Altered Development of the Dorsolateral Prefrontal Cortex in Chromosome 22q11.2 Deletion Syndrome: An In Vivo Proton Spectroscopy Study

By: Vandana Shashi, Aravindhan Veerapandiyan, Matcheri S. Keshavan, Michael Zapadka, Kelly Schoch, Thomas R. Kwapil, Stephen R. Hooper, Jeffrey A. Stanley


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Abstract:

Background

Chromosome 22q11.2 deletion syndrome (22q11DS), the most common microdeletion in humans, is associated with multiple medical features, almost universal cognitive deficits, and a high risk of schizophrenia. The metabolic basis of the psychological/psychiatric features is not well understood. Volumetric brain imaging studies have shown that gray matter abnormalities in the dorsolateral prefrontal cortex (DLPFC), an area that is believed to be integral for higher neurocognition, as well as being involved in schizophrenia, are associated with the psychological manifestations. However, studies have not characterized any possible metabolite alterations within the DLPFC of children with 22q11DS and their correlations with the psychological findings.

Methods

We conducted a short echo time, single-voxel, in vivo proton spectroscopy study involving children with 22q11DS (n = 26) and matched control subjects (n = 23).

Results

Absolute N-acetylaspartate (NAA) levels from the DLPFC were significantly elevated in children with 22q11DS compared with control subjects and the elevations were associated with poor global functioning and higher rates of comorbid attention-deficit/hyperactivity disorder. Children with 22q11DS had a lack of an age-associated decrease in NAA levels, a trend seen in
the control subjects. However, the results did not remain statistically significant after corrections for multiple comparisons were made.

Conclusions

These findings represent the first report of proton spectroscopy in children with 22q11DS. The elevated DLPFC NAA levels and the lack of decreasing trends in NAA with age in the 22q11DS group relative to control subjects suggest an alteration in cortical development. Also, such neuronal dysmaturation is associated with psychopathology in children with 22q11DS.

Keywords: ADHD | chromosome 22q11.2 deletion syndrome | DiGeorge syndrome | $^1$H MRS | NAA | N-acetylaspartate | neuropsychology | spectroscopy | velocardiofacial syndrome

Article:

Chromosome 22q11.2 deletion syndrome (22q11DS), also known as DiGeorge syndrome or velocardiofacial syndrome, is the most common chromosomal microdeletion syndrome in humans, occurring in approximately 1 in 1600 live births ([1] and [2]). It is associated with numerous and variable clinical manifestations, the more common of which are conotruncal heart abnormalities, palatal anomalies, hypoparathyroidism, and immune deficiency. Cognitive problems are seen in 80% to 100% of affected individuals and are highly variable, with deficits in visual-spatial processing, executive function, attention, verbal learning, working memory, arithmetic performance, language, and impaired functionality ([3],[4] and [5]). Behavioral and emotional problems occur in about 50% of children with 22q11DS; the most common are attention-deficit/hyperactivity disorder (ADHD), social impairments, somatic complaints, and anxiety problems ([5], [6], [7] and [8]). 22q11DS is also the most common identifiable genetic risk factor for schizophrenia; at least 25% of individuals with this deletion develop a schizophrenia-like psychosis by young adulthood ([9]). The exact pathophysiology of the cognitive/behavioral/emotional/psychiatric manifestations of the condition remains unexplained, although undoubtedly the hemizygous deletion of several genes predisposes to these deficits. In attempts to quantify the structural correlates of the neurocognitive manifestations, brain imaging studies have delineated several areas of gray matter (GM) volumetric reductions in individuals with 22q11DS, mainly in the posterior cortical regions and the cerebellum ([10]). An area of special interest is the left dorsolateral prefrontal cortex (DLPFC), which, together with its other cortical and subcortical circuits, plays an important role in attention, working memory, planning, reasoning, and execution (deficits that are integral to the neurocognitive phenotype in 22q11DS) ([11] and [12]). Voxel-based morphometric studies have shown volumetric reduction in the DLPFC gray matter volume in individuals with 22q11DS ([13] and [14]); this reduction is associated with deficits in sustained attention and executive function ([14]) and may be predictive of risk for schizophrenia-like psychotic symptoms in these patients in later life. Nonetheless, it is also evident that relative to other brain regions, the frontal lobes show less volumetric reduction in children with 22q11DS ([14] and [15]). This may suggest an alteration in the normal pruning of overproduced synaptic
connections, which in the DLPFC occurs in adolescence ([16], [17], [18], [19] and [20]). Thus, it is possible that pruning may occur later in children with 22q11DS than in typically developing children ([10]) and the delayed change in morphology of the frontal lobes may be associated with some of the neurocognitive abnormalities in the condition ([14]).

