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Hippocampal Volume Deficits and Shape Deformities in Young Biological Relatives of Schizophrenia Probands

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Abstract

Hippocampal volume decrement may be one of the changes that most closely pre-date schizophrenia onset. Studying hippocampal developmental morphology in adolescent or young adult biological relatives of schizophrenia probands has the potential to further our understanding of the neurodevelopmental etiology of schizophrenia, and to discover biomarkers that may aid its early identification. We utilized an artificial neural network segmentation algorithm to automatically define and reliably measure MRI hippocampus volumes. We compared 46 young, nonpsychotic biological relatives of probands against 46 healthy controls without family history of schizophrenia and 46 schizophrenia probands (Age range=13 to 28 years). We further contrasted hippocampal shape differences using spherical harmonic functions, and assessed how obstetric complications (a trigger for aberrant in utero neurodevelopment) may contribute to hippocampal abnormalities. Similar to schizophrenia probands, unaffected biological relatives of probands had significantly smaller hippocampus volumes than controls; which correspond to inward displacements in shape deformities principally in the anterior hippocampal sub-regions. Examination of hippocampus volume-age relationships indicate that hippocampus volume normally decreases with age during late adolescence through early adulthood. In contrast, relatives of probands did not show these age-expected changes. Deviant hippocampus volume-age relationships suggest aberrant hippocampal neurodevelopment among biological relatives. Relatives with a history of obstetric complications had significantly smaller left and right hippocampi than relatives without obstetrics complications, including a dose-relationship such that greater number of birth complications correlated with smaller hippocampus. Similar hippocampal volume deficits-obstetric complications relationships were observed among schizophrenia probands. Hippocampal abnormalities in schizophrenia are likely to be mediated by different neurobiological mechanisms, including factors associated with obstetric complications which occur during early neurodevelopment. Other brain maturational anomalies affecting the hippocampus in schizophrenia may manifest closer to illness onset in adolescence/early adulthood.

Keywords

neurodevelopment; adolescent brain maturation; genetics; magnetic resonance imaging; hippocampus surface anatomy; obstetric complications

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Introduction

Schizophrenia is characterized by well-replicated whole brain as well as frontotemporal brain volume deficits (Honea et al., 2005; Shenton et al., 2001; Steen et al., 2006). Hippocampus volume reduction, being ~4% smaller than healthy volunteers (Nelson et al., 1998; Wright et al., 2000), may be one of the largest in magnitude among the diffuse brain regional volume deficits in schizophrenia patients. Based on the few studies that have been able to investigate high-risk individuals before and after the onset of schizophrenia (Borgwardt et al., 2007; Job et al., 2005; Pantelis et al., 2003), the hippocampus also appears to be among the earliest brain regions in which volumetric reductions closely precede illness onset.

The neurobiological processes that underlie hippocampus volume changes around the first emergence of schizophrenia symptoms remain poorly understood. Abnormal brain maturation during late adolescence through early adulthood is a prime causative factor given the evidence supporting aberrant neurodevelopment in schizophrenia (Weinberger, 1996), onset of schizophrenia being typically in the late teens and early twenties, and our current understanding regarding normal brain maturation during this stage of life (Giedd et al., 1999; Gogtay et al., 2004; Sowell et al., 1999). However, it is a challenging task to assess whether hippocampus volume decrement around the onset of schizophrenia is indeed related to abnormal brain maturation during late adolescence/early adulthood. There are as yet informative experimental approaches to model brain maturation and to effectively test such hypotheses (Insel, 2007). Furthermore, studying adolescence/early adulthood brain maturation in schizophrenia patients is difficult since aberrant neurodevelopmental abnormalities may have already occurred by the time patients manifest the characteristic symptoms of schizophrenia.

A potentially fruitful research strategy to uncover the etiology of brain morphometric abnormalities (including hippocampus volume reductions) around the onset of schizophrenia is to study adolescent/young adult biological relatives of schizophrenia probands. Research involving such young biological relatives allows more direct quantification of brain maturation during a time period when the risk for developing schizophrenia is at its peak. Since biological relatives of schizophrenia probands share a common genetic vulnerability, familial high-risk studies are likely to further our understanding regarding how individual genetic variations may mediate specific neurobiological processes to affect abnormal brain development in schizophrenia (Keshavan et al., 2007). As a corollary, studying the brain developmental trajectory in adolescent/young adult relatives will help pinpoint the specific abnormal neurodevelopmental processes in schizophrenia.

The goal of this study is to examine hippocampal morphometry in unaffected, young biological relatives of schizophrenia probands. Using age-hippocampus volume relationship as a proxy measure for hippocampal developmental trajectory, we will assess if hippocampal maturation during late adolescence through early adulthood in biological relatives of schizophrenia probands differ from those in healthy volunteers without a family history of schizophrenia and in schizophrenia probands. In addition to contrasting age-hippocampus volume relationships, we will further compare differences in hippocampal shape. Besides being a potentially more sensitive method than volume measurements (Csernansky et al., 1998), quantification of surface deformations may also yield biologically meaningful information about the neurodevelopment of the hippocampus (Van Essen, 1997; Van Essen et al., 1998). There is evidence to suggest that physical tension arising from neurodevelopmental mechanisms may lead to shape variations. We hypothesize that biological relatives of schizophrenia probands will show deviant agehippocampus volume relationships and shape abnormalities that are suggestive of aberrant neurodevelopment. Since obstetric complications have been associated with hippocampal abnormalities in schizophrenia (DeLisi et al., 1988; Geddes et al., 1999) and environmental factors are likely to impact gross morphometric abnormalities in at-risk

individuals (Wood et al., 2005), we will also explore the effects of birth complications on hippocampal abnormalities in this study.

Materials and Methods

Subjects

Forty-six first- (N=30) or second-degree relatives of schizophrenia probands, 46 schizophrenia probands and 46 healthy normal volunteers (HNV) without family history of schizophrenia participated in this study. Study participants have been included in a previous report (Ho, 2007). Relatives were within the age range at-risk for developing schizophrenia (13 to 28 years), and have at least one first- or second-degree relative with schizophrenia (verified using Family History-Research Diagnostic Criteria (FH-RDC)(Andreasen et al., 1977)). The SCID-IV (Structured Clinical Interview for DSM-IV) was administered to exclude relatives with a lifetime diagnosis of schizophrenia, schizophreniform disorder, schizoaffective disorder, or delusional disorder, or substance abuse/dependence within the past year. Thirty-five relatives had no lifetime history of psychiatric disorders. The most common diagnosis in the remaining 11 relatives was major depressive disorder (Ho, 2007).

