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REDUCED N-ACETYL-ASPARTATE LEVELS IN SCHIZOPHRENIA PATIENTS WITH A YOUNGER ONSET AGE: A SINGLE-VOXEL ¹H SPECTROSCOPY STUDY.

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Abstract

Schizophrenia is widely considered a neurodevelopmental disorder. The timing of psychosis onset may determine the degree of functional and biological deficits. In this study, the association between age of onset of psychosis and *in vivo* biochemical levels was assessed in first-episode, antipsychotic-naïve (FEAN) schizophrenia subjects. We hypothesized greater biochemical deficits in the younger-onset FEAN subjects. *In vivo*, ¹H spectroscopy measurements of the left dorsolateral prefrontal cortex (DLPFC) were conducted on FEAN subjects (15 schizophrenia and 3 schizoaffective subjects) and healthy comparison subjects of comparable age and gender distribution (N=61). N-acetyl-aspartate was significantly lower in the left DLPFC of FEAN subjects as compared to healthy comparison subjects. However, there was a significant subject group-by-age interaction for N-acetyl-aspartate. Early-onset FEAN subjects showed lower N-acetyl-aspartate levels compared to the younger healthy comparison subjects, while adult-onset FEAN and older healthy comparison subjects did not differ. The lower N-acetyl-aspartate levels in the DLPFC of early-onset subjects suggest a reduction in functioning neurons or specifically a reduction in the proliferation of dendrites and synaptic connections, which is not apparent in the adult-onset schizophrenia subjects.

Keywords

proton magnetic resonance spectroscopy; dorsal lateral prefrontal cortex; schizophrenia

1. INTRODUCTION

Schizophrenia is a complex, debilitating brain disorder impairing perception, cognition, volition, social communication, emotions, and causing delusional and hallucinatory

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experiences. There is considerable evidence supporting a neurodevelopmental basis for schizophrenia (Church et al., 2002; Lipska and Weinberger, 2002; Murray and Lewis, 1987; Remschmidt, 2002; Weinberger, 1995). Premorbid antecedents (including birth complications, attention dysfunction and sensory and motor deficits) are possibly stable traits that can alter the normal course of brain development (Cornblatt et al., 2003; Remschmidt, 2002). Abilities that typically evolve over time in healthy development are impaired or delayed in schizophrenia (e.g., speech and language functions, and cognitive and psychosocial abilities). Consequently, it has been proposed that interaction/combination of these influences lead to the development of schizophrenia (Cornblatt et al., 2003; Isohanni et al., 2004; Remschmidt, 2002).

Neuroimaging studies of brain development show a heterochronous maturation behavior. This means that the timing of the maturation of different brain regions differs from each other with somatosensory and visual cortices developing ahead of higher-order cortices (Giedd et al., 1999; Gogtay et al., 2004; Huttenlocher and Dabholkar, 1997). This is consistent with the developmentally variable functions such as speech and language, and cognitive and psychosocial abilities, changing over time during childhood and adolescent years. Hence, the timing, or stage of development, of the onset of schizophrenia may play a role in the degree and nature of brain alterations. It is generally believed that earlier-onset schizophrenia subjects have more profound symptom severity and deficits in morphometric and biological measures compared to later-onset individuals (Alaghband-Rad et al., 1995; Asarnow et al., 1994; Badura et al., 2001; Bettes and Walker, 1987; DeLisi, 1992; Gogtay et al., 2004; Keshavan et al., 2000; Nicolson et al., 2003; Thompson et al., 2001). It has also been suggested that adult-onset schizophrenia is associated with more prominent deficits in the later maturing brain regions, such as the frontal and temporal lobes (Gogtay et al., 2004; Mehler and Warnke, 2002). In this light, studies of first-episode antipsychotic-naïve (FEAN) schizophrenia subjects, which are relatively free from the confounds of medication and aging effects are warranted.

