (Edwards Sihvola, Korhonen, Pulkkinen, Moilanen, Kaprio, Rose, & Dick. 2011). These age group differences in heritability are likely the result of complex epigenetic and gene x environment interactions upon brain development and maturation. Research has demonstrated that frontal areas continue to develop into early adulthood, with substantial individual differences in the rate of brain development (Casey, Geidd, & Thomas, 2000). These individual differences in brain development may indicate the importance of the unique environment in determining risk, in addition to the subtle, yet non-trivial biological aspects within the current sample. As these individuals have not begun or passed through the highest period of risk for developing depression, the full effects of the unique environmental influence upon genetic diathesis may have yet to occur and the window of adolescence into early adulthood may prove to be a very important window for using frontal EEG asymmetry as a predictive marker. This may, in part help to partially explain the results of age in the current study, and would suggest the utility of obtaining careful measures of environmental stressors during this period to investigate the interaction of FA and stressors in predicting depressive onset.

Given the large sample size, methodological specificity, and attention to important moderating factors of age and gender, the current study should provide the best estimates of the heritability of FA to date, and are within the low to moderate range. Although these estimates are within the lower range of those reported in the literature, the current estimates may represent more focal and representative heritability estimates of frontal asymmetry. This is due to larger

samples and varying reference montages, particularly for the CSD montage, as other montages will include substantial non-frontal influences.

Limitations:

A few limitations of the current study should be noted. First, negative or zero ICCs were observed in DZ males for several reference montages as well as negative ICCs for channel F1/F2 in females across all references. These negative relationships may have falsely inflated broad-sense heritability estimates among the affected references and/or channels. Although outlier analysis was computed and all outlier's greater than 3 standard deviations outside the mean were rejected (see figures A1-A3), the negative relationship persisted within the data. Although previous reports indicate that averaging across eyes open and eyes closed made no appreciable difference on calculating and measuring FA (Stewart et al. 2010), it is possible that during the eye open condition that the environmental stimulation of the recording room may have affected the alpha power non-systematically. Additionally, despite the large n, there was still significant data loss due to reasons outlined previously, most significantly was the loss of a dyad due to data integrity problems in one twin, necessitating the removal of the pair from the analysis. Although this is large sample, it derives from a parent investigation of long-term substance use with only minimal focus on depression risk. Prior research has indicated early alcohol and substance use significantly predicts later psychiatric disorders (Brook, Brook, Zhang, Cohen, & Whiteman, 2002) the current sample was not specifically oversampled to capture the full spectrum of depression. Lastly, the demographic distribution of the parent sample was made up of a primarily Caucasian sample. Unfortunately, individual racial and ethnicity characteristics were not included as potential model moderators. Given the racial/ethnic sample demographics of the sample, their inclusion is unlikely to have appreciably impacted the results. However, research has shown there can be important racial and ethnic differences related to mood disorder risk

(Breslau, Aguilar-Gaxiola, Kendler, Su, Williams, & Kessler, 2006) that, given the skewed sample demographics, may have been obscured within the genetic and electrophysiological results of the present study.

Conclusion:

In conclusion, this study extends previous findings with adolescent and adult populations, has highlighted the importance of recording reference, gender, and age to show how these aspects can affect estimates of heritability. The current study finds that FA is modestly heritable but with estimates at the low end of those previously published, with moderators including age, gender, and recording reference. Estimates of heritability, for both ACE and ADE models were higher for CSD and AVG reference montages compared to LM and CZ references. There were also significant gender effects with males demonstrating slightly higher overall heritability of FA, but primarily additive genetic contributions in ACE models where females exhibited greater common environmental heritability within he same models. For ADE models, male's estimates were again higher then females' but consisted primarily of non-additive genetic contributions; with females demonstrating more distributed additive and non-additive genetic contributions. The data also indicate substantial environmental influences on FA, with unique environmental influences accounting for the vast majority of variance across all models. However, age was also an important factor in estimating heritability, with the proportions of variance of genetic and environmental influences changing across age groups. Unlike previous reports (Bismark et al. 2010), no relationship was found between FA and specific serotonergic alleles. This evidence, considered collectively, suggests that FA may be a useful risk indicator, but that it may not index extensive genetic risk, instead indexing non-genetic factors particularly relating to early life history events.

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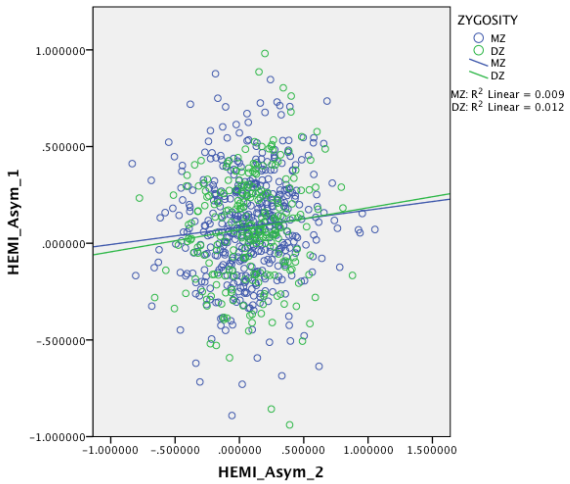
FIGURES APPENDIX:

Figure A1: Averaged aggregate (F7/8, F5/6, F3/4, F1/2) asymmetry correlations between twins for each zygosity at each reference montage. Panels represent reference montages: CSD(A), AVG (B), LM(C), and CZ(D).