Schizophrenia probands and HNV were selected from neuroimaging studies conducted at the Department of Psychiatry University of Iowa so as to match gender composition (M:F ratio=20:26), age (Mean=19.9 years (SD=4.1), 20.8 years (SD=4.5) and 20.9 years (SD=3.5) in relatives, probands and HNV respectively), and parental socioeconomic status of the relatives sample (Ho, 2007). Probands were evaluated using a semi-structured interview instrument, Comprehensive Assessment of Symptoms and History (CASH) (Andreasen et al., 1992), from which schizophrenia diagnosis meeting DSM-III-R or DSM-IV criteria was based. HNV were assessed using an abbreviated version of the CASH to exclude subjects with current or past psychiatric illnesses and substance misuse. FH-RDC was also used to confirm the absence of family history of schizophrenia in healthy controls.

MRI image acquisition and hippocampus volume/shape measurements

Each subject underwent a high-resolution anatomic MR imaging protocol using a 1.5 Tesla GE CVMRI scanner (General Electric Medical Systems, Milwaukee, Wisconsin) to acquire T1- and T2-weighted images of the whole brain. T1 sequence was obtained as a 3D volume in the coronal plane using a spoiled GRASS sequence with TE=6 ms, TR=20 ms, flip angle=30°, FOV=160×160×192 mm, matrix=256×256×124, NEX=2. T2 images were acquired using a 2D fast spin-echo sequence in the coronal plane with TE=85 ms, TR=4800 ms, slice thickness/gap=1.8/0.0 mm, FOV=160×160 mm, matrix=256×256, NEX=3, number of echoes=8, number of slices=124.

We utilized an artificial neural network (ANN) segmentation algorithm to automatically define and measure left and right hippocampus volumes (Ho et al., 2005; Magnotta et al., 1999; Pantel et al., 2000; Powell et al., 2008). The ANN is a three-layer fully connected feed-forward network trained utilizing the standard back propagation algorithm. It has high correspondence with manual tracing as evidenced by 3 different reliability measures: Mean spatial overlap=0.85 (SD=0.04); Mean relative overlap with manual tracing=0.74 (SD=0.04); Mean similarity index=0.85 (SD=0.03) (Powell et al., 2008). Spatial overlap is the ratio of intersection of manually traced and ANN-derived volumes to manually traced volume. Relative overlap is the ratio of intersection of manually traced and ANN-derived volumes to union of manually traced and ANN-derived volumes. Similarity index is the ratio of intersection of manually traced and ANN-derived volumes to half the sum of manually traced and ANN-derived volumes. Unlike intra-class correlations, another commonly used reliability metric which only reflects the correspondence between volumes, the 3 reliability measures we used

are more robust since these take into account volume as well as spatial location and shape differences between the ANN and hand-traced hippocampi (Powell et al., 2008).

To evaluate hippocampal shape differences between relatives and HNV, we used the SPHARMPDM shape analysis pipeline (Brechbuhler et al., 1995; Styner et al., 2006). This approach is based on spherical harmonic functions to describe 3 dimensional shapes. The ANN-delineated hippocampus provided a set of binary segmentation for use as input in the SPHARM-PDM analysis. These binary segmentations were converted into parametric surface meshes, and then mapped to a sphere using an area-preserving mapping algorithm that also minimizes angular distortions (Brechbuhler et al., 1995). To bring all surfaces of the SPHARM description into alignment, the spherical parametrizations were normalized using the first order ellipsoid from the spherical harmonic coefficients. We next performed a linear, uniform iconsahedron subdivision of the spherical parameterization thus providing the point distribution model (SPHARM-PDM). This point distribution model is the signed distance of the point on the template for each given subject. Hippocampal shape differences between relatives and HNV are then tested by comparing the point distributions of the two groups using the Hotelling T-square two-sample statistic. Next, we used KWMeshVisu to visualize shape differences (scalar values) between relatives and HNV with p-value and distance maps (Oguz et al., 2006). For schizophrenia probands, the ANN-delineated hippocampus was processed and analyzed similarly for shape comparison against HNV.

Obstetric complications

Obstetric complications were ascertained using the Birth History rating scale, a standardized interview administered to the mothers of relative subjects so to assess pregnancy complications as well as labor and delivery problems. Twenty-four specific obstetric complications within the Birth History scale were rated as either ‘Yes’ or ‘No’ based on maternal recall. Data regarding obstetric complications were available only on relatives and schizophrenia probands, but had not been collected from HNV.

Statistical analysis

Differences in left and right hippocampus volumes across the 3 groups were tested using ANCOVA (covariates: intracranial volume and gender). This is followed by pair-wise group comparisons to examine the effects of group membership (relatives versus HNV, probands versus HNV and relatives versus probands) and age on hippocampal volumes. In addition to intracranial volume and gender as covariates, we entered an age-by-group interaction term in the general linear models to test whether hippocampal volume-age relationships differed between pair-wise comparison groups. Hippocampus volume-age relationships were further examined using Pearson correlation coefficients (partialing for intracranial volume and gender). To assess group differences in hippocampal shape (i.e. relatives versus HNV and probands versus HNV), the SPHARM-PDM analysis utilizes the Hotelling T-square two-sample statistic. Significance maps summarizing raw p-values across the hippocampal surface (showing significant between-group differences), and the corresponding displacement maps (distance between the mean of hippocampal surfaces of relatives (or schizophrenia probands) displaced inward or outward relative to the mean of hippocampal surfaces of HNV) are presented to show groups differences in hippocampal shape. Given the skewed frequency distribution in the number of obstetric complications, rank-transformed data were used in the ANCOVA so as to test the effects of obstetric complications on hippocampus volume among relatives and schizophrenia probands (covariates intracranial volume, gender and age).

Results

Across the entire study sample of 138 study participants, there were significant group main effects on left and right hippocampal volumes (Table 1; $F \geq 9.83$, df=2,137, $p \leq .0001$). Compared to HNV, relatives had significantly smaller hippocampi (Main effects of group $F \geq 3.77$, df=1,91, $p \leq .05$) as were schizophrenia probands ($F \geq 14.93$, df=1,91, $p \leq .0002$). Hippocampal volumes in probands were also significantly smaller than relatives ($F \geq 16.84$, df=1,91, $p \leq .0001$). To assess sub-regions within the hippocampus where volumetric differences may be occurring, we examined hippocampal shape using SPHARM-PDM analysis (Figures 1 and 2). Compared to HNV, relatives of schizophrenia probands had significant shape differences primarily in the head of the hippocampus (Figures 1a and 1b; red and yellow regions ($p \leq .05$)). Displacement maps further indicate these shape differences in the heads of hippocampi correspond to inward displacements in relatives compared to HNV (Figures 1c and 1d). Compared to HNV, schizophrenia probands also showed similar inward displacement in the same anterior sub-regions of the hippocampus (Figure 2).