Previous ^1H spectroscopy studies of FEAN subjects have yielded mixed results to date. *In vivo* ^1H spectroscopy is a non-invasive neuroimaging approach capable of assessing, in localized brain regions, N-acetyl-aspartate (NAA; a marker of functioning neurons), phosphocreatine plus creatine (PCr+Cr; high-energy phosphate metabolites) and glycerophosphocholine plus phosphocholine (GPC+PC; catabolic and anabolic metabolite of membrane phospholipids). Several studies have shown no differences in NAA between FEAN subjects and controls in the left dorsolateral prefrontal cortex (DLPFC) (Molina et al., 2005; Ohrmann et al., 2006; Stanley et al., 1996), left prefrontal white matter (Fannon et al., 2003), left medial-temporal region (Bartha et al., 1999), left striatum (Bustillo et al., 2002; Gimenez et al., 2003), left anterior cingulate and left thalamus (Theberge et al., 2002). Additionally, Choe et al. (1996) reported no NAA/PCr+Cr differences in the right or left DLPFC in a mixture of FEAN and previously treated, but currently medication free subjects as compared to controls. Conversely, decreased NAA/PCr+Cr were reported in the DLPFC and temporal lobe (mixture of right and left measurements; Cecil et al., 1999) and prefrontal and hippocampal regions (Bertolino et al., 1998) of FEAN subjects compared to controls. The lack of consistency may be due to the variation in subject selection regarding early-versus adult-onset schizophrenia (Abbott and Bustillo, 2006).

The objective of this study was to assess the association of onset-age and NAA levels in schizophrenia by acquiring single voxel ^1H spectroscopy data in the left DLPFC of FEAN schizophrenia subjects and healthy control (HC) subjects. ^1H spectroscopy metabolites are sensitive to regional neurodevelopmental changes. For example, NAA is present in axons, dendrites and synaptic terminals and during the first several years of brain development NAA increases in grey matter (GM), cerebellum and thalamus are more prominent than in

white matter (WM) (Pouwels et al., 1999). This increase is consistent with the regional differences in early developmental formation of dendritic arborizations and synaptic connections suggesting NAA is a marker of functioning neuroaxonal tissue (Pouwels et al., 1999). The left DLPFC region was chosen because it is one of the later regions to develop and also because of its implication in schizophrenia (Barch et al., 2001). We hypothesized greater NAA deficits in the left DLPFC of early-onset schizophrenia subjects compared to the adult-onset subjects suggesting greater alterations or vulnerability in those patients with an earlier onset of psychotic symptoms.

2. MATERIALS AND METHODS

2.1. Subjects

Eighteen first-episode, antipsychotic-naïve schizophrenia subjects, recruited at the University of Pittsburgh Medical Center, participated in this study. Subjects either met DSM-IV criteria for schizophrenia (N=15) or schizoaffective disorder (N=3) based on the Structured Clinical Interview (SCID) for DSM-IV (American Psychiatric Association, 1994) and consensus diagnostic meetings using all clinical data. To clinically assess subjects younger than 15, the Schedule for Affective Disorders and Schizophrenia - Child Version (Ambrosini et al.) was used. Psychopathological ratings were carried out using the Brief Psychiatric Rating Scale (BPRS) (Overall and Gorham, 1962). Onset-age was assessed using all clinical information, including medical records, reports by family members or significant others, and the SCID interviews (Keshavan et al., 2003). The most likely date of onset of psychotic symptoms (hallucinations, delusions or disorganization of thinking; bizarre or catatonic behavior) was determined in consensus diagnostic conferences. The duration of untreated psychosis was defined as the time interval between onset of psychosis and the MR examination. The demographic information is included in Table 1. As a comparison group, 61 healthy comparison (HC) subjects of comparable age and gender distribution were recruited from Pittsburgh and the surrounding area (Table 1). The HC subjects had no history of psychiatric treatment and no Axis I psychopathology based on the SCID-NP interview (American Psychiatric Association, 1994). All subjects were initially screened prior to entering the study to exclude subjects with a significant past or current medical and/or neurological illness (e.g., hypertension, thyroid disease, diabetes, asthma requiring prophylaxis, seizures or significant head injury with loss of consciousness). All individuals provided signed informed consent approved by the University of Pittsburgh Institutional Review Board. For subjects aged less than 18, the parent/guardian also provided informed consent.

2.2. MRI Acquisition

All MR experiments were conducted on a 1.5T GE Signa Imaging System (GE Medical Systems, Milwaukee, WI) using a quadrature volume head coil. A set of sagittal and coronal scout MR images was first obtained to verify patient position and image quality. A 3D spoiled gradient recalled acquisition (TR=25ms, TE=5ms, flip-angle=400, FOV=240×180mm, slice-thickness=1.5mm, NEX=1, matrix=256×192, and scan-time=7min 44s) was performed in the coronal plane to obtain 124 images covering the entire brain for tissue segmentation analysis of the ¹H spectroscopy single voxel. A double spin echo sequence (2D fast spin-echo, TR=3,000ms, TE=17/102ms, echo-train length=8, FOV=240mm, approximately 24 slices, slice-thickness=5mm, gap=0mm, NEX=1, matrix=256×192, scan-time 5min 12s) was also used to obtain T₂ and proton density images in the axial plane to screen for neuroradiological abnormalities.