A



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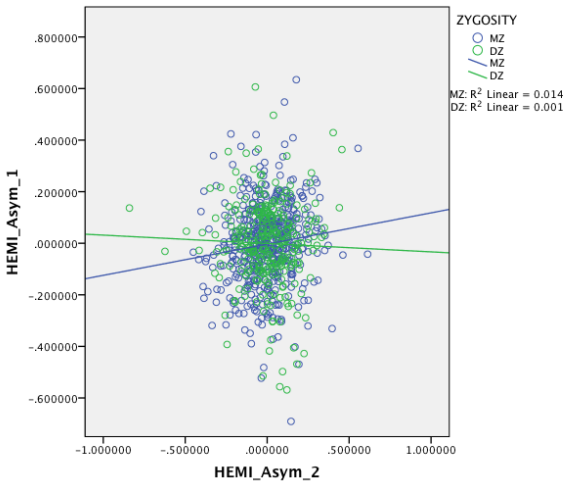

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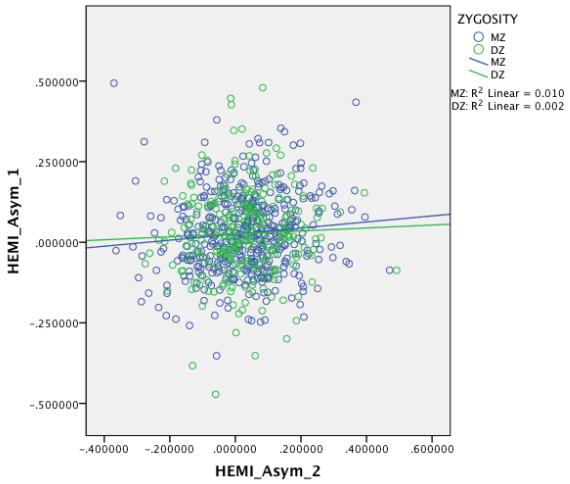

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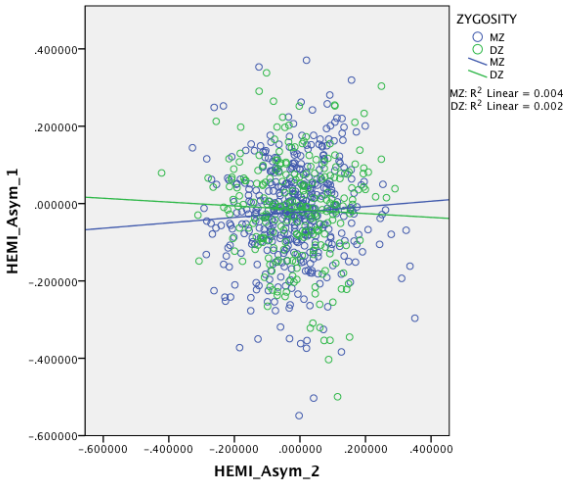
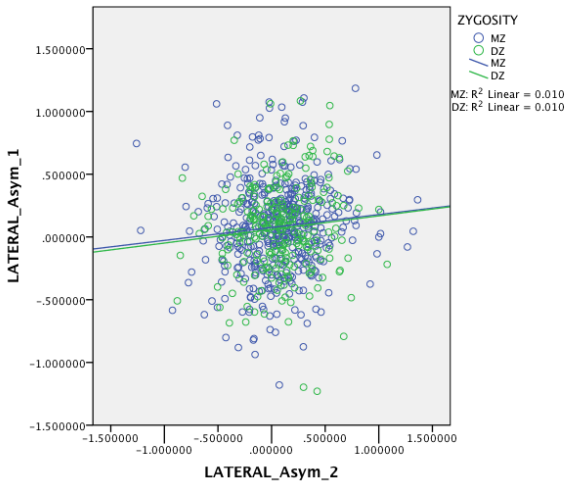


Figure A2: Averaged lateral (F7/8, F5/6) asymmetry correlations between twins for each zygosity at each reference montage. Panels represent reference montages: CSD(A), AVG (B), LM(C), and CZ(D).