Comparing relatives and HNV, there were statistically significant age-by-group interaction effects on left and right hippocampal volumes (Table 1; $F \geq 4.04$, $p \leq .05$) indicating that hippocampus volume-age relationships differed across the 2 groups. For HNV, left and right hippocampal volumes were inversely associated with age (Figures 3a and 3b; Pearson partial $r = -0.26$ and -0.17 respectively). These age-expected reductions in hippocampus volume during late adolescence through early adulthood in HNV were not observed among relatives (Figure 3; Pearson partial $r = 0.12$ and 0.22 for left and right hippocampus respectively). Hippocampal volume-age relationships among relatives also differed significantly to those in schizophrenia probands (Table 1; age-by-group interaction effects $F \geq 5.78$, $p \leq .02$). Although the hippocampal volume-age regression slopes in schizophrenia probands were steeper (Figure 3; Pearson partial $r = -0.40$ and -0.30 for left and right hippocampus respectively) than those in HNV, these were not significantly different (age-by-group interaction $F \leq 0.17$, $p \geq .68$).

When the analyses in Table 1 were conducted as an omnibus test using repeated measures ANCOVA with hemisphere as the repeated measure (i.e. left and right hippocampus volumes entered simultaneously as dependent measures), the results remained unchanged. The main effects of group and age-by-group interaction were still statistically significant ($F = 4.65$ and 4.74 respectively, $p \leq .03$). There were no significant hemisphere effects on age, group or age-by-group interactions ($F \leq 1.69$, $p \geq .20$).

Effects of obstetric complications on hippocampus volume in relatives of probands

Frequency distributions of obstetric complications in relatives and schizophrenia probands are summarized in Table 2. Approximately one-third of subjects in each group had no history of obstetric complications. The remaining relatives had at least one or as many as 11 obstetric complications each. The rest of schizophrenia probands had up to 4 obstetric complications each. The total number of obstetric complications had statistically significant effects on left and right hippocampus volumes among relatives ($F = 4.88$ and 4.76 respectively, $p \leq .03$) as well as in schizophrenia probands ($F = 9.85$ and 4.49 respectively, $p \leq .04$). Greater total number of obstetric complications was significantly associated with smaller hippocampus volumes (Figure 4a; Spearman partial $r \leq -0.34$, $p \leq .03$). Median number of obstetric complications in each group was 1 (25^{th}-75^{th} interquartile range=2.0). Median split comparison found that relatives with more than 1 obstetric complication had smaller left (Figure 4b; $F = 3.73$, $p = .06$) and right ($F = 5.58$, $p = .02$) hippocampal volumes than relatives with zero or 1 obstetric complication. Schizophrenia probands showed a similar pattern such that those with more than 1 obstetric complication had smaller hippocampal volumes than probands with zero or 1 obstetric complication (Figure 4c). The median split comparison in schizophrenia probands approached but did not achieve statistical significance ($F \leq 3.22$, $p \geq .08$).

Potential confounding effects of psychiatric diagnosis and familial clustering in biological relatives of probands

Thirty-five of the 46 relatives had no lifetime history of any psychiatric disorders. In the remaining 11 relatives, diagnoses included: major depressive disorder (6), depressive disorder, not otherwise specified (1), attention deficit hyperactivity disorder (2), panic disorder (1) and generalized anxiety disorder (1). Because psychiatric disorders (e.g. major depressive disorder and attention deficit hyperactivity disorder) have also been associated with brain volume deficits, we explored the potential confounding influence from psychiatric diagnosis on hippocampus volumes using 2 separate approaches. First, the relatives-HNV ANCOVA in Table 1 was repeated with psychiatric diagnosis entered as an additional covariate (i.e. as a class variable for the presence or absence of psychiatric disorders). Psychiatric diagnosis had no significant effects on hippocampus volumes ($F=0.15$ and 0.25 for left and right hippocampal volume respectively, $p \geq .62$). After statistically accounting for the effects of psychiatric disorders among relatives, the main effects of group (relatives of probands versus healthy controls) and age-by-group interaction were unchanged from the original results in Table 1. Second, the data were re-analyzed after excluding relatives with psychiatric disorders. Restricting the relatives sample to only those 35 relatives without any psychiatric disorders did not change the results reported in Table 1. The main effects of group and age-by-group interaction remained statistically significant ($F \geq 3.86$, $p \leq .05$).

Brain volumes are known to be correlated among family members. Schizophrenia probands and healthy controls in this report were unrelated and were derived from independent families. The 46 relatives were ascertained from 34 independent families: 21 families each contributed 1 relative subject, 4 families each contributed 1 relative subject and 1 schizophrenia proband, 7 families contributed 2 relatives subjects each, 1 family 3 relatives subjects and the last family 4 relatives subjects. To investigate for potential confounds that may have arisen due to correlations of brain volume among family members, we conducted a mixed model analysis in which pedigree was included as a random factor in the statistical models. In each mixed model, hippocampus volume was the dependent variable, group and age-by-group interaction as fixed factors and with covariates of intracranial volume, age, and gender. Again, the inclusion of family clustering factor in the analysis did not substantially change the results reported in Table 1; the main effects of group and age-by-group interaction were: Left hippocampus – $F=3.50$ and 3.91 respectively, $p \leq .06$; Right hippocampus - $F=3.13$ and 3.70 respectively, $p \leq .08$. There were no statistically significant effects of pedigree on hippocampus volumes ($F \leq 1.07$, $p \geq .40$).

Discussion

In this study, adolescent/young adult biological relatives of schizophrenia probands, who were still within the age range at-risk for developing schizophrenia, had hippocampus volume deficits when compared to age- and gender-equivalent healthy volunteers without a family history of schizophrenia. Hippocampal shape deformities (inward displacements) among relatives were primarily at the anterior sub-regions of the hippocampus where hippocampal CA1 neurons projecting to the medial prefrontal cortex are principally located. Relatives did not show the normal age-related decreases in hippocampus volumes expected during late adolescence into early adulthood. A history of obstetric complications among relatives of schizophrenia probands was further associated with smaller hippocampus volumes bilaterally. These hippocampal volume deficits, shape deformities and associations with obstetric complications among young, unaffected biological relatives of probands were similar to abnormalities seen in schizophrenia probands.

These findings extend our previous report of frontal gray matter (GM) volume deficits in young, unaffected biological relatives of schizophrenia probands (Ho, 2007). Although we previously

found nonsignificant temporal GM volume reductions in relatives, our prior lobar brain volume measures may not have been sensitive enough to detect volume deficits within smaller medial temporal structures such as the hippocampus. Our current findings of hippocampus volume deficits in relatives are consistent with MRI studies from other research groups that have examined genetic high-risk individuals still within the age range for developing schizophrenia (Keshavan et al., 2002;Lawrie et al., 1999) as well as studies involving older, unaffected biological relatives (Boos et al., 2007).