Further investigation using noninvasive neurochemical imaging techniques, such as in vivo proton magnetic resonance spectroscopy (1H MRS), could yield important information about the brain developmental alterations, but such studies are lacking in children with 22q11DS. Proton magnetic resonance spectroscopy allows for the in vivo analysis of neurometabolite levels, such as N-acetylaspartate (NAA), a neurometabolite present almost exclusively in the nervous system ([21] and [22]) and sensitive to brain development. In cortical areas, NAA increases with age, with changes most prominent in early postnatal development ([23] and [24]), followed by decreases in prefrontal NAA levels in adolescence and early adulthood ([25], [26] and [27]). Collectively, the increase followed by decreasing NAA levels with age in the prefrontal cortex may reflect the overproduction of dendrites and synaptic terminals, followed by the normal pruning of synapses that occur during cortical brain maturation ([20], [26], [28] and [29]), although conclusive evidence is lacking. In contrast, NAA levels have been reported as being relatively stable or increasing linearly with age in white matter (WM) tissue during brain development ([26] and [30]). Therefore, NAA is believed to be a marker of functioning neurons or, more specifically in the context of development, a marker of cortical maturation ([29]). NAA has been measured in several neurologic and psychiatric disorders, with the aim of understanding the pathophysiology of the particular disorder, including ADHD, wherein considerable variability in neurometabolites has been reported ([31]). To our knowledge, there have been no studies, so far, that have reported neurometabolite findings in DLPFC in children with 22q11DS. One recent study of 1H MRS in adults with 22q11DS found no differences in DLPFC metabolites between individuals with and without schizophrenia ([32]).

Proton magnetic resonance spectroscopy studies in individuals with schizophrenia in the general population have consistently reported decreased NAA within several brain regions, including the left DLPFC (both gray and white matter), in individuals with chronic schizophrenia and not in most first-episode studies ([33] and [34]). Decreased NAA in the DLPFC may suggest a reduction in functioning neurons in the cortex ([27]). A few 1H MRS studies in individuals at high risk for schizophrenia (other than 22q11DS) have shown varying reductions of NAA in prefrontal areas ([27] and [35]) and one reported increased NAA in prefrontal white matter in these individuals ([36]), but a recent meta-analysis reported that there were no NAA abnormalities in the frontal areas in individuals at high risk (offspring of individuals with schizophrenia) in the general population ([33]). Thus, there are no specific trends in the neurometabolites in these individuals at high risk for schizophrenia.

The aim of the current study was to investigate possible neurometabolite alterations in the DLPFC of nonpsychotic children with 22q11DS compared with healthy control subjects and to determine the neuropsychological correlates of these neurometabolites before the onset of psychosis. Additionally, due to significant changes in proton (1H) metabolite levels with age in typically developing individuals, possible age effects are also investigated. Based on our previous findings of a correlation between DLPFC gray matter volumetric reduction and neurocognition ([14]), as well as strong empirical evidence that the left DLPFC is involved in
schizophrenia and is abnormal in individuals at high risk for the disorder ([27], [34], [37] and [38]), we hypothesized that our cohort of children with 22q11DS (at high risk of psychosis) would show reduced NAA in the left DLPFC and that the reduced NAA would be correlated with neuropsychological functioning.

Methods and Materials

This cross-sectional study was approved by the Institutional Review Board of Wake Forest University Health Sciences. Participants included 26 patients with 22q11DS and 23 healthy control subjects. The 22q11DS subjects were enrolled in the study through the Genetics Clinic at Wake Forest University and the control subjects were recruited through the local pediatric practices and public school systems. The 22q11DS and control subjects were matched for age and gender. They were not matched for handedness, but there were no significant differences in handedness between the two groups ($\chi^2 = .002, p = .96$). Exclusion criteria for both groups were a psychotic disorder and age above 16 years; for the control subjects, exclusion criteria included the presence of a neurodevelopmental or genetic disorder. All children with 22q11DS had a molecularly confirmed deletion of the 22q11.2 interval, with either fluorescence in situ hybridization (Vysis, Gaithersburg, Maryland) or microarray analyses (Affymetrix, Santa Clara, California; www.affymetrix.com).