A



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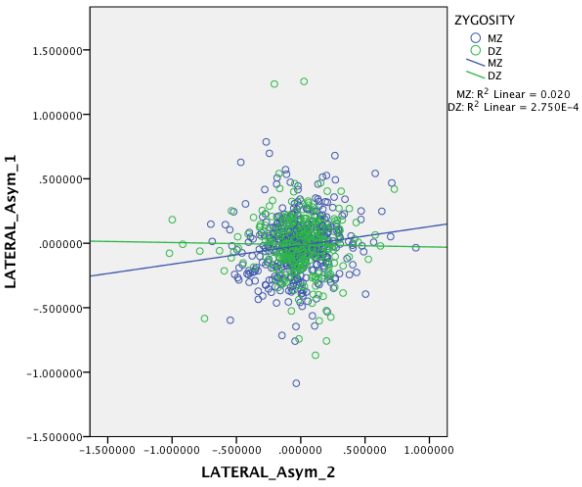

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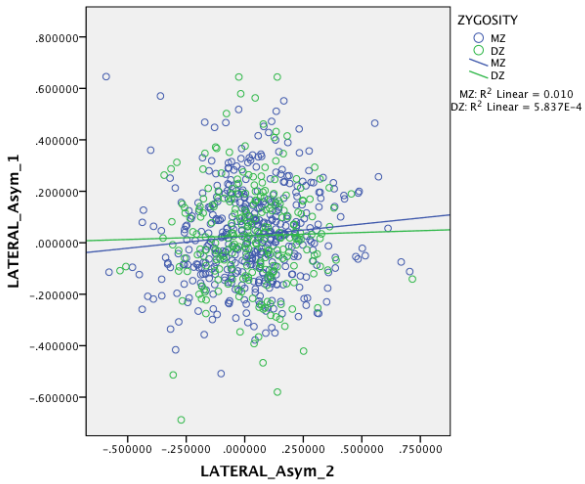

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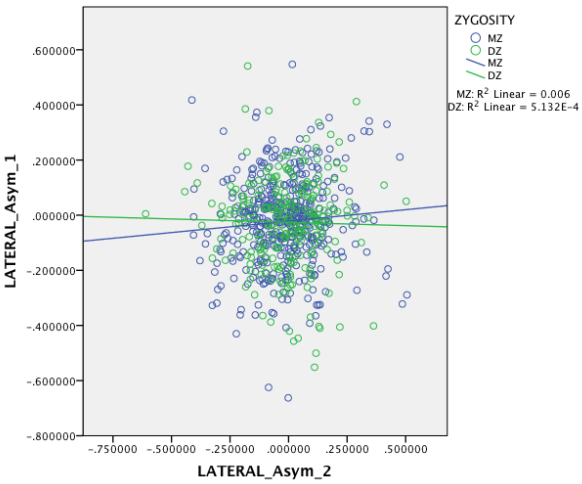
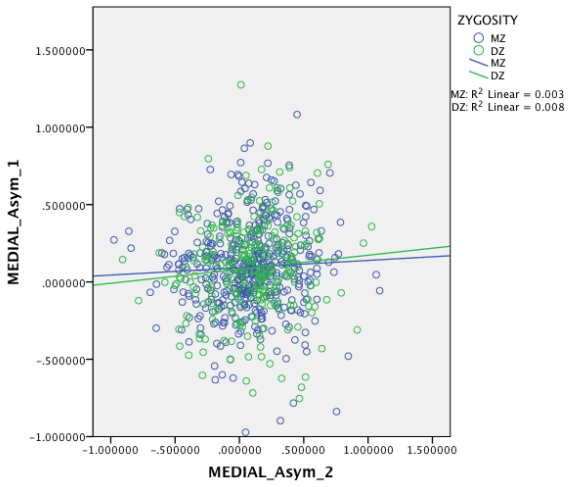


Figure A3: Averaged medial (F3/4, F1/2) asymmetry correlations between twins for each zygosity at each reference montage. Panels represent reference montages: CSD(A), AVG (B), LM(C), and CZ(D).

A



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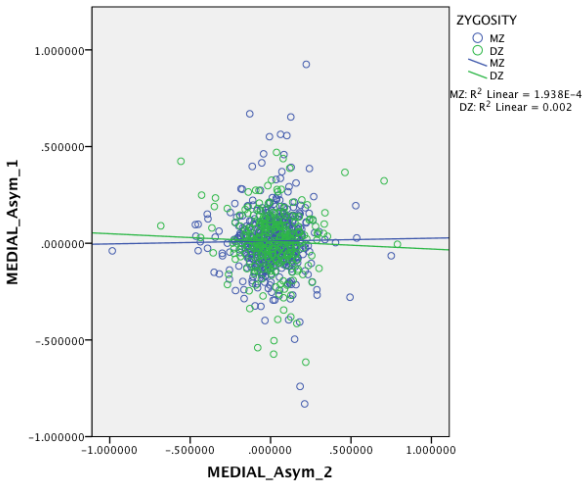

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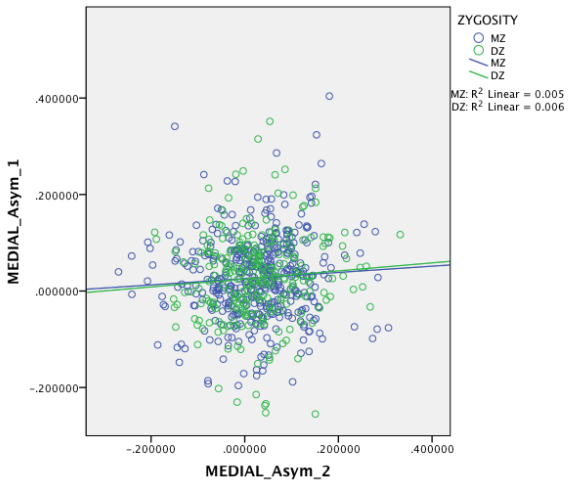