2.3. ^1H Spectroscopy Acquisition and Post-Processing

Based on the scout images, a $2 \times 2 \times 2 \text{ cm}^3$ single-voxel was positioned in the left DLPFC to acquire a single-voxel short TE ^1H spectrum using the stimulated acquisition mode (STEAM) sequence (Frahm et al., 1987) and the following parameters: TE=20ms, TM=13.6ms, TR=6sec., bandwidth=2kHz, 2,048 complex data points and 96 acquisitions. We chose the left DLPFC both because of some evidence suggesting lateralized prefrontal dysfunction in schizophrenia (Manoach et al., 1999) as well as time constraints. Additionally, for each water-suppressed measurement a water-unsuppressed spectrum also was collected for absolute quantification (TR=10sec. and 2 acquisitions). The position of the voxel was visually inspected and adjusted based on identifiable anatomical landmarks in reference to standard brain atlases as described in (Brambilla et al., 2004).

Post-processing and quantification steps were 100% automated. The quantification of the spectral metabolites NAA, glutamate, glutamine, *myo*-inositol (mI), PCr+Cr, GPC+PC, taurine, alanine, aspartate, gamma amino-butyric acid (GABA), glucose, and NAAG, as well as lipid resonances and macromolecule resonances (Seeger et al., 2003), was done using the Linear Combination (LC) Model software (version 6.1–4 and the non-simulated basis-set was included in the package; Provencher, 1993), an operator-independent fitting routine (Figure 1). The GM, WM, and cerebrospinal fluid (CSF) content in the MRS voxels of interest were determined by performing segmentation with a semi-automated histogram method, which was applied to the 3D SPGR data using the NIH Image software package, version 1.62 (National Institutes of Health, Bethesda, MD), as previously reported (Keshavan et al., 1995; Keshavan et al., 1994). The GM, WM and CSF voxel content values, along with the other appropriate correction factors were then utilized to obtain absolute quantification values with units of mmol/kg wet weight (Stanley et al., 1995).

2.4. Statistical Analyses

A generalized linear regression model (PROC GLM; SAS Institute Inc.) with subject-group, age and gender as the main effect terms, was used to statistically model the metabolite levels. A second model was applied to address the onset-age effect by subdividing the patients into early-onset (i.e., onset-age < 18 years of age) and adult-onset [i.e., onset-age = 18 years of age (McClellan et al., 1999)] groups as well as HC subjects into younger and older age groups (with similar age distributions as the FEAN subgroups) and by adding a subject group-by-onset/age group interaction term. Given the *a priori* hypothesis, the post-hoc tests of interest include adult-onset patients vs. older controls and early-onset vs. younger controls.

3. RESULTS

Regarding data quality, the mean (± 1 SD) signal-to-noise ratio (S/N) and full-width-half-maximum (FWHM) of the NAA peak for both groups as estimated by LC Model were 9.8 ± 1.8 vs 9.3 ± 1.8 and $3.4 \pm 0.7\text{Hz}$ vs $3.5 \pm 0.9\text{Hz}$, respectively. The metabolites with reasonable level of fitting confidence [i.e., the metabolites with an overall mean Cramer-Rao Lower Bound (CRLB) value of less than 20%] included NAA, PCr+Cr, GPC+PC, *myo*-inositol and glutamate. The CRLB values were $\leq 14\%$ for NAA, PCr+Cr and GPC+PC; $\leq 21\%$ for *myo*-inositol (mean CRLB of 10.4 ± 4.1) and $< 35\%$ for glutamate (mean CRLB of 17.5 ± 5.2). Metabolite values were not excluded from the analyses (i.e., based on their CRLB values) nor were there any significant group differences in S/N, FWHM or metabolite CRLB values. Therefore, the statistical analyses were limited to NAA, PCr+Cr, GPC+PC, *myo*-inositol and glutamate.