Neuropsychological Measures

The participants underwent a battery of neuropsychological tests; parental interviews were also conducted. These included the Wechsler Intelligence Scale for Children-Fourth Edition ([39]) (earlier participants were administered the Wechsler Intelligence Scale for Children-Third Edition [40]); Wechsler Individual Achievement Test-Second Edition (41); Wisconsin Cart Sorting Test (42); Continuous Performance Test, identical pairs and AX conditions (task in which the user presses the button when the letter X is preceded by the letter A) (43); and the Computerized Diagnostic Interview Schedule for Children (44). Global assessment of functioning by an examiner-rated scale (Global Assessment of Functioning [GAF]) was also ascertained for all participants, providing ratings of overall function, independent of specific mental health diagnoses (45).

Single-Voxel 1H Spectroscopy

Magnetic resonance images were conducted with a 1.5 T GE Signa imaging system (GE Medical Systems, Milwaukee, Wisconsin). A set of sagittal and axial scout images were first obtained to ascertain subject position and quality of images. A three-dimensional spoiled gradient recalled acquisition (repetition time [TR] = 25 msec, echo time [TE] =5 msec, flip angle = 400, field of view = 240 × 180 mm, slice thickness = 1.5 mm, matrix = 256 × 192) was performed in the coronal plane to obtain 124 images covering the entire brain for tissue segmentation. A fast spin echo sequence (two-dimensional fast spin echo; TR = 2500 msec; TE2 = 17/102 msec; echo train length = 8; field of view = 240 mm$^2$, with 24 slices; slice thickness = 5 mm; gap = 0 mm; number of excitations = 1; matrix = 256 × 192) was also obtained for T2 and proton density images in the coronal plane. Based on the scout images, a 2.0 × 2.0 × 2.0 cm$^2$ voxel was placed in the left DLPFC to acquire the in vivo singe-voxel, short echo time 1H spectroscopy using the
point-resolved spectroscopy sequence and the following parameters: TE = 35 msec, TR = 1500 msec, bandwidth = 2 kHz, 2048 complex data points, and 128 acquisitions. The superior frontal sulcus, the lateral fissure, and the genu of the corpus callosum were used as anatomical boundaries for the voxel placement in the DLPFC (Figure 1). Water-unsuppressed spectra were also acquired for spectral line shape correction (46) and for absolute quantification of $^1$H metabolite levels with millimoles per kilogram wet weight units. The $^1$H metabolites, glutamate, glutamine, myo-inositol, choline-containing compounds (glycerophosphocholine [GPC] + phosphocholine [PC]), phosphocreatine plus creatine (PCr + Cr), NAA, and N-acetylaspartylglutamate, as well as taurine, alanine, aspartate, gamma-aminobutyric acid, glucose, and lipid resonances and macromolecule resonances (47) were quantified using the Linear Combination Model software (LCMODEL Inc., Ontario, Canada) (48), an operator-independent fitting routine. The $^1$H metabolites with reasonable level of fitting confidence (i.e., the metabolites with an overall mean Cramer-Rao lower bound value of less than 20%) were the dependent variables in this analysis and included NAA, PCr + Cr, GPC + PC, myo-inositol, and glutamate. To address the variability in the tissue composition within the voxels of interest, the proportions of GM, WM, and cerebrospinal fluid (CSF) were estimated for each localized $^1$H spectroscopy voxel. In a fully automated procedure, the T1-weighted images were corrected for any B$_1$ field bias followed by extracting the brain tissue and segmenting the images into partial volume maps of GM, WM, and CSF space using FSL (FMRIB Analysis Group, Oxford, United Kingdom) tools (49). The tissue fractions within the voxels of interest were then extracted from the segmented images by matching the coordinates of the $^1$H spectroscopy voxel with the images using the FSL tools. The GM, WM, and CSF voxel content values, along with the other appropriate relaxation correction factors (23), were then utilized to obtain absolute quantification values, as described elsewhere (50).
A typical proton magnetic resonance spectroscopy spectrum is illustrated along with voxel placement in the subjects. Cr, creatine; GPC, glycerophosphocholine; myo-Ins, myo-inositol; NAA, N-acetylaspartate; PC, phosphocholine; PCr, phosphocreatine.