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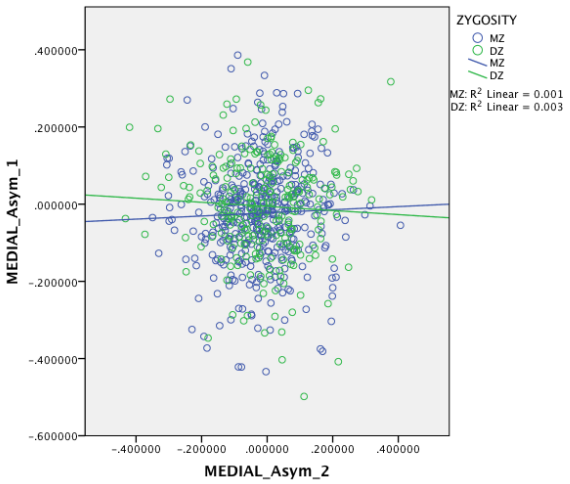

TABLES APPENDIX:

Table A1: Model fit statistics for ACE models of MALE twins estimating heritability of frontal asymmetry for the average hemisphere, medial and lateral channel pairs.

Reference	Model	Channel	X^2	df	-2LL	p	AIC	RMSEA
CSD	ACE	Aggregate	6.34	768	23.24	0.39	-5.66	0.01
		Medial	10.72		172.59	0.1	-1.28	0.04
		Lateral	8.56		356.03	0.2	-3.43	0.03
AVG	ACE	Aggregate	8.56	794	-747.43	0.2	3.43	0.03
		Medial	11.04		-807.41	0.09	-0.95	0.04
		Lateral	8.33		-279.96	0.21	3.67	0.03
CZ	ACE	Aggregate	3.75	754	-1063.32	0.71	8.24	
		Medial	10.98		-1009.59	0.09	1.02	0.04
		Lateral	1.41		-745.39	0.97	10.59	
LM	ACE	Aggregate	3.8	752	-986.14	0.7	8.20	
		Medial	7.89		-1525.92	0.25	4.11	0.03
		Lateral	1.92		-418.88	0.93	10.07	

For male twins, models of averaged lateral channels are better fitting than their medial averaged counterparts (Table 3). However, CSD, LM and CZ references show more consistent and better fitting models overall compared to AVG as estimated by chi-square values. For CZ and LM references, RMSEA estimates were inestimable for both the averaged hemisphere and lateral channels. All male ACE models were determined to have “good” to “excellent” model parsimony as judged by RMSEA statistics.

Table A2: Model fit statistics for ACE models of FEMALE twins estimating heritability of frontal asymmetry for the average hemisphere, medial and lateral channel pairs.

Reference	Model	Channel	X^2	df	-2LL	p	AIC	RMSEA
CSD	ACE	Aggregate	4.25	666	344.57	0.64	7.74	
		Medial	5.1		379.07	0.53	6.90	
		Lateral	4.4		654.09	0.62	7.60	
AVG	ACE	Aggregate	10.49	742	-535.13	0.11	1.51	0.04
		Medial	4.5		-577.55	0.61	7.49	
		Lateral	7.09		7.09	0.01	-3.82	0.06
CZ	ACE	Aggregate	17.08	620	-812.54	0.01	-5.07	0.07
		Medial	12.44		-777.35	0.05	-0.43	0.05
		Lateral	11.32		-490.33	0.08	0.68	0.05
LM	ACE	Aggregate	8.95	636	-830.72	0.18	3.05	0.03
		Medial	9.17		-1317.07	0.16	2.83	0.04
		Lateral	6.84		-286.43	0.34	5.16	0.02

For female twins, model fits were more consistent within reference montage than within region, highlighting the importance of reference choice. Within references, CSD and LM reference displayed relatively better model fit parameters compared to AVG and CZ. CZ overall was a poor fitting model. Within the better fitting models, the lateral channels showed better model fits than their medial counter parts (Table 4). All females ACE models were determined to have “good” to “excellent” model parsimony as judged by RMSEA statistics.

Table A3: Model fit statistics for ADE models of MALE twins estimating heritability of frontal asymmetry for the average hemisphere, medial and lateral channel pairs.