Besides volumetric differences, relatives as well as schizophrenia probands had hippocampal shape abnormalities which were characterized by relative inward displacements located at the anterior hippocampus bilaterally. These findings are in line with some but not all previous reports regarding hippocampal shape abnormalities in schizophrenia probands and their biological relatives. To-date, there have only been 2 studies on hippocampal shape in biological relatives (Connor et al., 2004; Tepest et al., 2003). Using high-dimensional mapping, Csernansky and colleagues found inward deformations in the head of hippocampus bilaterally among unaffected siblings (Tepest et al., 2003) as well as among first-episode schizophrenia patients (Csernansky et al., 1998; Csernansky et al., 2002). Similarly, more pronounced anterior hippocampal volume deficits in schizophrenia patients the have been reported by others (Narr et al., 2004; Nugent et al., 2007). However, Connor and colleagues found that hippocampal shape anomalies (located mostly in the posterior hippocampus) were more prevalent in patients with familial schizophrenia but not among unaffected first-degree relatives (Connor et al., 2004). Using spherical harmonics shape analysis similar to our study, Shenton and colleagues reported greater left-right asymmetry in the posterior amygdala-hippocampal complex among chronic schizophrenia patients (Shenton et al., 2002). Lee and colleagues found schizophrenia patients had shape deformities in both anterior as well as posterior subregions of the hippocampus (Lee et al., 2004). These conflicting findings may in part be related to differences in shape analytic methodologies, study population and small sample sizes.

Despite this lack of consensus in the current literature, our finding of deformations in the anterior sub-region of the hippocampus among relatives and schizophrenia probands may be of significance to schizophrenia for several reasons. First, shape assessments provide a potentially more sensitive method to detect subtle regional anatomical abnormalities in the hippocampus than the unitary measure from conventional volumetric analysis (Csernansky et al., 1998; Csernansky et al., 2004). Second, shape differences may yield biologically meaningful information regarding hippocampal neurodevelopment as well as the neurobiology of schizophrenia. It has been postulated that physical tension arising from neurodevelopmental processes and neuronal connectivity may lead to shape variations of brain structures (Van Essen, 1997; Van Essen et al., 1998). Projections from the hippocampus to the medial prefrontal cortex (primarily via the entorhinal cortex) are found predominantly from CA1 neurons in the head of the hippocampus (Barbas and Blatt, 1995; Goldman-Rakic et al., 1984). The hippocampus also receives reciprocal projections from the medial prefrontal cortex, including the anterior cingulate cortex, thereby forming neural networks important for various forms of memory and learning subserved by the hippocampus (Cabeza and Nyberg, 2000). Thus, anterior hippocampal shape abnormalities may be a marker for neurodevelopmental disruptions in frontal-temporal connections among schizophrenia probands and their biological relatives.

Postmortem and animal studies indicate that there is considerable elimination of cortical synapses during adolescence through early adulthood (Bourgeois et al., 1994; Huttenlocher and Dabholkar, 1997). Consistent with postmortem findings of dendritic ‘pruning’, in vivo MR imaging studies have found progressive GM reductions during this time period (e.g. (Gogtay et al., 2004; Sowell et al., 2003; Sowell et al., 2002)). Most pronounced GM reductions have been observed in the frontal and dorsal parietal brain regions (Giedd et al., 1999; Sowell et al.,

1999). Cross-sectional and longitudinal studies investigating the normal lifetime trajectory of hippocampal volume have been less consistent. Progressive reductions (Jernigan et al., 2001; Murphy et al., 1996), no changes (Gogtay et al., 2006), male-specific enlargements (Suzuki et al., 2005) or anterior sub-regional reductions with posterior sub-regional enlargements (Gogtay et al., 2006) have been reported.

In our study, we used hippocampus volume-age relationship as a proxy measure for hippocampus volume changes during late adolescence and early adulthood. Among HNV, hippocampus volumes were inversely correlated with age. This is consistent with the notion that hippocampus volume normally decreases during this age range, possibly as a result of maturational dendritic 'pruning'. Probands showed steeper hippocampal volume-age slopes than HNV (albeit non-significantly different) suggestive of accelerated dendritic pruning. Biological relatives of probands, on the other hand, had hippocampal volume-age relationships that were significantly different from those observed in HNV and in probands. While the lack of normal age-related hippocampus volume reductions among adolescent/young adult relatives of schizophrenia probands may be indicative of aberrant neurodevelopment and/or reduced dendritic elimination, these findings need to be interpreted with caution. More definitive inference regarding abnormal hippocampal maturation in biological relatives of schizophrenia probands will require additional studies with larger sample sizes and using within-subject longitudinal study designs.

Obstetric complications, particularly perinatal hypoxia and prenatal infections, have been associated with increased schizophrenia risk (Cannon et al., 2002a; Geddes et al., 1999), as well as with brain volume abnormalities (Cannon et al., 2002b) and smaller hippocampus volumes (DeLisi et al., 1988; Ebner et al., 2008) in schizophrenia probands. Risk for schizophrenia is elevated by 2-5 fold in the presence of obstetric complications, and disease susceptibility has been correlated with the number of obstetric complications (Cannon et al., 2000). Among high-risk individuals, some studies (but not all (Cannon et al., 2000)) have found excess rates of obstetric complications in biological relatives of probands (Sacker et al., 1996) and other at-risk individuals (Ballon et al., 2008). Unaffected relatives of schizophrenia probands with obstetric complications have also been shown to have smaller hippocampi (DeLisi et al., 1988; Ebner et al., 2008).

Reliability of maternal recall of obstetric events is difficult to establish in part because objective records of pregnancy and delivery are often incomplete and/or unavailable. Even though the accuracy of maternal recall of specific obstetric events varies greatly, maternal report of the total number of obstetric complications (the measure of interest in this study) has been shown to be more valid (Buka et al., 2000). Maternal recall bias (i.e. over-reporting obstetric complications in relatives) is not a major concern in this study since we have no pregnancy/birth history data on HNV. However, not having information regarding obstetric complications among HNV also limits our ability to assess the specificity of obstetric complications on diminished hippocampus volumes. Despite these limitations, we found associations between obstetric complications and smaller hippocampus volumes among biological relatives as well as in schizophrenia probands, including a dose-relationship such that greater total number of obstetric complications correlated with smaller hippocampus. Our findings are consistent with previous studies (DeLisi et al., 1988; Geddes et al., 1999; Stefanis et al., 1999; van Erp et al., 2004; Wood et al., 2005). Our results further support that besides genetic influence, environmental factors (such as obstetric complications) contribute to hippocampal volume deficits among familial high-risk individuals and in probands.