Statistical Methods

Statistical tests were performed with SPSS version 18.0 (IBM Corporation, Armonk, New York), with independent sample $t$ tests or univariate analysis of variance for continuous variables and Fisher's exact test for categorical variables to examine the differences in neuropsychological functioning between the two groups. The group differences in NAA, PCr + Cr, GPC + PC, glutamate, and myo-inositol levels were examined with independent sample $t$ tests. Medication status, age, and gender were used as covariates in univariate analyses of variance to determine if the results of the group differences were influenced by these factors. Pearson correlations were computed to determine associations between the neurometabolites and psychological functioning. Although our analyses were based on an a priori hypothesis, we present data before and after statistical corrections for multiple comparisons, although some studies involving 22q11DS do not adopt such corrections (32). To assess age effects, a group-by-age interaction model was also included.

Results

Demographics
There were no significant differences in age, gender, ethnicity, or parental socioeconomic status between the 22q11DS and control groups. The patient and control groups did not differ on sex composition (65% male and 54% male subjects, respectively), ethnic composition (92% Caucasian and 8% African American and 84% Caucasian, 8% African American, and 8% Hispanic, respectively), age (10.9 ± 2.6 years and 11.0 ± 2.2 years, respectively), handedness ($\chi^2 = .004, p = .99$), or socioeconomic status (SES) (SES in 22q11DS group = 32.8 [13.1] and SES in control group = 30.5 [12.3]). Medications in the 22q11DS and control groups consisted of stimulants for ADHD and anxiolytics, with 8 of 26 children with 22q11DS and 7 of 23 control children on a medication, with no significant group differences (Fisher's exact test $p = 1.0$).

Neuropsychological Measures
The 22q11DS group exhibited poorer neuropsychological performance than the control group, with significant differences in verbal comprehension, perceptual organization, working memory, processing speed, achievement, sustained attention, executive function, and verbal learning (Table 1) (some data previously published) (4). There was no difference in the incidence of anxiety and ADHD between the two groups. None of the subjects had a psychotic illness at the time of the study.

Table 1. Abnormalities in Neuropsychological Functioning in Children with 22q11DS Compared with Matched Control Subject

<table>
<thead>
<tr>
<th>Test</th>
<th>Group</th>
<th>Number</th>
<th>Mean (SD)</th>
<th>$t$ Statistic</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAF</td>
<td>22q11DS</td>
<td>26</td>
<td>66.3 (8.8)</td>
<td>$-2.07^a$</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>23</td>
<td>71.7 (9.3)</td>
<td></td>
</tr>
<tr>
<td>WISC Verbal Comprehension</td>
<td>22q11DS</td>
<td>25</td>
<td>75.0 (8.8)</td>
<td>$-8.35^b$</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>23</td>
<td>103.1 (14.0)</td>
<td></td>
</tr>
<tr>
<td>WISC Perceptual Organization</td>
<td>22q11DS</td>
<td>25</td>
<td>77.0 (10.1)</td>
<td>$-7.6^b$</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>23</td>
<td>104.3 (14.2)</td>
<td></td>
</tr>
<tr>
<td>WISC Working Memory</td>
<td>22q11DS</td>
<td>25</td>
<td>83.2 (12.2)</td>
<td>$-5.53^c$</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>23</td>
<td>103.3 (12.8)</td>
<td>$-5.9^b$</td>
</tr>
<tr>
<td>WISC Processing Speed</td>
<td>22q11DS</td>
<td>25</td>
<td>78.2 (12.7)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>23</td>
<td>99.2 (11.8)</td>
<td></td>
</tr>
<tr>
<td>WIAT Broad Reading</td>
<td>22q11DS</td>
<td>25</td>
<td>81.3 (14.1)</td>
<td>$-4.8^b$</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>23</td>
<td>101.2 (15.1)</td>
<td></td>
</tr>
<tr>
<td>WIAT Broad Mathematics</td>
<td>22q11DS</td>
<td>18</td>
<td>76.1 (18.3)</td>
<td>$-5.23^b$</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>22</td>
<td>103.09 (14.21)</td>
<td></td>
</tr>
<tr>
<td>WCST Perseverative Errors</td>
<td>22q11DS</td>
<td>26</td>
<td>89.8 (11.7)</td>
<td>$-3.14^c$</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>23</td>
<td>102.61 (14.7)</td>
<td></td>
</tr>
<tr>
<td>CPT AX Sustained Attention</td>
<td>22q11DS</td>
<td>25</td>
<td>1.54 (1.17)</td>
<td>$-3.37^c$</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>23</td>
<td>2.73 (1.21)</td>
<td></td>
</tr>
<tr>
<td>Test</td>
<td>Group</td>
<td>Number</td>
<td>Mean (SD)</td>
<td>t Statistic</td>
</tr>
<tr>
<td>------------------------------</td>
<td>----------------</td>
<td>--------</td>
<td>-----------</td>
<td>-------------</td>
</tr>
<tr>
<td>CPT IP Sustained Attention</td>
<td>22q11DS</td>
<td>25</td>
<td>.46 (.53)</td>
<td>−4.19c</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>23</td>
<td>1.25 (.76)</td>
<td></td>
</tr>
<tr>
<td>DISC Anxiety Disorders</td>
<td>22q11DS</td>
<td>26</td>
<td>10/26</td>
<td>Fisher's exact test p &lt; .01</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>23</td>
<td>1/23</td>
<td></td>
</tr>
<tr>
<td>DISC ADHD</td>
<td>22q11DS</td>
<td>26</td>
<td>8/26</td>
<td>Fisher's exact test ns</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>23</td>
<td>6/23</td>
<td></td>
</tr>
</tbody>
</table>