Reference	Model	Channel	X^2	df	-2LL	p	AIC	RMSEA
CSD	ADE	Aggregate	6.65	768	23.54	0.35	5.35	0.01
		Medial	10.05		171.91	0.12	1.95	0.04
		Lateral	10.13		357.6	0.12	1.86	0.04
AVG	ADE	Aggregate	6.03	794	-749.96	0.42	5.96	0.00
		Medial	10.21		-808.25	0.12	1.79	0.04
		Lateral	6.27		-282.02	0.39	5.73	0.01
CZ	ADE	Aggregate	3.19	754	-1063.88	0.78	8.80	
		Medial	10.85		-1009.72	0.09	1.15	0.04
		Lateral	0.94		-745.86	0.99	11.06	
LM	ADE	Aggregate	4.35	752	-985.59	0.63	7.65	
		Medial	8.25		-1525.55	0.22	3.74	0.03
		Lateral	2.13		-418.68	0.91	9.87	

For male twins using ADE models, models of lateral channels demonstrated better fit compared to their medial counterparts, with LM and CZ references displaying better overall model fits compared to the AVG and CSD references, consistent with the ACE model findings. For AVG and CSD, models of the average hemisphere activity were the best fitting model. For CZ and LM references, the averaged lateral channels displayed the best model fit. All male ADE models were determined to have “good” to “excellent” model parsimony as judged by RMSEA statistics.

Table A4: Model fit statistics for ADE models of FEMALE twins estimating heritability of frontal asymmetry for the average hemisphere, medial and lateral channel pairs.

Reference	Model	Channel	X^2	df	-2LL	p	AIC	RMSEA
CSD	ADE	Aggregate	4.73	666	345.05	0.58	7.26	
		Medial	6.24		380.21	0.4	5.76	0.01
		Lateral	4.38		654.07	0.63	7.62	
AVG	ADE	Aggregate	10.55	742	-535.06	0.1	1.44	0.04
		Medial	4.5		-577.55	0.61	7.49	
		Lateral	6.93		6.93	0.02	-3.66	0.06
CZ	ADE	Aggregate	17.11	620	-812.5	0.01	-5.11	0.07
		Medial	12.75		-777.03	0.05	-0.75	0.06
		Lateral	11.25		-490.4	0.08	0.75	0.05
LM	ADE	Aggregate	8.73	636	-830.94	0.19	3.27	0.03
		Medial	9.23		-1317	0.16	2.76	0.04
		Lateral	6.63		-286.64	0.36	5.37	0.01

For female ADE models, CSD and LM displayed the best overall model fit, with lateral channels achieving the best fit, consistent with female ACE models. In contrast, neither the CZ or AVG models were a particularly good fit, with the CZ models having relatively poor fit as judged by chi-square statistics (Table 6). All females ADE models were determined to have "good" to "excellent" model parsimony as judged by RMSEA statistics.

Table A5: Model fit statistics for ACE models of MALE twins estimating heritability of frontal asymmetry.

Reference	Model	Channel	X^2	df	-2LL	p	AIC	RMSEA
CSD	ACE	F7/8	15.33	768	360.51	0.02	-3.32	0.06
n=386		F5/6	5.99		613.84	0.42	6.01	0.00
		F3/4	8.56		494.96	0.2	3.44	0.03
		F1/2	12.07		225.75	0.06	-0.07	0.05
AVG	ACE	F7/8	10.79	752	-238.25	0.10	94.93	0.04
n=378		F5/6	5.80		-202.49	0.45	-35.25	0.00
		F3/4	6.56		-418.31	0.36	28.53	0.01
		F1/2	19.85		-2740.04	0.00	-1071.73	0.07
CZ	ACE	F7/8	3.36	760	-673.98	0.76	12.04	0.00
n=382		F5/6	2.53		-542.8	0.87	29.71	0.00
		F3/4	7.11		-536.94	0.31	25.46	0.02
		F1/2	6.87		-1129.5	0.33	30.15	0.02
LM	ACE	F7/8	4.67	752	-282.33	0.59	7.32	0.00
n=378		F5/6	4.93		-322.51	0.55	7.06	0.00
		F3/4	5.96		-990.82	0.43	6.04	0.00
		F1/2	14.00		-1978.99	0.03	994.21	0.05

When investigating each channel pair separately, regardless of gender or model type (ACE vs ADE), the CSD reference montage generally allowed for the preservation of more data for analysis. Overall, there was a gender by reference interaction, where male ACE models fit better for CZ and LM reference montages while female data fit best to the CSD and AVG references. Within male twins, both ACE and ADE models fit the data well (all p's >.05), with model parsimony judged to be either good (RMSEA < .10) or excellent (RMSEA<.05) for nearly all channel pairs (Tables 7 & 9).

For females, models fit best for the CSD and AVG reference montages with fit statistics indicating good or excellent fit. Like males, both ACE and ADE models fit the data well, with models generally fitting better to lateral channels compared to medial channels, and model parsimony judged to be either good (RMSEA < .10) or excellent (RMSEA<.05) for nearly all channel pairs (Tables 8 & 10). The LM reference displayed inconsistent model fit, with lateral channel pairs having good model fit, while medial channel pairs displaying poor model fit,

particularly F1/2. Models of the CZ reference, displayed the poorest fit to the data, with the relative worst modal parsimony amongst all female ACE models across references.

Table A6: Model fit statistics for ACE models of FEMALE twins estimating heritability of frontal asymmetry.