Compared to the HC subjects, NAA ($p=0.0035$) levels were significantly lower in the left DLPFC of FEAN schizophrenia subjects (Figure 2 and Table 2a). There were no significant PCr+Cr, GPC+PC, glutamate and myo-inositol level differences between FEAN and HC subjects. The subject group-by-age interaction was significant for NAA ($p=0.0004$), (Figure 2 and Table 2). This is supported by a significant positive NAA-age correlation in FEAN schizophrenia subjects ($r=0.53$; $p=0.024$) and a negative NAA-age correlation in HC subjects ($r=-0.39$; $p=0.0022$). The GPC+PC levels positively correlated with age in the FEAN schizophrenia ($r=0.56$; $p=0.016$), which was not significant in the HC subjects. Regarding the subject group-by-onset/age group interaction term, NAA ($p=0.0007$) were significant and post-hoc analyses showed lower NAA levels in the left DLPFC of early-onset FEAN subjects ($p<0.0001$) compared to the younger HC subjects (Table 2; Figure 2).

When comparing duration of untreated psychosis, BPRS negative and positive symptom scores, and total BPRS scores, there were no significant differences between early-onset and adult-onset subjects. There were no age differences between FEAN and HC subjects as well as when comparing the two younger and older subgroups (Table 1). Regarding the left DLPFC voxel tissue composition, there were no significant differences in the percent GM or cerebral spinal fluid (CSF) tissue content between FEAN and HC subjects (GM: $41\pm14\%$ vs. $37\pm11\%$ and $p=0.31$; CSF: $3.1\pm0.4\%$ vs. $2.5\pm0.8\%$ and $p=0.75$) or between early-onset FEAN versus younger HC subjects and adult-onset FEAN versus older HC subjects (GM: interaction term $p=0.52$; CSF: interaction term $p=0.69$).

4. DISCUSSION

In this study, NAA levels were significantly lower in the left DLPFC of FEAN subjects compared to the HC subjects. Additionally, the subject group-by-age interaction was significant for NAA levels. By subdividing the FEAN schizophrenia subjects into early-onset and adult-onset groups, only the early-onset schizophrenia subjects showed a significant (13%) decrease in NAA compared to the younger HC subjects. Studying first-episode, antipsychotic-naïve schizophrenia subjects minimizes the confounding effects of medication and illness chronicity. The segmentation results showed no significant group differences or age-by-group interactions for percent GM tissue or percent CSF, suggesting that tissue heterogeneity could not account for the ^1H metabolite differences. Additionally, the use of a short-TE and a long sequence repetition time (TR) assures that MR signal relaxation effects could not have accounted for the ^1H metabolite differences.

NAA, which is the second most abundant free amino acid next to glutamate, is synthesized in neuronal mitochondria from acetyl-CoA and aspartate by the membrane bound enzyme L-aspartate N-acetyltransferase and the principal metabolizing enzyme, N-acetyl-L-aspartate aminohydrolase II (aspartoacylase) predominates in WM, with highest activity in oligodendrocytes (Baslow, 2003). Cell culture studies have shown that NAA is localized in neurons, immature oligodendrocytes and in O-2A progenitor cells (Urenjak et al., 1993). Additionally, recent reinvestigations have revealed localization of NAA in mature oligodendrocytes (Bhakoo and Pearce, 2000) and evidence of inter-compartmental cycling of NAA between neurons and oligodendrocytes (Baslow, 2003; Bhakoo and Pearce, 2000; Chakraborty et al., 2001). Thus, NAA may be a marker of functioning neuroaxonal tissue that includes functional aspects of the formation and/or maintenance of myelin (Chakraborty et al., 2001).

Numerous studies have shown that *in vivo* ^1H spectroscopy can detect and monitor metabolite changes as a function of normal brain development and aging. Early in postnatal brain development, levels of NAA (or NAA/PCr+Cr ratios) are low. This is followed by dramatic increases in NAA, which then plateau as the brain reaches maturation (van der

Knaap et al., 1992). Specifically, the brain regions showing greatest NAA elevations during development include GM, cerebellum and the thalamus (Pouwels et al., 1999). This provides additional evidence that NAA does not merely reflect the number of neurons but is a marker of functioning neurons or in the context of development a marker of dendritic and synaptic proliferation (Pouwels et al., 1999).