22q11DS, chromosome 22q11.2 deletion syndrome; ADHD, attention-deficit/hyperactivity disorder; AX, ; CPT, Continuous Performance Test; DISC, Diagnostic Interview Schedule for Children; GAF, Global Assessment of Functioning; IP, identical pairs; ns, nonsignificant; WCST, Wisconsin Cart Sorting Test; WIAT, Wechsler Individual Achievement Test; WISC, Wechsler Intelligence Scale for Children.

a
p < .05.
b
p < .001.
c
p < .01.

Neurometabolites and Overall Quality of the \(^1\)H MRS

Figure 1 includes the spectral modeling using Linear Combination Model of a typical in vivo \(^1\)H spectrum from the left DLPFC. The mean full width at half maximum of the NAA for 22q11DS and control subjects were 2.7 ± .7 Hz and 2.5 ± .6 Hz and the mean signal-to-noise ratios were 10.0 ± 1.6 and 9.9 ± 1.8, respectively; both were nonsignificant. The mean Cramer-Rao lower bound values of NAA, NAA + N-acetylaspartylglutamate, glutamate, glutamate + glycine, myo-inositol, PCr + Cr, and GPC + PC are noted in Table 2. Additionally, there were no significant differences in gray matter fraction within the \(^1\)H spectroscopy voxel between the 22q11DS and control groups, as presented in Table 2. NAA levels were significantly higher in the 22q11DS group relative to the control group and this group difference in NAA remained significant when ADHD medication status was used as a covariate (univariate analysis of variance, F = 4.5, p < .05), as well as using age, gender as covariates (univariate analysis of variance, F = 4.1, p < .05). Other neurometabolites did not significantly differ between the two groups.

Table 2. Comparison of \(^1\)H Metabolite Levels Within the Left DLPFC in Children with 22q11DS and Control Subjects