Reference	Model	Channel	X^2	df	-2LL	p	AIC	RMSEA
CSD	ACE	F7/8	5.58	666	660.29	0.47	6.41	0.00
n=335		F5/6	4.47		859.03	0.61	7.52	0.00
		F3/4	5.03		703.27	0.54	6.97	0.00
		F1/2	2.60		354.55	0.86	9.40	0.00
AVG	ACE	F7/8	6.82	626	-164.43	0.34	266.94	0.02
n=315		F5/6	2.83		-96.72	0.83	202.91	0.00
		F3/4	7.46		-469.48	0.28	277.06	0.02
		F1/2	15.55		-1120.17	0.02	210.84	0.07
CZ	ACE	F7/8	25.06	632	-281	0.00	-119.45	0.10
n=318		F5/6	5.37		-180.5	0.50	36.87	0.00
		F3/4	12.39		-338.09	0.05	39.01	0.05
		F1/2	11.66		-947.57	0.07	43.34	0.05
LM	ACE	F7/8	5.41	636	-96.17	0.49	6.58	0.00
n=320		F5/6	6.90		-108.51	0.33	5.10	0.02
		F3/4	12.57		-826.3	0.05	-0.56	0.05
		F1/2	11.89		-1708.77	0.06	881.90	0.05

Table A7: Model fit statistics for ADE models of MALE twins estimating heritability of frontal asymmetry.

Reference	Model	Channel	X^2	df	-2LL	p	AIC	RMSEA
CSD	ADE	F7/8	15.42	768	360.61	0.02	-3.42	0.06
n=386		F5/6	7.87		615.73	0.25	4.121	0.02
		F3/4	8.06		494.46	0.23	3.94	0.03
		F1/2	11.36		225.04	0.08	0.63	0.04
AVG	ADE	F7/8	7.63	752	-241.41	0.27	98.09	0.02
n=378		F5/6	4.81		-203.47	0.57	-34.27	0.00
		F3/4	5.30		-419.58	0.51	29.80	0.00
		F1/2	19.85		-2740.04	0.00	-1071.73	0.07
CZ	ADE	F7/8	2.74	760	-674.6	0.84	12.66	0.00
n=382		F5/6	2.31		-543.76	0.89	29.93	0.00
		F3/4	6.51		-537.53	0.37	26.05	0.01
		F1/2	6.87		-1129.5	0.33	30.15	0.02
LM	ADE	F7/8	4.75	752	-282.24	0.58	7.23	0.00
n=378		F5/6	4.83		-322.61	0.57	7.16	0.00
		F3/4	6.09		-990.69	0.41	5.91	0.00
		F1/2	14.29		-1978.7	0.03	993.92	0.06

Table A8: Model fit statistics for ADE models of FEMALE twins estimating heritability of frontal asymmetry.

Reference	Model	Channel	X^2	df	-2LL	p	AIC	RMSEA
CSD	ADE	F7/8	5.06	666	659.76	0.54	6.94	0.00
n=335		F5/6	4.57		859.12	0.6	7.43	0.00
		F3/4	5.95		704.19	0.43	6.05	0.00
		F1/2	2.82		354.77	0.83	9.18	0.00
AVG	ADE	F7/8	6.75	626	-164.49	0.34	267.00	0.02
n=315		F5/6	2.73		-96.82	0.84	203.01	0.00
		F3/4	7.92		-469.01	0.24	276.59	0.03
		F1/2	15.92		-1119.8	0.01	210.47	0.07
CZ	ADE	F7/8	25.28	632	-280.78	0.00	-119.67	0.10
n=318		F5/6	5.37		-180.5	0.50	-1263.12	0.00
		F3/4	12.17		-338.31	0.06	39.23	0.05
		F1/2	11.93		-947.3	0.06	43.07	0.05
LM	ADE	F7/8	5.60	636	-96.36	0.47	6.39	0.00
n=320		F5/6	6.83		-108.58	0.34	5.10	0.02
		F3/4	12.56		-826.31	0.05	-0.55	0.05
		F1/2	11.92		1708.74	0.06	881.87	0.05

When evaluating the ADE models, there is again a reference by gender interaction that indicates better model fitting for males (compares to females) at references AVG, CZ, and LM, while models for CSD data fit female data better. Within male twins, all models describe the data accurately, with fit statistics in the good to excellent range. However, models for references CZ, LM, and AVG have relatively better fit compared to CSD. Similarly to ACE models, ADE models of individual channel pairs generally display better fit for channels F3/4, F5/6 and F7/8, compared to F1/2.

For females, models of the CSD reference most accurately fit the data compared to AVG, CZ and LM references, which display more inconsistent indices of model fit. However, across references, channels F5/6 and F7/8 have the most consistent measures of model fit compared to F3/4 and F1/2.

Table A9: Model fit statistics for ACE models for 11 y/o FEMALE twins estimating heritability of frontal asymmetry.

