Dougherty MK, Gu H, Bizzell J, Ramsey S, Gerig G, Perkins DO, Belger A., Differences in subcortical structures in young adolescents at familial risk for schizophrenia: A preliminary study. Psychiatry Res. 2012 Nov 9. doi:pii: S0925-4927(12)00094-7. 10.1016/j.pscychresns.2012.04.016. [Epub ahead of print], PMID: 23146250

Differences in Regional Subcortical Volumes in Young Adolescents at Familial High Risk for Schizophrenia

Michael Dougherty, Hongbin Gu, Joshua Bizzell, Guido Gerig, Diana Perkins, Aysenil Belger

Introduction

Although the pathophysiological mechanisms underlying schizophrenia remain unknown, the disorder has long been associated with significant and widespread alterations in brain structure (Shenton et al. 2001). Volumetric differences from healthy comparisons have also been reported as early as the first episode of psychosis (Ellison-Wright et al. 2008; Vita et al. 2006), in adolescents with schizophrenia (Rapoport and Gogtay 2010), prodromal individuals, and in unaffected relatives (Boos et al. 2007; Fusar-Poli et al. 2011). Few studies, however, have examined cohorts of asymptomatic adolescent relatives in the pre-morbid phase before the period of psychosis risk. Studies of brain maturation in children and adolescents with familial risk for schizophrenia are challenged by the difficulty of controlling for normal developmental effects during a period of rapid cerebral change. It is nevertheless likely that the dynamic functional and structural brain changes that occur during puberty and the periadolescent period represent in and of themselves a factor increasing vulnerability for neuropsychiatric disorders (Paus et al. 2008), and thereby constitute a critical period for targeted investigations into the pathogenesis of schizophrenia.

There are several brain regions that are particularly promising candidates for investigation in the neurodevelopmental hypothesis of schizophrenia. While parts of the isocortex are undoubtedly involved in schizophrenic pathology (Ellison-Wright et al. 2008), alterations in deeper, non-isocortical regions are also key features of the illness. Among these are medial temporal lobe (MTL) structures such as the amygdala and hippocampus, and the basal ganglia (BG). The hippocampus is among the most studied structures in schizophrenia research, and has been consistently associated with volumetric reduction in first-episode patients (Vita et al. 2006). Regarding high-risk (HR) cohorts, there is now a general consensus that at least some of these subjects experience hippocampal reduction before psychosis onset, exhibiting an "intermediate phenotype" between first-episode patients and healthy comparisons (Boos et al. 2007; Fusar-Poli et al. 2011). The trend during this time period is complex however, with separate studies supporting environmental (Buehlmann et al. 2009; Lawrie et al. 2001; Wood et al. 2005) and hereditary (Goldman et al. 2008; Narr et al. 2002) factors as the dominant force behind pre-psychotic hippocampal reduction. Furthermore, amygdala-hippocampus effects may vary depending on the presence of an affective component of psychosis (Velakoulis et al. 2006), and comparisons across studies of MTL structures are made more difficult by methodological differences, such as voxel-based morphometry (VBM) versus region-of-interest (ROI) approaches, and the fact that many older studies examined the amygdala and hippocampus as one complex.

While there is comparatively less literature on the basal ganglia in schizophrenia, what findings exist have been more consistent. Caudate volumes have typically been found to be smaller at disease onset, whereas the putamen and globus pallidus have more often been found unchanged (Brandt and Bonelli 2008; Ellison-Wright et al. 2008). In contrast, all three regions of basal ganglia have been reported to be enlarged with disease progression, a finding predominantly attributed to medication effects (Brandt and Bonelli 2008; Ellison-Wright et al. 2008). The limited investigations in HR subjects have provided modest support for a BG defect, including abnormalities of shape (Mamah et al. 2008), function (Fusar-Poli et al. 2010), and molecular composition (Keshavan et al. 2009), as well as correlations with neurocognitive deficits (Bhojraj et al. 2011b; Hannan et al. 2010). Volumetric studies of relatives of schizophrenia patients are conflicting (Goldman et al. 2008; Rajarethinam et al. 2007), leaving the contribution of genetic risk in BG uncertain.

Despite the significant uncertainty concerning the exact role of the above regions in schizophrenia, there is much reason to believe in the general prospect of a striato-limbic pathogenesis of the disease. Here we examined volumetric differences in the regions of the hippocampus, amygdala, putamen, globus pallidus, and caudate nucleus, in non-help-seeking child and adolescent relatives of schizophrenia patients versus healthy comparisons. We further examined whether observed differences are modulated by age, reflecting possible alterations in peripubertal neurodevelopment. Finally, we also explored the correlation between regional volumes and the severity of any prodromal psychotic symptoms in the HR group. We hypothesized that subcortical differences would be present in familial high risk (FHR) children and would correlate with the severity of their subsyndromal clinical symptomatology.

Experimental/Materials and methods

Participants

The current study presents the baseline structural MRI data for the first sample recruited in an ongoing multimodal, longitudinal study of adolescents with FHR for schizophrenia by the University of North Carolina (UNC) Conte Center. Identification and recruitment of 36 FHR subjects between ages 9-18 years was done through the specialized schizophrenia treatment services of UNC hospitals and affiliated clinics, as well as through consumer organizations (North Carolina National Alliance on Mental Illness and Mental Health Association). Healthy comparison (HC) subjects (n = 79) were recruited from the same local communities as the FHR subjects, via email advertisements to databases of UNC students and employees as well as county public schools. Subjects were matched for gender, age, and ethnicity. In order to yield a relatively continuous distribution of subjects in each group across the age range, recruitment was structured into three age brackets: 9-11, 12-14 and 15-18 years old.

For all subjects, FHR was defined as family history of a psychotic disorder in a first degree relative, and was confirmed with the Family Interview for Genetic Studies (FIGS) (Maxwell 1996). For the FHR subjects, diagnosis of the affected relative with schizophrenia or schizoaffective disorder was confirmed using either the Structured Clinical Interview for DSM IV disorders (SCID) (adults) or the Washington University Kiddie Schedule for Affective Disorders and Schizophrenia (WASH-U-KSADS) (children) (Geller 1996). All subjects had a WASH-U-KSADS. Any Axis I disorder in a healthy comparison or their first degree relatives resulted in exclusion; FHR subjects meeting criteria for a psychotic disorder or bipolar disorder

were excluded. Subjects were also excluded if they had a serious medical or neurological disorder, or a history of antipsychotic treatment within three months of enrollment. Four FHR and 24 HC subjects did not meet inclusion criteria. All included subjects underwent a baseline clinical evaluation for the presence and severity of positive, negative, disorganized, and general symptoms, and scores were assigned on each dimension using the Scale of Prodromal Symptoms (SOPS) (Miller et al. 1999). None of the FHR subjects were treatment-seeking at the time of recruitment into the study.

Subjects under 18 years of age gave verbal assent to participation in the study, while legal guardians of minors and 18-year-old subjects provided informed consent. The UNC Biomedical Institutional Review Board approved the study.