The neurobiological mechanisms underlying how obstetric complications may mediate brain volumetric abnormalities (including smaller hippocampi) and increased schizophrenia susceptibility are still not well understood (Cannon et al., 2002b). Current evidence implicate

gene-environment interaction (i.e. detrimental effects on the developing brain from obstetric complications interact with disease genes to cause schizophrenia) and/or additive gene and environmental influences (i.e. genetic and obstetric risk factors for schizophrenia act independently but additively) (Ellman and Cannon, 2008; Mittal et al., 2008). Recent studies further indicate that schizophrenia candidate genes (e.g. *AKT1*, *BDNF*, *DTNBP1* and *GRM3*), which contribute to regulating the response of neural cells to birth hypoxia, interact significantly with severe obstetric complications in influencing schizophrenia susceptibility (Cannon et al., 2008; Nicodemus et al., 2008). Thus, inadequate production of neurotrophins (such as BDNF) in reaction to perinatal hypoxia may potentially lead to dendritic atrophy and smaller hippocampi. Such early neurodevelopmental abnormalities may predispose the individual to schizophrenia through having diminished neural reserve against normal adolescent/early adulthood dendritic pruning. Alternatively, other schizophrenia susceptibility genes may have additive effects on reducing hippocampal volumes during adolescent brain maturation that are independent of obstetric complications.

In conclusion, schizophrenia likely involves different abnormal brain maturational processes; some occurring in early neurodevelopment while others manifest during adolescence/early adulthood closer to illness onset.

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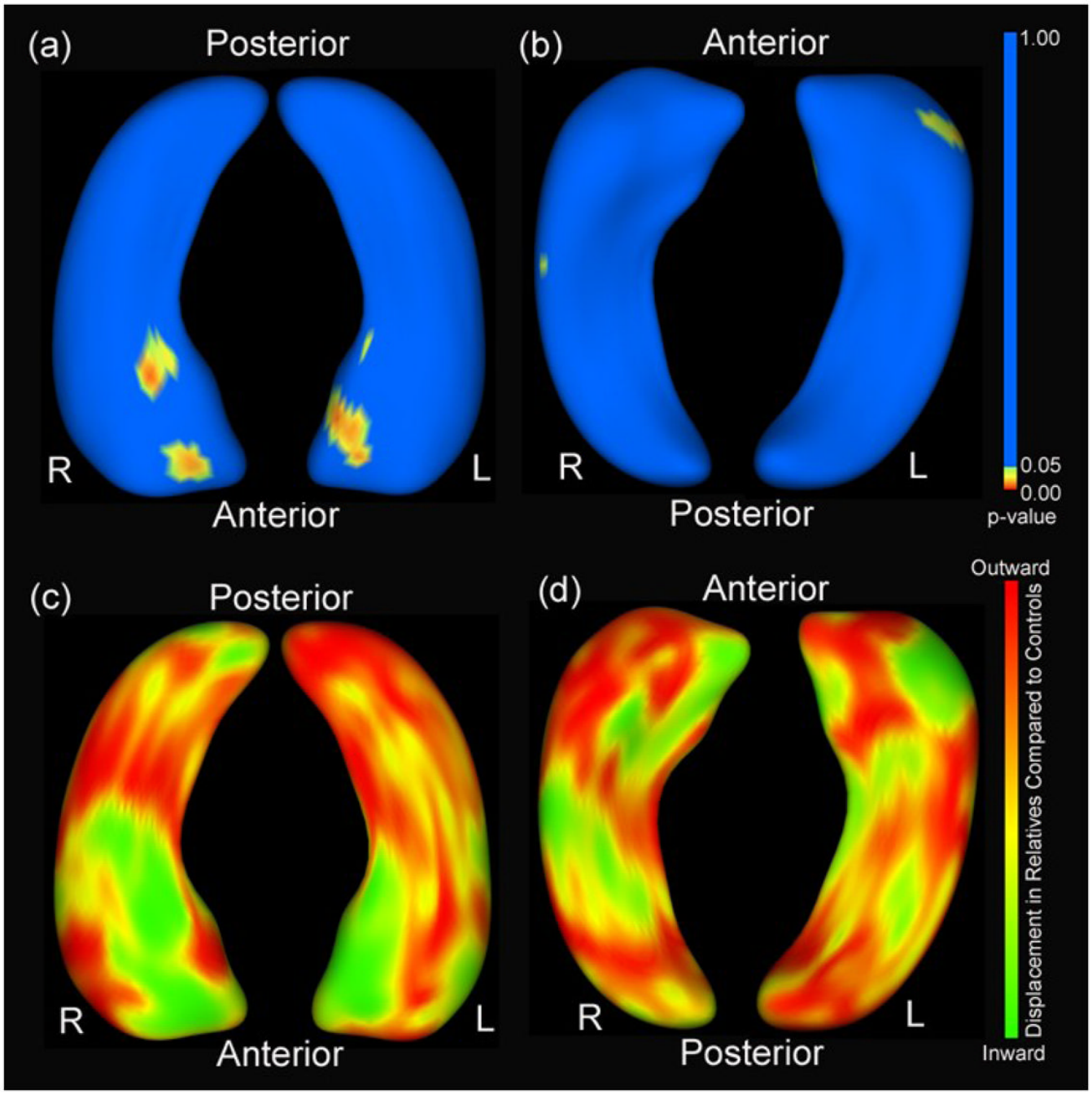


Figure 1.

Comparison of hippocampal shape between 46 relatives of schizophrenia probands and 46 healthy volunteers using spherical harmonic analysis: T-maps reveal significant shape differences in the heads of bilateral hippocampi primarily over superior **(a)** and less so in the inferior **(b)** surfaces (red and yellow areas; raw $p \leq .05$); Displacement maps over superior **(c)** and inferior **(d)** surfaces (mean distances displaced inward (green) or outward (red) in relatives compared to healthy volunteers) further indicate that sub-regions where relatives differed significantly from healthy volunteers correspond to inward displacements.