The inverse relationship between NAA levels and age in the HC subjects is in line with MRI volumetric data showing GM reductions over this age range (Giedd et al., 1999; Gogtay et al., 2004). This also is consistent with the normal synaptic pruning processes that occur during cortical brain maturation (Huttenlocher et al., 1982; Rakic et al., 1986). In contrast, NAA levels are relatively constant in WM and in the basal ganglia during brain development (Pouwels et al., 1999). The 13% lower NAA in the left DLPFC of early-onset FEAN schizophrenia subjects may reflect excessive synaptic pruning as previously hypothesized (Feinberg, 1982; Pettegrew et al., 1991). However, one may argue that the lower NAA, which appears to parallel the NAA levels of the younger HC subjects (Figure 2), may be due to a reduction of functioning neurons or more specifically a reduction in the proliferation of dendrites and synaptic connections (i.e., collectively reduced neuropil), as supported in post-mortem studies (Selemon and Goldman-Rakic, 1999). With the ability to detect NAA decreases as small as 8% (as estimated in a power analysis calculation; alpha of 0.05, 80% power and two tailed), this reduction appears absent in the adult-onset schizophrenia subjects when compared to the older HC subjects. Additionally, the evidence of NAA localized in oligodendrocytes and the inclusion of WM in the localized spectroscopy voxel also raises the possibility of the reduced NAA reflecting decreased glial density and/or underdeveloped myelin. This infers that the deviation in development is present prior to the illness. Thus, the maturation of the later developing DLPFC may be compromised in early-onset schizophrenia. Considering the evidence of greater premorbid deficits in earlier-onset subjects with schizophrenia as noted earlier, these results further support the presence of greater neurobiological abnormalities in patients with an earlier age of onset. There is evidence for genetic susceptibility in earlier-onset schizophrenia (Alda et al., 1996; Ritsner et al., 2003; Ross and Pearlson, 1996). In contrast, schizophrenia subjects with a later-onset who presumably have a relatively more mature brain, have relatively fewer alterations in metabolite levels at least in the DLPFC. Interestingly, symptom severity did not differ between the early-onset and adult-onset subjects, though NAA findings were different. The sample size is a limitation; further investigation is needed to better characterize the NAA-by-age interaction behavior and to determine whether alterations in other developmental markers (e.g., speech and language delays, and cognitive and psychosocial impairments) are associated with the NAA differences between the two onset-age groups.

As alluded earlier, the spectroscopy literature in schizophrenia is problematic with variability in stage of illness, medication status, localized brain areas and in inadequate sensitivity to detect reasonable group differences (Abbott and Bustillo, 2006; Keshavan et al., 2000; Stanley, 2002; Steen et al., 2005). However, recent studies contrasting first-episode versus chronic schizophrenia subjects show no significant differences in prefrontal NAA/PCr+Cr ratios or NAA levels of first-episode patients but reduced prefrontal NAA/PCr+Cr ratios or NAA levels in chronic schizophrenia patients both compared to healthy controls (Molina et al., 2005; Ohrmann et al., 2006). Also, Fannon et al. (2003) demonstrated no prefrontal NAA/PCr+Cr ratio differences between first-episode schizophrenia patients and controls. This lack of significant difference in NAA in these studies where the mean age and mean onset-age of patients were either similar if not older than that of the older-onset FEAN subjects of this study, which also showed no significant NAA differences, is very consistent across studies. At the other extreme of comparing prior ¹H spectroscopy studies of children and adolescents with schizophrenia, there also is

evidence of consistency. Reduced NAA/PCr+Cr ratios have been reported in the left prefrontal white matter (Brooks et al., 1998), medial prefrontal cortex (Thomas et al., 1998) and in the prefrontal cortex bilaterally (Bertolino et al., 1998) in children and adolescents with schizophrenia. The recent study by O'Neill and colleagues (2004) reported no prefrontal NAA differences between children and adolescents with schizophrenia, which may be due to the large variability in the measurements. The study by Bertolino et al. (1998) also stands out because the bilateral reduction of NAA/PCr+Cr ratios in the prefrontal cortex was observed not only in childhood-onset schizophrenia patients but also in schizophreniform patients (Bertolino et al., 2003) and chronic schizophrenia patients (Bertolino et al., 1996). Collectively, studies by others and our data show consistency of greater prefrontal NAA deficits in the schizophrenia patients with a younger onset-age compared to those with an older onset-set age.

In summary, lower NAA levels were observed in the DLPFC of FEAN schizophrenia subjects compared to HC subjects, which were more pronounced in the younger-onset FEAN subjects and suggest a reduction in the proliferation of dendrites and synaptic connections as well as the integrity of glia. This association between NAA levels and onset-age in the left DLPFC is not confounded by effects due to medication, aging processes, voxel heterogeneity, MR relaxation or expressing the measurements, which are factors that may account, at least in part, for the lack of consistency or reporting positive findings in prior ¹H spectroscopy studies (Keshavan et al., 2000; Stanley, 2002). More importantly, these results provide direct evidence that the degree of alterations in metabolite levels specifically in the DLPFC may depend on the timing of the onset of schizophrenia. Longitudinal studies of young relatives at risk for schizophrenia prospectively followed through the age of risk may provide additional evidence for the timing of metabolite alterations prior to the symptomatic onset of schizophrenia.