Image acquisition and analysis

All subjects were scanned on a 3 T General Electric short-bore scanner at the Duke-UNC Brain Imaging Analysis Center. Multi-contrast high resolution MRI pulse sequences were used to allow multi-channel segmentation for optimal fidelity, including T1 weighting (IRprepped 3-D FSPGR, TR 7.5 ms, TE 3.0 ms, inversion preparation time 450 ms, flip angle 12^{o}, bandwidth/pixel 244 Hz, imaging matrix 256 x 256, FOV 256 x 256 mm, slice thickness 1 mm) and a double-echo dual-contrast FSE sequence (TR 3000 ms, TE 25.1 and 87.7 ms, flip angle 90^{o}, bandwidth/pixel 122.1 Hz, imaging matrix 256 x 192, FOV 256 x 256 mm, slice thickness 2 mm) for optimized proton density and T2 weighting. Total brain and tissue volumes were obtained with an automatic, expectation-maximization scheme (EMS) brain segmentation tool which used all three MRI contrasts and an atlas prior (Prastawa et al. 2003). A pipeline for automated subcortical segmentation, developed by investigators at the Neuro Image Research and Analysis Laboratories (NIRAL) at UNC and based on an unbiased population atlas embedding probabilistic models of anatomical structures (see details in Gouttard et al. 2007), was used to delineate subcortical ROI, including the caudate nucleus, putamen, globus pallidus, amygdala, and hippocampus. Two HC subjects voluntarily withdrew before image acquisition, and 14 scans (4 FHR and 10 HC) could not be processed due to excessive motion, orthodontic device interference, or other technical difficulty. The final data set (26 FHR and 43 HC) consisted of those scans on which automatic processing was successfully completed, and each of these images was visually inspected for gross segmentation errors by two blinded raters. In the interest of preserving the objectivity of the automated method, it was elected to neither exclude nor manually correct the frequent but minor errors in boundaries produced by the automated, probabilistic algorithm.

Statistical Analysis

Demographic variables were analyzed by two non-parametric tests, the Fischer Exact Test for categorical variables and the Wilcoxon Two-Sample Rank Test for continuous variables. The categorical variables included gender, race, handedness, and highest level of parental education, which was used as a rough proxy for socioeconomic status and home environment. The continuous variables analyzed were each subject's age and SOPS scores (Miller et al. 1999).

The group and age related differences in total brain tissue volume (TBV=total gray matter [GM] + total white matter [WM]) were first examined in an ANCOVA model with group, gender, age and age by group interaction. We then normalized the five subcortical ROI—

amygdala, hippocampus, caudate, putamen, and globus pallidus—by dividing each by the TBV to correct for individual differences in head size (corrected volume=ROI volume/TBV *100,000). For the remainder of the manuscript, ROI "volume" will refer to "TBV-corrected volume" unless otherwise specified. We tested for group differences in hemispheric asymmetry by group X hemisphere interaction in a MANCOVA model with group (FHR, HC), age (9-18 years), and gender (male, female) as between-subject variables and hemisphere (left, right) as a within-subject variable. With no group X hemisphere interactions detected, we combined corresponding left and right subcortical structures and modeled the total volume with ANCOVA models, which include group, gender, and age as well as group by age interaction. The volumetric differences associated with age were estimated by separate age slopes for the FHR and HC groups. Group differences in the age-related changes were tested in the interaction between age and group.

Within the high-risk group, Pearson's *r* was used to test the association of each SOPS domain (positive, negative, disorganized, general, and total scores) with corrected volume of each subcortical ROI. As a sensitivity analysis to evaluate the potential confounding effect of age, we also calculated the partial correlation between SOPS scores and ROI volume while controlling for age.

All tests were two-tailed at significance level of .05. Considering the unique function and significance of each subcortical structure, no multiple comparison correction was applied in this analysis.

Results

Sample Demographics

The sociodemographic profile of the sample is shown in Table 1. The familial risk group did not differ significantly from the comparison group in age, adolescent stage, gender, ethnicity, handedness, or last grade completed. Parents of the HC group had significantly higher levels of education than those of the FHR group ($p<.01$). SOPS scores were significantly higher in the FHR group in all dimensions, although the scores still fell well below a level that could be considered "prodromal" (Miller et al. 1999).

Table 1. Demographic Characteristics of Familial High Risk (FHR) and Comparison Groups

	Comparison (n=43)		FHR (n=26)		p-value[a]
Age [mean (SD)]	14.22	(2.52)	14.49	(2.35)	0.85
Late Adol. Stage[b] [N (%)]	26	(60%)	16	(62%)	1.00
Female [N (%)]	25	(58%)	15	(58%)	1.00
Caucasian [N (%)]	34	(79%)	19	(73%)	0.39
Right-handed[c] [N (%)]	32	(76%)	22	(88%)	0.69
Last grade completed [mean (SD)]	7.65	(2.50)	7.42	(2.55)	0.71
Highest Parental Education					**<0.01**
Without high school degree [N (%)]	0	(0%)	4	(15%)	
High school diploma or GED [N (%)]	4	(9%)	8	(31%)	
College degree [N (%)]	11	(26%)	6	(23%)	

Graduate Degree [N (%)]	28	(65%)	8	(31%)	
SOPS Scores					
Positive [mean (SD)]	1.11	(1.67)	2.34	(2.29)	< **.01**
Negative [mean (SD)]	0.91	(1.44)	3.20	(3.04)	< **.0001**
Disorganization [mean (SD)]	0.51	(0.92)	1.46	(1.74)	<**.001**
General [mean (SD)]	0.63	(1.14)	1.97	(2.32)	< **.001**
Total [mean (SD)]	3.15	(3.68)	8.97	(6.70)	< **.0001**

[a] p-values are based on Fisher's exact tests for categorical variables and Wilcoxon two-sample rank tests for continuous variables. **Bold** values are p<.05.
[b] Late Adolescent is defined as 14 and older.
[c] Two subjects were missing handedness data (total n = 67), and of the non-right-handed subjects, one was ambidextrous.

Volumetric Analysis

Total Brain Volume. The ANCOVA model of total brain tissue volume (Table 2, first column) confirmed significantly larger total volume in the male adolescents than the females. The FHR and HC groups had similar TBV overall and in relation to age, with both study groups showing modest annualized differences of less than 1% in TBV (Figure 1). The increase, however, was only significant in the HC group in the post hoc analysis of the slope of TBV-age relationship.

Hemisphere Asymmetry. MANCOVA models found significant hemispheric asymmetry in all subcortical structures (p<.01), but no significant differences between groups (p>.50). For both FHR and HC, amygdala and caudate were about 3% larger on the right than the left, while hippocampus, putamen, and globus pallidus were larger on the left by 5%, 2% and 1% respectively (Supplementary Table).

Subcortical Structures. With no evidence for group differences in hemispheric asymmetry, data for each ROI was collapsed across both hemispheres to test for group- and age-related differences (Table 2). Effect of gender was significant at p<.05 only in the amygdala. Pairwise comparisons revealed that males had greater volumes in most structures except the globus pallidus, where females tended to be larger. Averaging across the age range, the FHR group showed smaller volumes than the HC group in all the subcortical structures. The difference was significant in the hippocampus. In the analysis of group differences in age effects however, the groups showed significantly different volume-age relationships in nearly all subcortical structures (p=.08 for amygdala, and p<.05 for caudate, hippocampus, putamen, and globus pallidus; see Table 2 and Figure 1). Post hoc analysis of each group revealed that the TBV-corrected volumes were largely stable across adolescence in the HC group, with non-significant (p>.18) slopes in all ROIs. In contrast, significant positive-sloping volume-age relationships (p<.05) were observed in all subcortical structures for the FHR group (Table 2 and Figure 1).