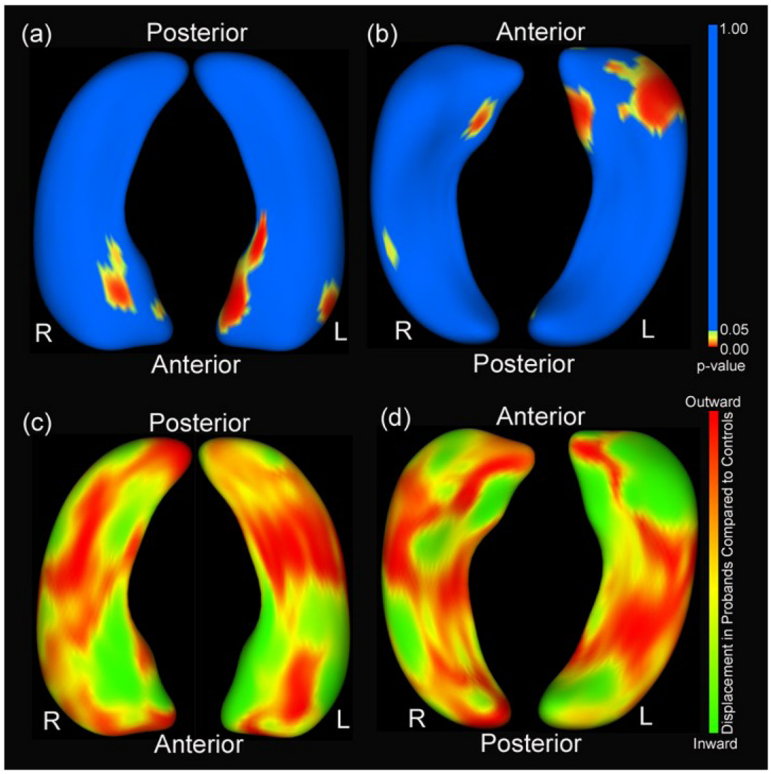


Figure 2.

Comparison of hippocampal shape between 46 schizophrenia probands and 46 healthy volunteers using spherical harmonic analysis: T-maps reveal significant shape differences in the heads of bilateral hippocampi primarily over both superior **(a)** as well as inferior **(b)** surfaces (red and yellow areas; raw $p \leq .05$); Displacement maps over superior **(c)** and inferior **(d)** surfaces (mean distances displaced inward (green) or outward (red) in probands compared to healthy volunteers) further indicate that sub-regions where schizophrenia probands differed significantly from healthy volunteers correspond to inward displacements.