Reference	Model	Channel	X^2	df	-2LL	*p*	AIC	RMSEA
CSD	ACE	F7/8	14.19	240	175.11	0.03	-304.89	0.11
		F5/6	2.03		239.61	0.92	-240.39	
		F3/4	9.44		190.23	0.15	-289.77	0.07
		F1/2	4.35		85.66	0.62	-394.34	
AVG	ACE	F7/8	17.11	248	-60.31	0.01	-556.31	0.12
		F5/6	56.68		68.55	0	-427.45	0.26
		F3/4	11.84		-81.37	0.07	-577.37	0.09
		F1/2	24.88		-341.68	0	-837.68	0.16
CZ	ACE	F7/8	5.59	230	-248.06	0.47	-708.06	
		F5/6	7.12		-164.57	0.31	-624.57	0.04
		F3/4	10.09		-228.36	0.12	-688.36	0.08
		F1/2	20.69		-394.14	0	-854.19	0.14
LM	ACE	F7/8	3.34	204	35.67	0.77	-443.67	
		F5/6	56.68		68.55	0	-427.45	0.26
		F3/4	17.44		-296.89	0.01	-704.89	0.14
		F1/2	---		---	---	---	---

Table A10: Model fit statistics for ACE models for 17 y/o FEMALE twins estimating heritability of frontal asymmetry.

Reference	Model	Channel	X^2	df	-2LL	*p*	AIC	RMSEA
CSD	ACE	F7/8	10.44	422	472.74	0.11	-371.26	0.06
		F5/6	3.96		604.17	0.68	-239.83	
		F3/4	1.65		497.96	0.95	-346.04	
		F1/2	5.37		258.1	0.5	-585.9	
AVG	ACE	F7/8	32.56	490	338.84	0	-641.16	0.13
		F5/6	5.32		276.24	0.5	-703.76	
		F3/4	30.99		100.56	0	-879.44	0.13
		F1/2	48.02		-329.14	0	-1309.14	0.17
CZ	ACE	F7/8	11.37	386	-203.4	0.08	-975.4	0.07
		F5/6	5.11		-35.93	0.53	-807.93	
		F3/4	9.19		-127.96	0.16	-899.96	0.05
		F1/2	22.39		-544.34	0	-1316.34	0.12
LM	ACE	F7/8	4.23	428	-63.08	0.65	-919.08	
		F5/6	5.7		-52.58	0.46	-908.58	
		F3/4	10.72		-532.15	0.1	-1388.15	0.06
		F1/2	---		---	---	---	---

Table A11: Model fit statistics for ADE models for 11 y/o FEMALE twins estimating heritability of frontal asymmetry.

Reference	Model	Channel	X^2	df	-2LL	*p*	AIC	RMSEA
CSD	ADE	F7/8	13.97	240	174.9	0.03	-305.1	0.10
		F5/6	2.05		239.62	0.92	-240.38	
		F3/4	11.31		192.11	0.08	-287.89	0.09
		F1/2	4.39		85.69	0.62	-394.31	
AVG	ADE	F7/8	17.11	248	-60.31	0.01	-556.31	0.12
		F5/6	56.5		68.37	0	-427.63	0.26
		F3/4	11.86		-81.36	0.07	-577.36	0.09
		F1/2	24.88		-341.68	0	-837.68	0.16
CZ	ADE	F7/8	5.59	230	-248.06	0.47	-708.06	
		F5/6	7.12		-164.57	0.31	-624.57	0.04
		F3/4	10.33		-228.12	0.11	-688.12	0.08
		F1/2	20.84		-394.04	0	-854.04	0.15
LM	ADE	F7/8	3.35	204	-35.66	0	-443.66	
		F5/6	56.5		68.37	0	-427.63	0.26
		F3/4	17.37		-296.96	0.01	-704.96	0.13
		F1/2	---		---	---	---	---

Table A12: Model fit statistics for ADE models for 17 y/o FEMALE twins estimating heritability of frontal asymmetry.

Reference	Model	Channel	X^2	df	-2LL	*p*	AIC	RMSEA
CSD	ADE	F7/8	10.15	422	472.46	0.12	-371.54	0.06
		F5/6	4.03		604.25	0.67	-239.75	
		F3/4	1.72		498.03	0.94	-345.97	
		F1/2	5.46		258.2	0.49	-585.8	
AVG	ADE	F7/8	32.2	490	338.48	0	-641.52	0.13
		F5/6	5.32		276.24	0.5	-703.76	
		F3/4	30.99		100.56	0	-879.44	0.13
		F1/2	48.02		-329.14	0	-1309.14	0.17
CZ	ADE	F7/8	12.01	386	-202.76	0.06	-974.76	0.07
		F5/6	5.11		-35.93	0.053	-807.93	
		F3/4	8.8		-128.35	0.19	-900.35	0.05
		F1/2	22.39		-544.34	0	-1316.34	0.12
LM	ADE	F7/8	4.4	428	-62.91	0.62	-918.91	
		F5/6	5.7		-52.5	0.46	-908.58	
		F3/4	10.73		-532.15	0.1	-1388.15	0.06
		F1/2	---		---	---	---	---

Table A13: Model fit statistics for ACE models for 11 y/o MALE twins estimating heritability of frontal asymmetry.