<table>
<thead>
<tr>
<th>(^1)H Metabolites</th>
<th>Group</th>
<th>Total Number</th>
<th>Mean Level ± 1 SD (CV%) mmol/kg w.w.</th>
<th>t Statistic</th>
<th>CRLB ± 1 SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA</td>
<td>22q11DS</td>
<td>26</td>
<td>11.3 ± .9 (8.1)</td>
<td>2.14b</td>
<td>7.0 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>23</td>
<td>10.7 ± .9 (8.7)</td>
<td></td>
<td>7.2 ± 1.3</td>
</tr>
<tr>
<td>NAA +</td>
<td>22q11DS</td>
<td>26</td>
<td>11.7 ± .95 (8.2)</td>
<td>2.68b</td>
<td>6.0 ± .52</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>23</td>
<td>10.9 ± .95 (8.2)</td>
<td></td>
<td>7.2 ± 1.3</td>
</tr>
<tr>
<td><strong>1H Metabolites</strong></td>
<td><strong>Group</strong></td>
<td><strong>Total Number</strong></td>
<td><strong>Mean Level ± 1 SD (CV%) mmol/kg w.w.</strong></td>
<td><strong>tStatistic</strong></td>
<td><strong>CRLB ± 1 SD</strong></td>
</tr>
<tr>
<td>-------------------</td>
<td>-----------</td>
<td>-----------------</td>
<td>----------------------------------------</td>
<td>---------------</td>
<td>----------------</td>
</tr>
<tr>
<td>NAAG</td>
<td>Control</td>
<td>21</td>
<td>10.9 ± .96 (8.8)</td>
<td></td>
<td>6.4 ± .74</td>
</tr>
<tr>
<td></td>
<td>22q11DS</td>
<td>26</td>
<td>10.2 ± 1.3 (12.8)</td>
<td>−.13</td>
<td>9.8 ± 1.5</td>
</tr>
<tr>
<td>Glu + Gln</td>
<td>Control</td>
<td>23</td>
<td>10.2 ± 1.24 (12.2)</td>
<td>9.74 ± 1.7</td>
<td>9.74 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>22q11DS</td>
<td>26</td>
<td>3.4 ± .6 (17)</td>
<td>−.94</td>
<td>12.9 ± 2.8</td>
</tr>
<tr>
<td>Myo-inositol</td>
<td>Control</td>
<td>23</td>
<td>3.6 ± .5 (14)</td>
<td></td>
<td>12.2 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>22q11DS</td>
<td>26</td>
<td>6.7 ± .7 (11)</td>
<td>1.28</td>
<td>8.3 ± .8</td>
</tr>
<tr>
<td>PCr + Cr</td>
<td>Control</td>
<td>23</td>
<td>6.5 ± .7 (11)</td>
<td></td>
<td>8.6 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>22q11DS</td>
<td>26</td>
<td>1.4 ± .2 (17)</td>
<td>−.51</td>
<td>9.0 ± 1.4</td>
</tr>
<tr>
<td>GPC + PC</td>
<td>Control</td>
<td>23</td>
<td>1.5 ± .2 (16)</td>
<td></td>
<td>9.0 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>22q11DS</td>
<td>26</td>
<td>45.9 ± 10.4</td>
<td>.04</td>
<td></td>
</tr>
<tr>
<td>Gray Matter Voxel Fraction</td>
<td>Control</td>
<td>23</td>
<td>46.1 ± 9.9</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

When corrections for multiple comparisons are made, the group differences in NAA are no longer significant. 22q11DS, chromosome 22q11.2 deletion syndrome; Cr, creatine; CRLB, Cramer-Rao lower-bound value; CV, coefficient of variation in percentages; DLPFC, dorsolateral prefrontal cortex; Gln, glutamine; Glu, glutamate; GPC, glycerophosphocholine; 1H, proton; NAA, N-acetylaspartate; NAAG, N-acetylaspartylglutamate; PC, phosphocholine; PCr, phosphocreatine; SD, standard deviation; w.w., wet weight.

a  

$p < .05$.

**Age Effects**

There were no significant correlations with age for the metabolites, except for myo-inositol, which was negatively correlated with age in the 22q11DS group (Pearson $r = −.47, p < .05$) but not in the control group (Pearson $r = .20, p = .35$). The control group showed a trend toward decreasing NAA levels with age, although not significant (Pearson $r = −.32$, medium effect size), which was absent in the 22q11DS group ($r = −.030$). The relationship between age and NAA levels in the two groups is illustrated in Figure 2.
Figure 2. The linear relation of N-acetylaspartate (NAA) and age did not differ significantly between the two groups, although there was a trend toward lower levels with age in the control group ($r = -0.32$) not seen in the chromosome 22q11.2 deletion syndrome (22q11DS) group ($r = -0.030$). w.w., wet weight.