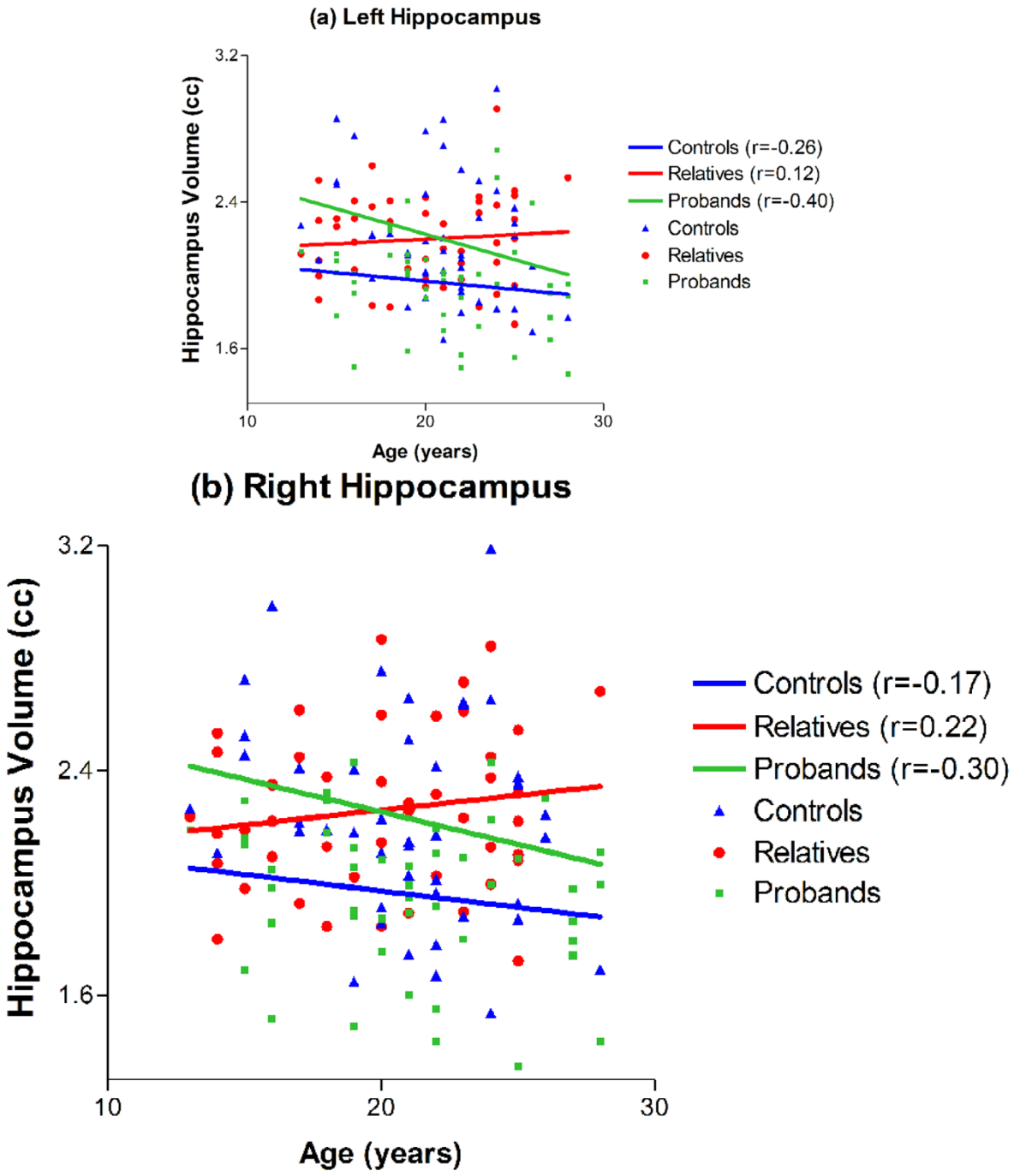
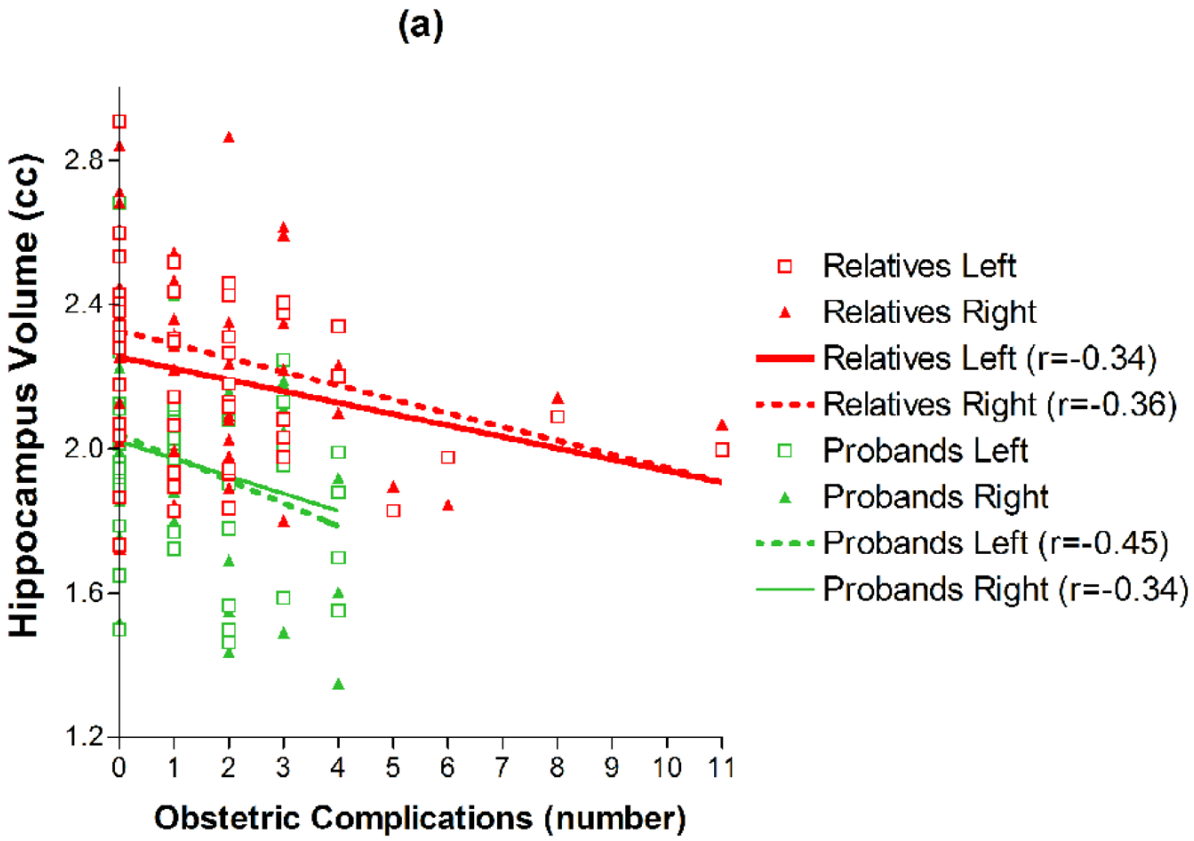
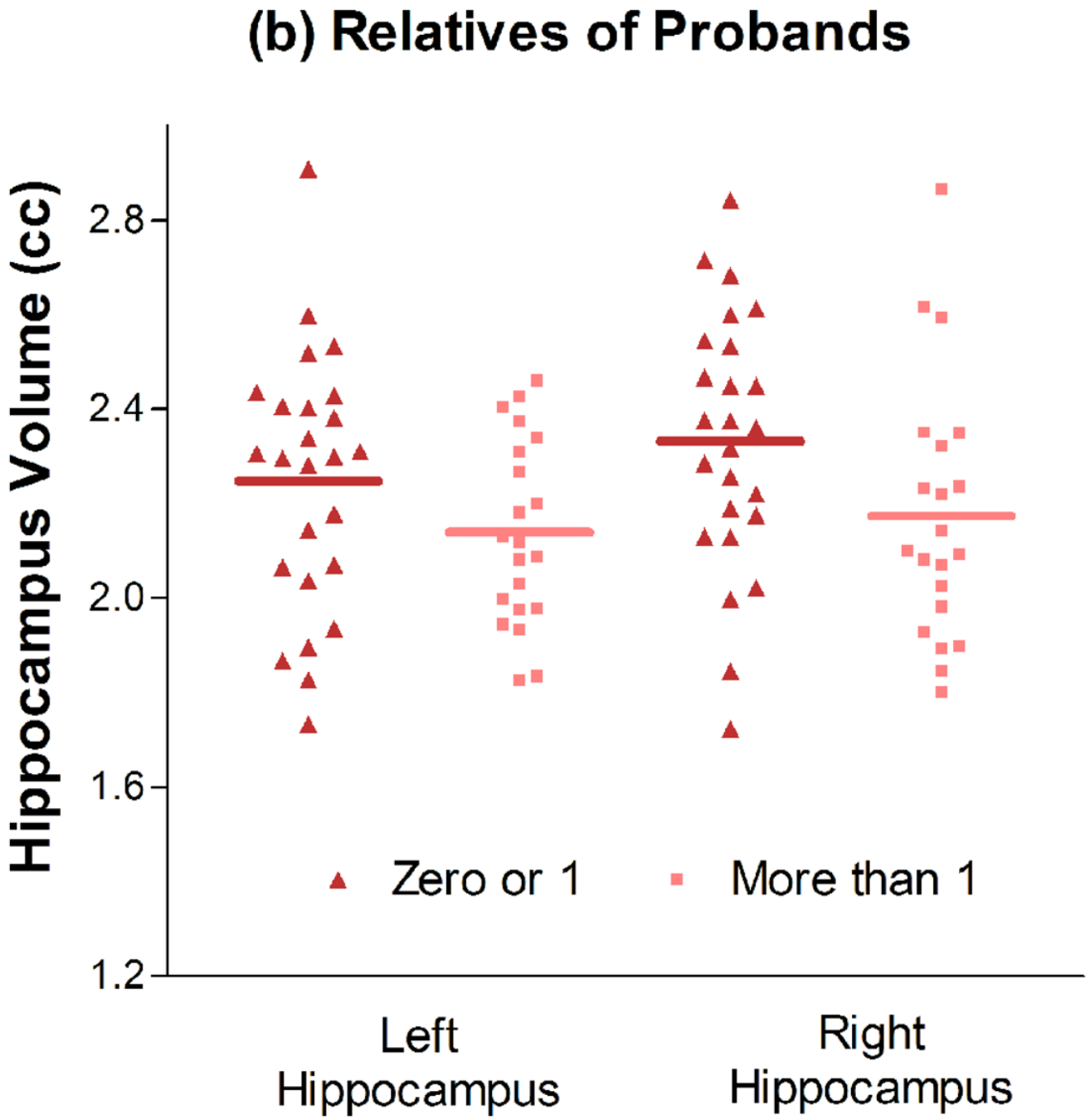


Figure 3.
Comparison of left **(a)** and right **(b)** hippocampus volume-age correlations in healthy volunteers without family history of schizophrenia, biological relatives of schizophrenia probands and schizophrenia probands.