Reference	Model	Channel	X^2	df	-2LL	*p*	AIC	RMSEA
CSD	ACE	F7/8	4.3	456	174.01	0.64	-737.99	
		F5/6	13.07		311.37	0.04	-600.63	0.07
		F3/4	7		280.33	0.32	-631.67	0.03
		F1/2	8.59		88.43	0.2	-823.57	0.04
AVG	ACE	F7/8	16.65	478	-77.44	0.01	-1033.44	0.09
		F5/6	5.39		-120.76	0.49	-1076.76	
		F3/4	7.23		-261.91	0.3	-1217.91	0.03
		F1/2	82.89		-367.9	0	-1323.9	0.23
CZ	ACE	F7/8	5.28	448	-453.87	0.51	-1349.87	
		F5/6	2.99		-368.97	0.81	-1264.97	
		F3/4	5.02		-362.91	0.54	-1258.91	
		F1/2	7.44		-708.75	0.28	-1604.75	0.03
LM	ACE	F7/8	9.39	440	-214.78	0.15	-1094.78	0.05
		F5/6	10.59		-224.76	0.1	-1104.76	0.06
		F3/4	7.17		-663.27	0.31	-1543.27	0.03
		F1/2	---		---	---	---	---

Table A14: Model fit statistics for ACE models for 17 y/o MALE twins estimating heritability of frontal asymmetry.

Reference	Model	Channel	X^2	df	-2LL	*p*	AIC	RMSEA
CSD	ACE	F7/8	4.3	456	174.01	0.64	-737.99	
		F5/6	13.07		311.37	0.04	-600.63	0.07
		F3/4	7		280.33	0.32	-631.67	0.03
		F1/2	8.59		88.43	0.2	-823.57	0.04
AVG	ACE	F7/8	16.65	478	-77.44	0.01	-1033.44	0.09
		F5/6	5.39		-120.76	0.49	-1076.76	
		F3/4	7.23		-261.91	0.3	-1217.91	0.03
		F1/2	82.89		-367.9	0	-1323.9	0.23
CZ	ACE	F7/8	5.28	448	-453.87	0.51	-1349.87	
		F5/6	2.99		-368.97	0.81	-1264.97	
		F3/4	5.02		-362.91	0.54	-1258.91	
		F1/2	7.44		-708.75	0.28	-1604.75	0.03
LM	ACE	F7/8	9.39	440	-214.78	0.15	-1094.78	0.05
		F5/6	10.59		-224.76	0.1	-1104.76	0.06
		F3/4	7.17		-663.27	0.31	-1543.27	0.03
		F1/2	---		---	---	---	---

Table A15: Model fit statistics for ADE models for 11 y/o MALE twins estimating heritability of frontal asymmetry.

Reference	Model	Channel	X^2	df	-2LL	*p*	AIC	RMSEA
CSD	ADE	F7/8	4.32	456	174.02	0.63	-737.98	
		F5/6	14.43		312.73	0.03	-599.27	0.08
		F3/4	7.02		280.35	0.32	-631.65	0.03
		F1/2	8.1		87.93	0.23	-824.07	0.04
AVG	ADE	F7/8	16.03	478	-78.06	0.01	-1034.06	0.08
		F5/6	5.37		-120.78	0.5	-1076.78	
		F3/4	6.71		-262.43	0.35	-1218.43	0.02
		F1/2	82.99		-367.81	0	-1323.81	0.23
CZ	ADE	F7/8	4.97	448	-454.17	0.55	-1350.17	
		F5/6	3.07		-368.89	0.8	-1264.89	
		F3/4	4.9		-363.03	0.56	-1259.03	
		F1/2	7.44		-708.75	0.28	-1604.75	0.03
LM	ADE	F7/8	9.82	440	-214.35	0.13	-1094.35	0.05
		F5/6	10.49		-224.85	0.11	-1104.85	0.06
		F3/4	7.53		-662.91	0.27	-1542.91	0.03
		F1/2	---		---	---	---	---

Table A16: Model fit statistics for ADE models for 17 y/o MALE twins estimating heritability of frontal asymmetry.

Reference	Model	Channel	X^2	df	-2LL	*p*	AIC	RMSEA
CSD	ADE	F7/8	4.32	456	174.02	0.63	-737.98	
		F5/6	14.43		312.73	0.03	-599.27	0.08
		F3/4	7.02		280.35	0.32	-631.65	0.03
		F1/2	8.1		87.93	0.23	-824.07	0.04
AVG	ADE	F7/8	16.03	478	-78.06	0.01	-1034.06	0.08
		F5/6	5.37		-120.78	0.5	-1076.78	
		F3/4	6.71		-262.43	0.35	-1218.43	0.02
		F1/2	82.99		-367.81	0	-1323.81	0.23
CZ	ADE	F7/8	4.97	448	-454.17	0.55	-1350.17	
		F5/6	3.07		-368.89	0.8	-1264.89	
		F3/4	4.9		-363.03	0.56	-1259.03	
		F1/2	7.44		-708.75	0.28	-1604.75	0.03
LM	ADE	F7/8	9.82	440	-214.35	0.13	-1094.35	0.05
		F5/6	10.49		-224.85	0.11	-1104.85	0.06
		F3/4	7.53		-662.91	0.27	-1542.91	0.03
		F1/2	---		---	---	---	---