Correlations with Prodromal Symptoms

Table 3 shows Pearson's r with corresponding correlations between subcortical structures and SOPS symptoms. The volumes of amygdala, hippocampus and putamen were negatively correlated with disorganization scores, indicating an association of smaller volumes with higher symptom ratings. The putamen was further negatively correlated with total SOPS score.

Notably, in a sensitivity analysis controlling for age, partial correlations with disorganized symptoms remained significant in the putamen (r = -0.42, p = .04) and hippocampus (r = -.46, p = .02).

Table 2. ANCOVA for Subcortical Regions-of-Interest.

	Total Brain Volume	Amy. Corr. Vol.[a]	Hipp. Corr. Vol.[a]	Caudate Corr. Vol.[a]	Putamen Corr. Vol.[a]	Glob. Pal.
Gender						
Male [LSM]	1249341	312.5	324.4	487.4	629.0	327.1
Female [LSM]	1141799	306.4	319.7	478.0	623.9	332.1
Difference [p-value]	**<.01**	**.03**	.14	.30	.35	.08
Group						
Healthy Comparison [LSM]	1199581	309.6	325.7	483.5	629.9	330.4
Familial High Risk [LSM]	1191559	309.2	318.4	482.0	622.9	328.9
Difference [p-value]	.74	.87	**.02**	.86	.20	.61
Group X Age						
Healthy Comparison [Slope, p-value]	12490 **.04**	0.02 .98	-0.09 .91	0.89 .69	-0.92 .49	-0.95 .18
Familial High Risk [Slope, p-value]	3846 .64	2.18 **.03**	3.12 **.01**	8.87 **.01**	6.50 **<.01**	1.91 **.05**
Difference [p-value]	.40	.08	**.02**	**.04**	**<.01**	**.02**

[a] Corrected Volume = (total ROI volume /total brain volume) *100,000. Total ROI volume is the sum of left and right ROI volume. Total brain volume is the sum of total gray matter and total white matter. **Bold** values are p<.05. LSM = Least Square Mean.

Table 3. Correlation Analysis of SOPS Scores with Volumes of Subcortical Regions-of-Interest in Familial High-Risk Adolescents.

	Positive		Negative		Disorganized		General		Total	
Amygdala [r, p-value]	-0.25	.21	-0.03	.85	**-0.47**	**.02**	-0.19	.34	-0.28	.17
Hippocampus [r, p-value]	-0.17	.38	-0.08	.68	**-0.60**	**<.01**	-0.10	.61	-0.28	.16
Caudate [r, p-value]	-0.16	.42	-0.03	.87	-0.25	.21	0.01	.95	-0.12	.53
Putamen [r, p-value]	-0.34	.09	-0.22	.28	**-0.58**	**<.01**	-0.20	.34	**-0.42**	**.03**
Globus Pallidus [r, p-value]	-0.23	.26	-0.22	.26	-0.35	.08	-0.29	.15	-0.36	.07

[a] **Bold** values are p<.05.

Discussion

Our findings reveal that relative to HC subjects, FHR subjects showed smaller volumes in the hippocampus and BG structures within the context of a significant group by age interaction, such that the largest reductions occurred in early adolescence. Furthermore, the volumes of several regions were negatively correlated with SOPS disorganization scores, indicating that subjects with greater disorganization showed smaller volumes in the amygdala, hippocampus, and putamen.

This study's cohort is unique in that subjects span the entire range of adolescence, including early adolescence. Most studies report on FHR subjects starting at ages 18, 16, or 13, yet individuals who go on to develop schizophrenia exhibit neuropsychological abnormalities

much earlier in childhood (Jones et al. 1994; Reichenberg et al. 2005). Indeed, brain morphology is in a dynamic state throughout childhood, likely experiencing a particularly dramatic neurodevelopmental shift at puberty (Bramen et al. 2010; Lenroot et al. 2007), which may precipitate the onset of a spectrum of psychopathology (Paus et al. 2008). Meanwhile, studies of childhood-onset schizophrenia (COS) must necessarily study the early adolescent age group, but these patients are severe extremes of the schizophrenia phenotype with well-established structural morphologic abnormalities. Therefore, in order to characterize biological markers of risk for schizophrenia in an early premorbid stage, the current study encompassed the entire range of puberty in a non-prodromal FHR cohort.

[insert Figure 1]

Indeed, our results can only be interpreted in a developmental context. Hippocampal size has been shown to exhibit little change relative to intracranial volume (ICV) during healthy adolescence (Mattai et al. 2011; Ostby et al. 2009), as was the case in our healthy comparisons. In contrast, hippocampal volume has been reported to be smaller in COS subjects relative to healthy comparisons throughout adolescence (Nugent III et al. 2007). Additionally, relatives of schizophrenia patients have also been shown to have intermediate volume reductions (Boos et al. 2007; Fusar-Poli et al. 2011). However, a recent study using a semi-automated technique similar to ours failed to detect a significant difference from healthy comparison subjects in unaffected siblings of COS patients (Mattai et al. 2011). However, while Mattai et al. failed to find group differences at the older, median age of their sample, their plotted trajectories do seem to differ in slope between groups, perhaps allowing for a volumetric difference at earlier ages that correspond to our sample. Thus, the inclusion of a broader age range in our study allows us to reconcile these findings, by showing that volume differences may be present in younger familial risk children but absent in later adolescence.

While our only significant findings in the MTL were hippocampal, the group X age interaction approached significance in the amygdala. Although little data is available on longitudinal changes of amygdala volume during adolescence, the HC group from one study showed that, much like the hippocampus, the amygdala also normally exhibits a relatively flat growth trajectory (Giedd et al. 1999). Conflicting studies of early-onset schizophrenia have demonstrated either no difference from comparison subjects in trajectory or baseline volume (Giedd et al. 1999), or a stably smaller amygdala volume relative to comparisons across adolescence (Frazier et al. 2008). Although amygdala reduction has been well-associated with adult-onset disease (Ellison-Wright et al. 2008), two recent meta-analyses of VBM studies in HR individuals have yielded opposing findings (Chan et al. 2011; Fusar-Poli et al. 2011), failing to clarify the conflicting results from previous studies employing multiple methodologies (Bhojraj et al. 2011a; Velakoulis et al. 2006). The trend-level group X age interaction detected over a younger age range in our sample suggests that such age-mediated effects could reconcile some of the inconsistencies in these studies. The absence of statistical significance in the amygdala may be attributable to greater inter-subject variability in this structure relative to other subcortical ROI's, or may indeed reflect a smaller effect size for this structure, both of which could further explain the discrepancies between the findings of other studies of the amygdala.