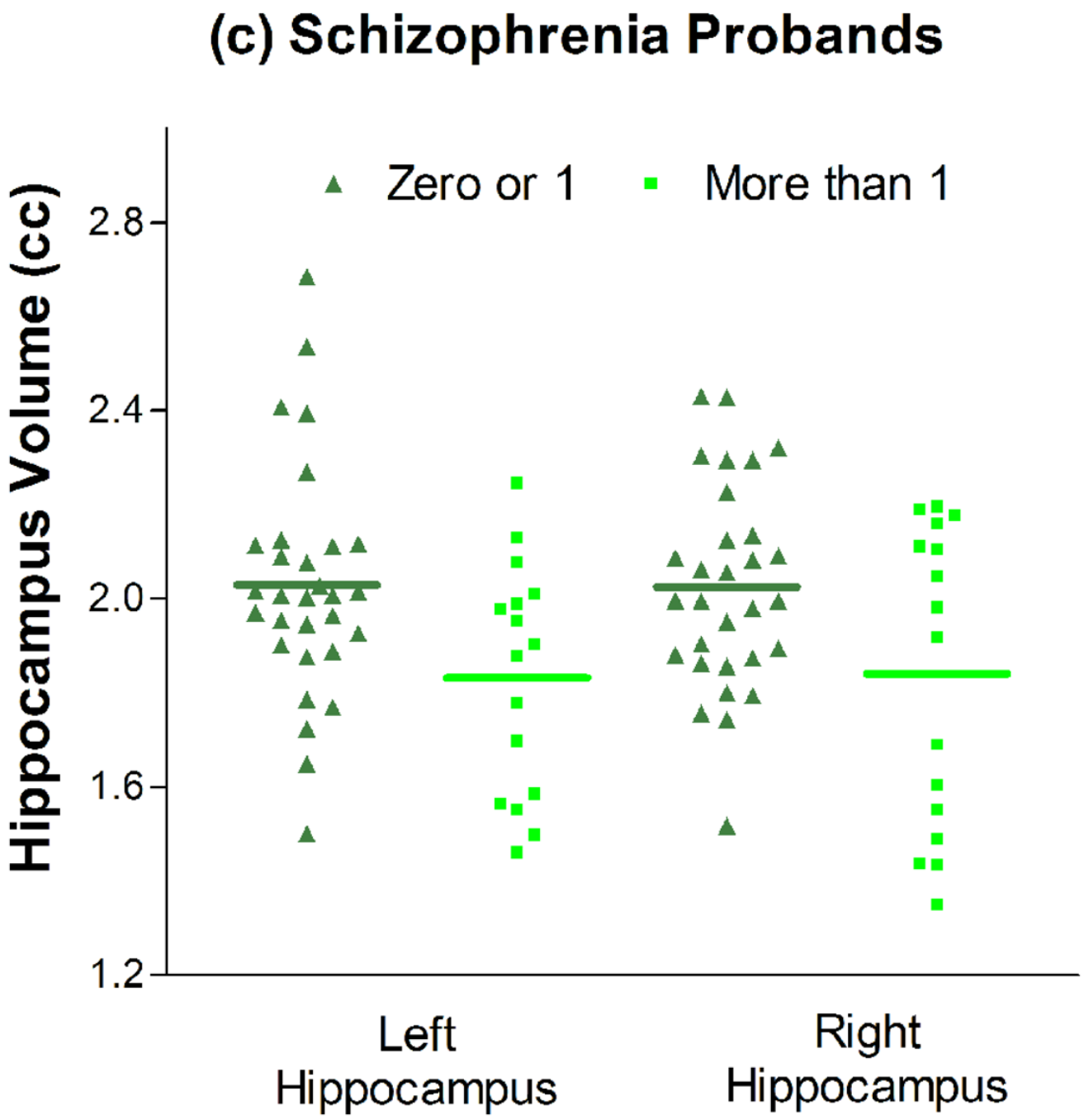


Figure 4.

Effects of obstetric complications on hippocampus volume in biological relatives of schizophrenia probands and in schizophrenia probands: **(a)** Correlations between hippocampus volumes and total number of obstetric complications; **(b)** Comparison of hippocampus volumes between relatives subgrouped by median number of obstetric complications: Zero or 1 obstetric complication (N=25) versus More than 1 obstetric complication (N=21); **(c)** Comparison of hippocampus volumes between schizophrenia probands subgrouped by median number of obstetric complications: Zero or 1 obstetric complication (N=30) versus More than 1 obstetric complication (N=16).

Table 1

Comparison of MRI hippocampus volumes (Mean (SD) (cubic centimeters)) between healthy volunteers without family history of schizophrenia (HNV), biological relatives of schizophrenia probands and schizophrenia probands

	HNV (N=46)	Relatives (N=46)	Probands (N=46)	Group [a] $F_{2,137}$ (p)	Pair-wise Group Comparison [a] $F_{1,91}$ (p)								
					Relatives versus HNV			Probands versus HNV			Relatives versus Probands		
					Group	Age	Interaction [b]	Group	Age	Interaction [b]	Group	Age	Interaction [b]
Left	2.22 (0.26)	2.19 (0.24)	1.96 (0.21)	11.19 (<.0001)	4.53 (.04)	0.65 (.42)	4.38 (.04)	20.22 (<.001)	8.54 (.004)	0.04 (.85)	16.99 (<.0001)	2.87 (.09)	5.78 (.02)
Right	2.26 (0.26)	2.25 (0.27)	2.00 (0.26)	9.83 (.0001)	3.77 (.05)	0.01 (.92)	4.04 (.05)	14.93 (.0002)	4.38 (.04)	0.17 (.68)	16.84 (<.0001)	0.79 (.38)	6.02 (.02)

[a] Covariates for main effects: Intracranial volume and gender

[b] Age × Group

Table 2

Frequency of obstetric complications among 46 biological relatives of schizophrenia probands and 46 schizophrenia probands

Total Number of Obstetric Complications per Subject	**Relatives of Probands (N)**	**Schizophrenia Probands (N)**
None	16	18
1	9	12
2	10	6
3	5	6
4	2	4
5	1	0
6	1	0
8	1	0
11	1	0
Pregnancy Complications	Relatives of Probands (N)	Schizophrenia Probands (N)
Preterm vaginal bleeding	6	2
Premature uterine contractions	6	6
Hypertension/Toxemia of pregnancy	5	13
Convulsions	1	0
Rhesus incompatibility	2	3
Rubella infection	0	0
Other serious medical illness	2	2
Anxiety Disorders	4	6
Depressive Disorders	4	5
Other emotional problems	0	0
Radiation exposure	3	2
Labor/Delivery Complications		
Premature delivery	16	8
Multiple births	4	1
Breech delivery	1	0
Forceps delivery	4	9
Cord around fetus neck	4	3
Fetus blue at birth	2	2
Fetal slow heart beat	2	1
Fetus did not breathe at first	0	2
Fetal convulsions	0	0
Fetus required additional oxygen	7	0
Fetus required blood transfusion	1	0
Fetus required incubator	4	2
Fetal birth defects	3	0