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Figure 1.

An example of quantifying a ^1H spectrum acquired from the DLPFC of a HC subjects. The modeled spectrum (thick line) is superimposed on the acquired spectrum, and the residual and the individual modeled curves of the main metabolites are shown below.

Figure 2. Scatter plot of NAA levels versus age for a) HC subjects (○) and b) FEAN schizophrenia subjects (●). The regression lines are displayed, which have been “smoothed” using the LOWESS procedure (LOWESS = 66) (HC: dashed line; FEAN: solid line), to illustrate the metabolite level-age associations. The color black symbols signify adult-onset/older subjects and the color grey symbols signify the early-onset/younger subjects.

Table 1

Subject Group Characteristics.

Item	HC Subjects			FEAN Schizophrenia Subjects		
	Younger	Older	Total	Early-Onset	Adult-Onset	Total
Sample Size	27	34	61	8	10	18
Mean Age $\pm$ SD (years)	18.3 $\pm$ 3.0	28.5 $\pm$ 4.9	24.0 $\pm$ 6.6	17.5 $\pm$ 2.1 ^a	28.0 $\pm$ 4.6 ^a	23.4 $\pm$ 6.5 ^a
Age Range (years)	12.0 - 21.7	22.5 - 38.4	12.0 - 38.4	14.4 - 21.8	22.0 - 35.8	14.4 - 35.8
Gender	18 males	21 males	39 males	5 males	8 males	13 males
Distribution	9 females	13 females	22 females	3 females	2 females	5 females
Handedness	89% right	95% right	92% right	88% right	90% right	89% right
Onset-Age of Psychotic Symptoms (years)				15.5 $\pm$ 1.8	25.7 $\pm$ 4.0	21.1 $\pm$ 6.1
Duration of Untreated Psychosis (years)				2.0 $\pm$ 1.9	2.3 $\pm$ 1.7 ^b	2.2 $\pm$ 1.8
BPRS				8.3 $\pm$ 2.7	5.9 $\pm$ 1.9 ^b	6.9 $\pm$ 2.5
Negative Symptoms BPRS				13.1 $\pm$ 2.7	16.0 $\pm$ 4.2 ^b	14.7 $\pm$ 3.8
Positive Symptoms BPRS Total				53.1 $\pm$ 8.7	50.2 $\pm$ 9.5 ^b	52.1 $\pm$ 8.9

^aNot significantly different when compared to the respective HC group.^bNot significantly different when compared to early-onset schizophrenia subjects.

Table 2

Mean Metabolite Levels and Statistical Analysis Results.

Absolute Metabolite Levels in mmol/kg wet weight (coefficient of variation as a percent)						
	NAA	PCr+Cr	GPC+PC	glutamate	myo-inositol	
a) All Subjects HC (N=61)						
	7.66 (8.1%)	5.2 (11.7%)	1.37 (16.7%)	6.66 (21.3%)	3.88 (20.8%)	
FENM (N=18)		5.04 (10.5%)	1.39 (15.2%)	6.17 (17.0%)	3.48 (16.3%)	
p-values from the main effect model (group term)	0.0035	0.28	0.79	0.15	0.18	
p-values from the group-by-age interaction model (group-by-age term)	0.0004	0.13	0.16	0.16	0.64	
b) Dichotomization of FEAN Schizophrenia Subjects						
p-values from the group-by-age group interaction model (group-by-age group term)	0.0007	0.22	0.34	0.29	0.41	
Younger HC (N=27)	7.84 (6.5%)	5.17 (12.6%)	1.35 (15.7%)	6.91 (19.0%)	3.78 (18.9%)	
Early-onset FEAN (N=8)	6.81 (9.2%)	4.81 (9.7%)	1.24 (14.9%)	5.82 (15.8%)	3.21 (7.7%)	
p-values from the least squares means post-hoc analysis	<0.0001					
Older HC (N=34)	7.52 (9.0%)	5.23 (11.1%)	1.39 (17.5%)	6.45 (23.0%)	3.96 (22.1%)	
Adult-onset FEAN (N=10)	7.42 (8.5%)	5.22 (10.1%)	1.50 (10.3%)	6.42 (17.3%)	3.90 (15.3%)	
p-values from the least squares means post-hoc analysis	0.67					