From longitudinal studies of pediatric neurodevelopment at the National Institutes of Mental Health, we have learned that in healthy adolescents the caudate nucleus appears to follow the same "inverted U" trajectory as other gray matter (GM) structures across adolescence (Lenroot et al. 2007). Less certain are the trajectories of the lenticular nuclei, but they may

follow a similar, declining trajectory (Giedd et al. 1996; Ostby et al. 2009). This pattern of subtle decline is consistent with our comparison data, but in stark contrast to the often sharply positively-sloped BG trajectories of the FHR group. The group X age interactions of BG structures in the FHR group are consistent with other reports in the literature, which include smaller caudates in another adolescent FHR study (Rajarethinam et al. 2007) but failure to find volumetric reductions in unaffected adult relatives (Goldman et al. 2008; Hannan et al. 2010; Mamah et al. 2008).

Our study supports the assertion that the trajectory of cerebral development as indicated by group X age interactions may be a more accurate marker of psychopathology than static average volumes compared between groups, at least during adolescence (Shaw et al. 2010). In our study, trajectories in each subcortical ROI showed distinct group differences. Interpretation of these trajectories is difficult due to an absence of literature on subcortical structures in peripubertal FHR cohorts, although the general principle of aberrant trajectories as a marker of psychiatric disease has been demonstrated in the striatum of developmental cohorts with autism (Langen et al. 2009) and ADHD (Castellanos et al. 2002). The deviant trajectories of cortical maturation associated with early-onset schizophrenia have been attributed to an abnormal acceleration of normal back-to-front cortical thinning during adolescence (Rapoport and Gogtay 2010), a process which may also take place in adult conversion (Thompson et al. 2009). Such a decline in gray matter has not yet been demonstrated for subcortical structures, and any study of such trajectories would likely face significant confounding effects of medication. Nevertheless, volumetric and/or gray matter decline would be a reasonable expectation in the subcortex of young adolescents with schizophrenia, based on the likelihood that adolescent cerebral maturational processes tend to involve a significant degree of synaptic pruning (Rapoport and Gogtay 2008), and the fact that the subcortical regions in question generally exhibit slightly declining trajectories in normally developing adolescents (Mattai et al. 2011; Ostby et al. 2009). An accelerated decline would yield the reduced volumes seen at psychotic conversion. Paradoxically, our data suggest not a decrease but rather a sharp *increase* in subcortical GM of FHR adolescents with age, insofar as we can speculate on such patterns from cross-sectional data. One explanation for these findings may lie in the "natural" attrition of the high-risk cohort during adolescence, such that those who are more symptomatic or who convert will not be included in the older portion of our FHR group. In other words, it is possible that older FHR individuals in our study represent a slightly lower risk group by virtue of having moved later in the risk-age window without converting. Alternatively, the group X age interaction in our study may represent a "natural" normalization process that procures "resilience" during adolescence. While subcortical volume increase has yet to be shown in a study of subjects at familial high-risk for schizophrenia, such a process is plausible given the findings of the largest study of COS siblings to date (Gogtay et al. 2007). In that study, siblings of COS patients demonstrated intermediate deficits in prefrontal and temporal cortices, which normalized by age 20 and correlated with improved overall functioning. The trajectories in Mattai et al's recent study suggest the possibility of a similar process of volumetric convergence in the hippocampi of healthy siblings, albeit less dramatically. Such a normalization is intuitive in FHR samples, as only a minority of individuals (6-21% depending on type) will actually go on to develop psychosis (Kendler et al. 1993). This percentage is substantially less than the 20-50% rates of conversion reported in clinical high risk samples (Cannon 2010). Our FHR sample is characterized by low (within normal limits) SOPS scores and a conversion rate of 1/26 after 1-4 years of follow-up. The question may therefore be posed whether such a sample might not be

better labeled "intermediate" risk when considering the spectrum of schizophrenia risk from population-wide rates to the "ultra-high" risk of prodromal individuals. Though it would be unwarranted to blindly assume that developmental trajectories are identical for cortical and subcortical regions, it is interesting to speculate that our group X age interactions may represent an analogous "normalization" in the subcortex to that demonstrated by Gogtay and colleagues in the cortex of COS siblings.

Despite this "intermediate" degree of risk in FHR individuals, the significant group X age effects still suggest that younger adolescent FHR subjects in particular display clear structural abnormalities on MRI. That the subcortical regions examined here would show such abnormalities is congruent with current theories of the pathogenetic mechanisms of schizophrenia, which include prominent, if not central, roles for the same (Howes and Kapur 2009; Lodge and Grace 2010; Simpson et al. 2010; Tamminga et al. 2010). One particularly interesting deficit receiving much recent attention as a potential unifying framework of neurodevelopmental pathogenesis in schizophrenia is that of parvalbumin-containing fast-spiking GABA interneurons (FSGI). This particular class of neurons appears to be partially responsible for gamma-band oscillatory activity, which has been reported to be markedly disturbed and associated with poorer cognitive functioning in schizophrenia. Postmortem studies of schizophrenia patients indicate that FSGI are not only prominently disrupted in the prefrontal cortex, a core region associated with the pathophysiology of cognitive deficits in schizophrenia (Gonzalez-Burgos et al. 2010), but are also decreased across all subregions of the schizophrenic hippocampus (Zhang and Reynolds 2002). Early FSGI dysfunction in the hippocampus has been linked to neonatal infection (Meyer and Feldon 2009), schizophrenia-associated genes (Fazzari et al. 2010), and redox dysregulation with concomitant NMDA receptor hypofunction (Steullet et al. 2010). Psychostimulant abuse and psychological stress (Lodge and Grace 2010), perhaps via the action of adrenal and gonadal hormones (McEwen 2010), may also exacerbate the aberrant circuitry in adolescence. Disruption of this GABA- and glutamatergic circuitry could actually impair normal neuroplasticity and lead to the loss of synaptic and dendritic density in the neuropil that is likely the cause of volumetric reductions in most cerebral regions in schizophrenia (Harrison 1999; Woo et al. 2010).

Intriguingly, rodent models of schizophrenia have provided evidence for a possible link between FSGI and striatal dysfunction, by implicating ventral hippocampal (corresponding to anterior hippocampus in humans) FSGI in the regulation of subcortical dopaminergic systems (Lodge and Grace 2010). Decreased GABA inhibition of hippocampal efferents could lead to hyperactive circuitry to the ventral tegmental area through the nucleus accumbens—part of the ventral striatum—resulting in aberrant attribution of salience to stimuli (Lodge and Grace 2010). The construct of salience misattribution has been proposed as a framework for both the positive and negative symptoms of schizophrenia as a final common pathway of dopaminergic dysfunction (Howes and Kapur 2009). And while abnormalities of the ventral, "limbic" striatum (nucleus accumbens and some ventral caudate and putamen) could potentially contribute to volumetric deficiencies in our sample, recent functional imaging studies have shown that the dorsal, "associative" striatum (pre-commissural caudate and putamen) may be most disrupted in prodromal individuals (Fusar-Poli et al. 2010) as well as unmedicated schizophrenics (Kegeles et al. 2010), suggesting a greater functional overlap of ventral and dorsal striatum than previously suspected. Indeed, the location of the volumetric reduction detected in the present study is most consistent with dorsal striatum. Thus, our findings are compatible with a developmental

dysregulation of striatolimbic circuitry that has been identified as abnormal in the schizophrenia-spectrum.

Our findings of a significant correlation between severity of disorganization and the volumes of subcortical ROI's suggest a specific role for these regions in the emergence of the symptomatology of schizophrenia in FHR individuals. Although the domain of cognitive disorganization has been previously associated with structural deficits of prefrontal cortex in FHR samples (Bhojraj et al. 2011a; Harms et al. 2010), there is little data on such associations in subcortical regions. Nevertheless, striatal volume abnormalities have been linked to affective flattening and hallucinations in patients with schizophrenia, and caudate shape abnormality (but not volume decrease) was correlated with thought disorganization (Ballmaier et al. 2008; Mamah et al. 2008). Conceptual disorganization and thought disorder have also shown correlations with decreased volume of MTL structures, but these were in diagnosed schizophrenics (Bogerts et al. 1993; Rajarethinam et al. 2001), and not as frequent as those with positive and negative dimensions.

This study's finding of a correlation between subcortical volumes and disorganized symptoms in the absence of similar correlations with positive or negative symptom severity may indicate that the cognitive domain is preferentially affected in young adolescent FHR individuals. A recent factor analysis of psychopathology in the "at-risk mental state" has demonstrated that disorganized and cognitive symptoms load together in their own dimension (Demjaha et al. 2010). Furthermore, neurocognitive deficits are among the earliest symptomatic manifestations of disease and/or disease risk (Jones et al. 1994; Reichenberg et al. 2005). As such, disorganized features may be more prominent than the traditional clinical positive and negative symptoms of psychosis in a young, non-help-seeking, "intermediate" risk cohort like ours. Under this framework, our finding of a correlation between severity of disorganization and smaller regional subcortical volumes is consistent with previously reported associations between cognitive dysfunction and subcortical volumes in high-risk samples (Hannan et al. 2010; van Erp et al. 2008). In fact, a recent FHR study has actually shown bilateral hippocampal and striatal volumes to be among the regions most associated with overall neurocognitive deficit, even including several prefrontal areas (Bhojraj et al. 2011b). As these correlations were only exploratory analyses and SOPS scores in the study group were overall too low to permit any strong conclusions, further studies incorporating specific measures of disorganized symptoms would help to clarify the relationship of these to cognitive dysfunction in young FHR subjects, as well as the role of cortical and subcortical abnormalities in this symptom domain.

Limitations of this study include a relatively small sample size, which could have reduced the statistical significance of some results. The somewhat broad ROI's investigated here could also mask more striking effects at the subregional level, for example in anterior hippocampus or caudate. Further, the lack of a group X hemisphere interaction in our preliminary analysis could be a product of our smaller, cross-sectional sample, as Mattai et al's NIMH cohort seems to show a disproportionate deficit in the left hippocampus of siblings (Mattai et al. 2011). Our choice of strict exclusion criteria to create a "super-normal" comparison group, while preserving contrasts between groups that could otherwise be confounded by unrelated disease or medication factors, may limit generalizability in studies of clinical applications. However, even if such criteria magnify subtle differences undetected in other studies, that any differences exist at all in a pre-symptomatic sample argues strongly for a neurodevelopmental pathogenesis of schizophrenia. Overall, potential confounding differences between groups were minimal, with only maximum

degree of parental education significant. By definition, a much larger portion of the FHR group had parents with persistent mental illness, a characteristic associated with significantly less education. Nevertheless, since we employed this demographic variable as a rough proxy for home environment and socioeconomic status, such environmental differences cannot be discarded as an explanation for some of the variance between groups.

Confounding influences of sex must be considered in a study of brain structure during adolescence, and gender effects were detected, significantly in the amygdala and at trend-level in the globus pallidus. The ANCOVA attempts to separate these effects from the principal investigation of group differences, but may not completely eliminate the possibility of type I and II errors secondary to gender-based confounders, especially considering the complexity of such interactions during adolescence. Indeed, there is evidence that the MTL is differentially more affected than the BG by puberty and the associated hormonal milieu, perhaps due to different receptor densities for gonadal hormones in the hippocampus and amygdala (Bramen et al. 2010; Neufang et al. 2009), or even disease-specific interactions with gender (Frazier et al. 2008). However, BG structures also exhibit gender effects (Frazier et al. 2008; Giedd et al. 1996; Neufang et al. 2009), and follow different trajectories with earlier peaks in females (Lenroot et al. 2007). The sexual dimorphism of subcortical trajectories raises the possibility that sex interactions could have enhanced or diminished our group- and age-related findings in a study powered to investigate more sources of variance. Tanner stage and hormone blood levels were not collected, but could have provided additional insights.

It is also possible that a loss of precision due to our image processing methods may have weakened our results. While an automated method eliminates some of the subjective bias inherent in manual tracings, manual segmentation is much more able to capture individual variability than current automated methods. The subcortical segmentation method for this study has been shown to correlate well with manual segmentations (Gouttard et al. 2007) and be highly reproducible between scans with human phantoms (Gouttard et al. 2008). In a multi-site study of traveling phantoms using the same segmentation method, all structures demonstrated a high intra-site ICC (0.94 and higher), except for the right amygdala which showed greater variability with ICC of 0.61 (Gerig and Gu, personal communication). The segmented images for the present study were visually inspected by two separate, blinded raters, and none adjusted or discarded, suggesting that any errant results are not the product of subjective bias and would tend to regress toward the mean, leading to a greater proportion of type II rather than type I error. Although greater variability in measurement could possibly explain the failure to detect differences at $p<.05$ in the amygdala, this is less likely given our preliminary null finding of a group X hemisphere interaction ($F(1,64)=0.03$, $p=.86$) in the presence of a very good ICC (0.97) in the left amygdala.

Finally, we must re-emphasize the limits of interpretations of group X age interactions in cross-section, as we have not truly measured intra-subject change, which is likely to be quite variable in the peri-pubertal period. In light of recent reports from longitudinal cohorts, such variability may indeed have led to a type I overestimate of our already-subtle findings (Mattai et al. 2011). As previously mentioned, this estimate may also be affected by the exclusion of higher-risk older subjects, who by virtue of their age had developed more severe prodromal symptoms. Nonetheless, the present study is the first to our knowledge to examine these particular regional

trajectories in relatives of patients with schizophrenia during the dynamic period of adolescent development from 9-13 years of age. We believe the present study to be a valuable exploratory investigation of a cohort traditionally difficult to analyze, and indicative of promising areas for future research regarding the neurodevelopment of subcortical circuitry in schizophrenia-spectrum disorders.

In summary, we have demonstrated subcortical volumetric deficiencies in a sample of non-prodromal adolescents with familial risk for schizophrenia, which also correlate with low-level disorganization symptoms. These findings are in structures that characteristically exhibit a broad range of abnormalities in schizophrenia-spectrum populations, but in our sample appear to be normalizing (increasing) in post-pubertal, late adolescence. Our data are consistent with current theories of the pathogenesis of schizophrenia, in that an initial (likely genetic in an FHR cohort) insult in populations of neurons in key subcortical regions may represent a vulnerability phenotype for psychosis in late adolescence and early adulthood. However, it is likely that a "second-hit" such as stress or substance abuse during adolescence is necessary to push an individual over the threshold of psychosis. In the absence of triggers, the majority of FHR individuals may be able to engage restitutive mechanisms to normalize their brain volumes, perhaps by modulating neurodevelopmental processes such as synaptic pruning. Such normalization has been demonstrated to occur and to correlate with clinical and functional improvement in multiple adolescent psychiatric disorders (Rapoport and Gogtay 2008). Longitudinal data as well as documentation of conversion status will further inform about the significance of the differences in subcortical trajectories detected in this study. Understanding the factors at work in the pre-symptomatic period may assist in the identification of "triggers" of psychosis and the development of targeted, early interventions.

Acknowledgements
The neuroimaging studies and analyses were funded by the NIMH Silvio O. Conte Center grant MH064065, as well as by the National Alliance for Medical Image Computing (NA-MIC) U54 EB005149. Further support for individual authors includes Mr. Dougherty's support from the Howard Holderness Distinguished Medical Scholars fellowship, Dr. Belger from the FOH ( ) and a NARSAD Independent Investigator Award, Mr. Bizzell, Dr. Perkins, ….We would like to thank Stacey Ramsey, Kathleen Monforton, and Erin Douglas for subject recruitment and collection of demographic data, Susan Music, Zoe Englander, and Mary Agnes McMahon for image acquisition, Joe Blocher for image pre-processing, and Robert Hamer for comments on statistical approach and on the final manuscript.

Conflicts of Interest
None reported.

References

Ballmaier, M., Schlagenhauf, F., Toga, A. W., et al. Regional patterns and clinical correlates of basal ganglia morphology in non-medicated schizophrenia. *Schizophrenia Research* (2008) 106(2-3):140-147.

Bhojraj, T. S., Francis, A. N., Montrose, D. M., et al. Grey matter and cognitive deficits in young relatives of schizophrenia patients. *NeuroImage* (2011b) 54(Supplement 1):S287-S292.

Bhojraj, T. S., Sweeney, J. A., Prasad, K. M., et al. Gray matter loss in young relatives at risk for schizophrenia: Relation with prodromal psychopathology. *NeuroImage* (2011a) 54(Supplement 1):S272-S279.

Bogerts, B., Lieberman, J. A., Ashtari, M., et al. Hippocampus-amygdala volumes and psychopathology in chronic schizophrenia. *Biological Psychiatry* (1993) 33(4):236-246.

Boos, H. B. M., Aleman, A., Cahn, W., et al. Brain Volumes in Relatives of Patients With Schizophrenia: A Meta-analysis. *Arch Gen Psychiatry* (2007) 64(3):297-304.

Bramen, J. E., Hranilovich, J. A., Dahl, R. E., et al. Puberty Influences Medial Temporal Lobe and Cortical Gray Matter Maturation Differently in Boys Than Girls Matched for Sexual Maturity. *Cerebral Cortex* (2010).

Brandt, G. N., and Bonelli, R. M. Structural neuroimaging of the basal ganglia in schizophrenic patients: a review. *WMW Wiener Medizinische Wochenschrift* (2008) 158(3):84-90.

Buehlmann, E., Berger, G. E., Aston, J., et al. Hippocampus abnormalities in at risk mental states for psychosis? A cross-sectional high resolution region of interest magnetic resonance imaging study. *Journal of Psychiatric Research* (2009) 44(7):447-453.

Cannon, T. D. Prediction of Psychosis Through the Prodromal Syndrome. In W. F. Gattaz and G. Busatto, eds., *Advances in Schizophrenia Research 2009*. Springer New York, 2010.

Castellanos, F. X., Lee, P. P., Sharp, W., et al. Developmental Trajectories of Brain Volume Abnormalities in Children and Adolescents With Attention-Deficit/Hyperactivity Disorder. *JAMA: The Journal of the American Medical Association* (2002) 288(14):1740-1748.

Chan, R. C. K., Di, X., McAlonan, G. M., et al. Brain Anatomical Abnormalities in High-Risk Individuals, First-Episode, and Chronic Schizophrenia: An Activation Likelihood Estimation Meta-analysis of Illness Progression. *Schizophrenia Bulletin* (2011) 37(1):177-188.

Demjaha, A., Valmaggia, L., Stahl, D., et al. Disorganization/Cognitive and Negative Symptom Dimensions in the At-Risk Mental State Predict Subsequent Transition to Psychosis. *Schizophrenia Bulletin* (2010).

Ellison-Wright, I., Glahn, D. C., Laird, A. R., et al. The Anatomy of First-Episode and Chronic Schizophrenia: An Anatomical Likelihood Estimation Meta-Analysis. *Am J Psychiatry* (2008) 165(8):1015-1023.

Fazzari, P., Paternain, A. V., Valiente, M., et al. Control of cortical GABA circuitry development by Nrg1 and ErbB4 signalling. *Nature* (2010) 464(7293):1376-1380.

Frazier, J. A., Hodge, S. M., Breeze, J. L., et al. Diagnostic and Sex Effects on Limbic Volumes in Early-Onset Bipolar Disorder and Schizophrenia. *Schizophrenia Bulletin* (2008) 34(1):37-46.

Fusar-Poli, P., Borgwardt, S., Crescini, A., et al. Neuroanatomy of vulnerability to psychosis: A voxel-based meta-analysis. *Neuroscience & Biobehavioral Reviews* (2011) In Press, Corrected Proof.

Fusar-Poli, P., Howes, O. D., Allen, P., et al. Abnormal Frontostriatal Interactions in People With Prodromal Signs of Psychosis: A Multimodal Imaging Study. *Arch Gen Psychiatry* (2010) 67(7):683-691.

Geller, B., Zimerman, B., Williams, M., Frazier, J., 1994, revised 1996. WASH-U-KSADS (Washington University at St. Louis Kiddie and Young Adult Schedule for Affective Disorders and Schizophrenia — Lifetime and Present Episode Version for DSM-IV). Washington University School of Medicine, St. Louis, MO. WASH-U-KSADS

(Washington University at St. Louis Kiddie and Young Adult Schedule for Affective Disorders and Schizophrenia — Lifetime and Present Episode Version for DSM-IV). 1996.

Giedd, J. N., Jeffries, N. O., Blumenthal, J., et al. Childhood-onset schizophrenia: progressive brain changes during adolescence. *Biological Psychiatry* (1999) 46(7):892-898.

Giedd, J. N., Snell, J. W., Lange, N., et al. Quantitative Magnetic Resonance Imaging of Human Brain Development: Ages 4â€“18. *Cerebral Cortex* (1996) 6(4):551-559.

Gogtay, N., Greenstein, D., Lenane, M., et al. Cortical Brain Development in Nonpsychotic Siblings of Patients With Childhood-Onset Schizophrenia. *Arch Gen Psychiatry* (2007) 64(7):772-780.

Goldman, A. L., Pezawas, L., Mattay, V. S., et al. Heritability of Brain Morphology Related to Schizophrenia: A Large-Scale Automated Magnetic Resonance Imaging Segmentation Study. *Biological Psychiatry* (2008) 63(5):475-483.

Gonzalez-Burgos, G., Hashimoto, T., and Lewis, D. Alterations of Cortical GABA Neurons and Network Oscillations in Schizophrenia. *Current Psychiatry Reports* (2010) 12(4):335-344.

Gouttard, S., Styner, M., Joshi, S., et al. Subcortical structure segmentation using probabilistic atlas priors. Presented at conference, "Medical Imaging 2007: Image Processing." San Diego, CA, USA, 2007.

Gouttard, S., Styner, M., Prastawa, M., et al. Assessment of Reliability of Multi-site Neuroimaging Via Traveling Phantom Study, *Medical Image Computing and Computer-Assisted Intervention – MICCAI 2008*, Vol. 5242, *Lecture Notes in Computer Science*. Springer Berlin / Heidelberg, 2008.

Hannan, K. L., Wood, S. J., Yung, A. R., et al. Caudate nucleus volume in individuals at ultra-high risk of psychosis: A cross-sectional magnetic resonance imaging study. *Psychiatry Research: Neuroimaging* (2010) 182(3):223-230.

Harms, M. P., Wang, L., Campanella, C., et al. Structural abnormalities in gyri of the prefrontal cortex in individuals with schizophrenia and their unaffected siblings. *The British Journal of Psychiatry* (2010) 196(2):150-157.

Harrison, P. J. The neuropathology of schizophrenia. *Brain* (1999) 122(4):593-624.

Howes, O. D., and Kapur, S. The Dopamine Hypothesis of Schizophrenia: Version IIIâ€"The Final Common Pathway. *Schizophrenia Bulletin* (2009) 35(3):549-562.

Jones, P., Rodgers, B., Murray, R., et al. Child Developmental Risk-Factors for Adult Schizophrenia in the British 1946 Birth Cohort. *Lancet* (1994) 344(8934):1398-1402.

Kegeles, L. S., Abi-Dargham, A., Frankle, W. G., et al. Increased Synaptic Dopamine Function in Associative Regions of the Striatum in Schizophrenia. *Arch Gen Psychiatry* (2010) 67(3):231-239.

Kendler, K. S., McGuire, M., Gruenberg, A. M., et al. The Roscommon Family Study: I. Methods, Diagnosis of Probands, and Risk of Schizophrenia in Relatives. *Arch Gen Psychiatry* (1993) 50(7):527-540.

Keshavan, M. S., Dick, R. M., Diwadkar, V. A., et al. Striatal metabolic alterations in non-psychotic adolescent offspring at risk for schizophrenia: A 1H spectroscopy study. *Schizophrenia Research* (2009) 115(1):88-93.

Langen, M., Schnack, H. G., Nederveen, H., et al. Changes in the Developmental Trajectories of Striatum in Autism. *Biological Psychiatry* (2009) 66(4):327-333.

Lawrie, S. M., Whalley, H. C., Abukmeil, S. S., et al. Brain structure, genetic liability, and psychotic symptoms in subjects at high risk of developing schizophrenia. *Biological Psychiatry* (2001) 49(10):811-823.

Lenroot, R. K., Gogtay, N., Greenstein, D. K., et al. Sexual dimorphism of brain developmental trajectories during childhood and adolescence. *NeuroImage* (2007) 36(4):1065-1073.

Lodge, D. J., and Grace, A. A. Developmental pathology, dopamine, stress and schizophrenia. *International Journal of Developmental Neuroscience* (2010) In Press, Corrected Proof.

Mamah, D., Harms, M. P., Wang, L., et al. Basal Ganglia Shape Abnormalities in the Unaffected Siblings of Schizophrenia Patients. *Biological Psychiatry* (2008) 64(2):111-120.

Mattai, A., Hosanagar, A., Weisinger, B., et al. Hippocampal Volume Development in Healthy Siblings of Childhood-Onset Schizophrenia Patients. *Am J Psychiatry* (2011):appi.ajp.2010.10050681.

Maxwell, M. E. Family Interview for Genetic Studies: Clinical Neurogenetics Branch, Intramural Research Program. NIMH., 1996.

McEwen, B. S. Stress, sex, and neural adaptation to a changing environment: mechanisms of neuronal remodeling. *Annals of the New York Academy of Sciences* (2010) 1204:38-59.

Meyer, U., and Feldon, J. Neural basis of psychosis-related behaviour in the infection model of schizophrenia. *Behavioural Brain Research* (2009) 204(2):322-334.

Miller, T. J., McGlashan, T. H., Woods, S. W., et al. Symptom Assessment in Schizophrenic Prodromal States. *Psychiatric Quarterly* (1999) 70(4):273-287.

Narr, K. L., van Erp, T. G. M., Cannon, T. D., et al. A Twin Study of Genetic Contributions to Hippocampal Morphology in Schizophrenia. *Neurobiology of Disease* (2002) 11(1):83-95.

Neufang, S., Specht, K., Hausmann, M., et al. Sex Differences and the Impact of Steroid Hormones on the Developing Human Brain. *Cerebral Cortex* (2009) 19(2):464-473.

Nugent III, T. F., Herman, D. H., Ordonez, A., et al. Dynamic mapping of hippocampal development in childhood onset schizophrenia. *Schizophrenia Research* (2007) 90(1-3):62-70.

Ostby, Y., Tamnes, C. K., Fjell, A. M., et al. Heterogeneity in Subcortical Brain Development: A Structural Magnetic Resonance Imaging Study of Brain Maturation from 8 to 30 Years. *J. Neurosci.* (2009) 29(38):11772-11782.

Paus, T., Keshavan, M., and Giedd, J. N. Why do many psychiatric disorders emerge during adolescence? *Nat Rev Neurosci* (2008) 9(12):947-957.

Prastawa, M., Bullitt, E., Moon, N., et al. Automatic brain tumor segmentation by subject specific modification of atlas priors. *Academic radiology* (2003) 10(12):1341-1348.

Rajarethinam, R., DeQuardo, J. R., Miedler, J., et al. Hippocampus and amygdala in schizophrenia: assessment of the relationship of neuroanatomy to psychopathology. *Psychiatry Research: Neuroimaging* (2001) 108(2):79-87.

Rajarethinam, R., Upadhyaya, A., Tsou, P., et al. Caudate volume in offspring of patients with schizophrenia. *The British Journal of Psychiatry* (2007) 191(3):258-259.

Rapoport, J. L., and Gogtay, N. Brain Neuroplasticity in Healthy, Hyperactive and Psychotic Children: Insights from Neuroimaging. *Neuropsychopharmacology* (2008) 33(1):181-197.

Rapoport, J. L., and Gogtay, N. Childhood onset schizophrenia: support for a progressive neurodevelopmental disorder. *International Journal of Developmental Neuroscience* (2010) In Press, Corrected Proof.

Reichenberg, A., Weiser, M., Rapp, M. A., et al. Elaboration on Premorbid Intellectual Performance in Schizophrenia: Premorbid Intellectual Decline and Risk for Schizophrenia. *Arch Gen Psychiatry* (2005) 62(12):1297-1304.

Shaw, P., Gogtay, N., and Rapoport, J. Childhood psychiatric disorders as anomalies in neurodevelopmental trajectories. *Human Brain Mapping* (2010) 31(6):917-925.

Shenton, M. E., Dickey, C. C., Frumin, M., et al. A review of MRI findings in schizophrenia. *Schizophrenia Research* (2001) 49(1-2):1-52.

Simpson, E. H., Kellendonk, C., and Kandel, E. A Possible Role for the Striatum in the Pathogenesis of the Cognitive Symptoms of Schizophrenia. *Neuron* (2010) 65(5):585-596.

Steullet, P., Cabungcal, J.-H., Kulak, A., et al. Redox Dysregulation Affects the Ventral But Not Dorsal Hippocampus: Impairment of Parvalbumin Neurons, Gamma Oscillations, and Related Behaviors. *J. Neurosci.* (2010) 30(7):2547-2558.

Tamminga, C. A., Stan, A. D., and Wagner, A. D. The Hippocampal Formation in Schizophrenia. *Am J Psychiatry* (2010) 167(10):1178-1193.

Thompson, P. M., Bartzokis, G., Hayashi, K. M., et al. Time-Lapse Mapping of Cortical Changes in Schizophrenia with Different Treatments. *Cerebral Cortex* (2009) 19(5):1107-1123.

van Erp, T. G. M., Therman, S., Pirkola, T., et al. Verbal recall and recognition in twins discordant for schizophrenia. *Psychiatry Research* (2008) 159(3):271-280.

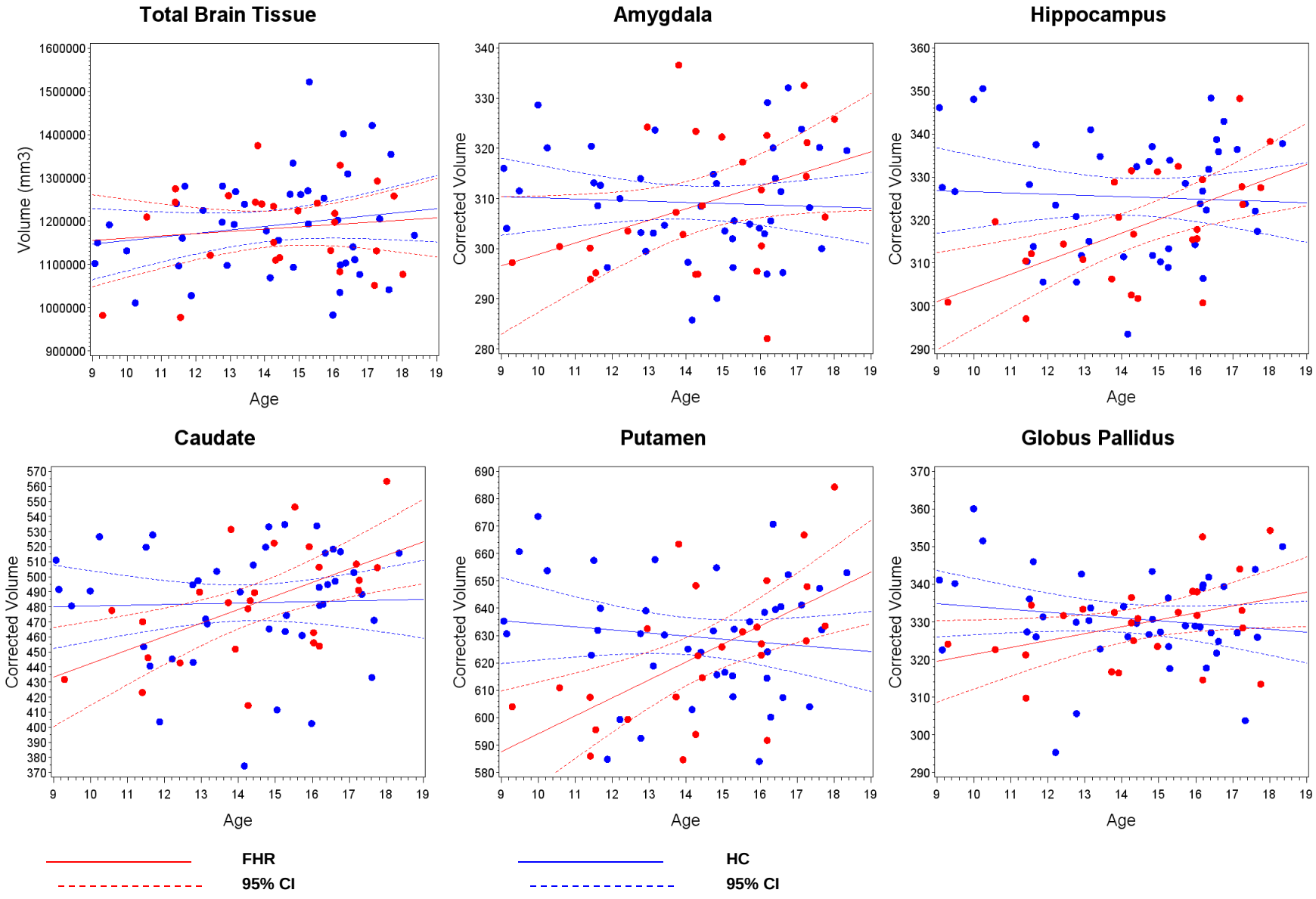
Velakoulis, D., Wood, S. J., Wong, M. T. H., et al. Hippocampal and Amygdala Volumes According to Psychosis Stage and Diagnosis: A Magnetic Resonance Imaging Study of Chronic Schizophrenia, First-Episode Psychosis, and Ultra-High-Risk Individuals. *Arch Gen Psychiatry* (2006) 63(2):139-149.

Vita, A., De Peri, L., Silenzi, C., et al. Brain morphology in first-episode schizophrenia: A meta-analysis of quantitative magnetic resonance imaging studies. *Schizophrenia Research* (2006) 82(1):75-88.

Woo, T.-U. W., Spencer, K., and McCarley, R. W. Gamma Oscillation Deficits and the Onset and Early Progression of Schizophrenia. *Harvard Review of Psychiatry* (2010) 18(3):173-189.

Wood, S. J., Yücel, M., Velakoulis, D., et al. Hippocampal and anterior cingulate morphology in subjects at ultra-high-risk for psychosis: the role of family history of psychotic illness. *Schizophrenia Research* (2005) 75(2-3):295-301.

Zhang, Z. J., and Reynolds, G. P. A selective decrease in the relative density of parvalbumin-immunoreactive neurons in the hippocampus in schizophrenia. *Schizophrenia Research* (2002) 55(1-2):1-10.


Total Brain Tissue
Volume (mm3)
1600000
1500000
1400000
1300000
1200000
1100000
1000000
900000
9 10 11 12 13 14 15 16 17 18 19
Age
Amygdala
Corrected Volume
340
330
320
310
300
290
280
9 10 11 12 13 14 15 16 17 18 19
Age
Hippocampus
Corrected Volume
360
350
340
330
320
310
300
290
9 10 11 12 13 14 15 16 17 18 19
Age
Caudate
Corrected Volume
570 560 550 540 530 520 510 500 490 480 470 460 450 440 430 420 410 400 390 380 370
9 10 11 12 13 14 15 16 17 18 19
Age
Putamen
Corrected Volume
690 680 670 660 650 640 630 620 610 600 590 580
9 10 11 12 13 14 15 16 17 18 19
Age
Globus Pallidus
Corrected Volume
370 360 350 340 330 320 310 300 290
9 10 11 12 13 14 15 16 17 18 19
Age
FHR
95% CI
HC
95% CI