We divided the 22q11DS children and the control subjects in two groups based on the median age in each group (10.6 years in the 22q11DS group and 11.1 years in the control group). A significantly higher mean NAA level was seen in younger control subjects, compared with the older ones (11.16 [±.96] and 10.22 [±.65], respectively, $t$ statistic $= 2.60$, $p < .05$). There was no significant difference in the mean NAA levels in children with 22q11DS who were above and below the median age 11.29 (.93) and 11.31 (.93), respectively. There was also a significantly higher mean NAA level in older children with 22q11DS relative to older control subjects (11.29 [.93] and 10.27 [.64], respectively, $F = 9.3$, $p < .01$). In contrast, the NAA levels were not significantly different between the younger 22q11DS and control children (11.31 [.93] and 10.27 [.64], respectively). These results confirm the divergence in NAA levels with increasing age, as illustrated in Figure 2.

When Bonferroni corrections were applied due to the multiple comparisons that were used, the differences in the groups and age on NAA and myo-inositol levels were no longer significant.

### Relationship between Neuropsychological Functioning and NAA Levels

We then examined the relationship between NAA levels and neuropsychological functioning, since NAA differed between the two groups. A significant relationship was found between NAA and global functioning (GAF) in the 22q11DS group (Table 3 and Figure 3). The interaction term of group by NAA was not significant for GAF scores. The mean NAA level was significantly higher in children with 22q11DS who had ADHD compared with those that did not have this diagnosis (Table 3). No such relationships between ADHD diagnosis, GAF, or other neuropsychological measures and NAA levels were evident in the control group. To account for the effects of ADHD medication on the correlation between NAA and global functioning, we performed Pearson partial correlations with ADHD medications as a covariate and found that the
correlations between NAA levels and GAF ($r = -0.43, p < .05$) remained significant. We did not find a significant correlation between full-scale IQ, verbal comprehension, or perceptual organization indices and NAA levels in either the 22q11DS or control groups.

Table 3. Relationship of NAA Levels and Neuropsychological Findings in Children with 22q11DS

<table>
<thead>
<tr>
<th>Dependent Measure</th>
<th>Pearson Correlation/ $t$ Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAF</td>
<td>$-0.43^a$</td>
</tr>
<tr>
<td>DISC Inattention Symptoms</td>
<td>$0.156$</td>
</tr>
<tr>
<td>DISC Hyperactive Symptoms</td>
<td>$0.16$</td>
</tr>
<tr>
<td>DISC Any Diagnosis</td>
<td>$0.035$</td>
</tr>
<tr>
<td>WISC Verbal Comprehension Factor</td>
<td>$0.116$</td>
</tr>
<tr>
<td>WISC Working Memory</td>
<td>$-0.314$</td>
</tr>
<tr>
<td>WISC Perceptual Organization Factor</td>
<td>$-0.019$</td>
</tr>
<tr>
<td>WISC Processing Speed Factor</td>
<td>$-0.222$</td>
</tr>
<tr>
<td>WIAT Broad Reading</td>
<td>$-0.151$</td>
</tr>
<tr>
<td>WIAT Broad Mathematics</td>
<td>$-0.383$</td>
</tr>
<tr>
<td>WCST Perseverative Errors</td>
<td>$-0.076$</td>
</tr>
<tr>
<td>CPT AX Sustained Attention</td>
<td>$-0.072$</td>
</tr>
<tr>
<td>CPT IP Sustained Attention</td>
<td>$0.128$</td>
</tr>
<tr>
<td>DISC ADHD (NAA Levels of Subjects with and without ADHD)</td>
<td>$t$ statistic $= -2.50^a$</td>
</tr>
<tr>
<td>DISC Any Anxiety Disorder (NAA Levels of Subjects with and without Anxiety Disorder)</td>
<td>$t$ statistic $= 0.488$</td>
</tr>
</tbody>
</table>

Effect size: $.1 =$ small, $.3 =$ moderate, and $≥.5 =$ large. 22q11DS, chromosome 22q11.2 deletion syndrome; ADHD, attention-deficit/hyperactivity disorder; AX, task in which the user presses the button when the letter X is preceded by the letter A; CPT, Continuous Performance Test; DISC, Diagnostic Interview Schedule for Children; GAF, Global Assessment of Functioning; IP, identical pairs; NAA, $N$-acetylaspartate; ns, nonsignificant; WCST, Wisconsin Cart Sorting Test; WIAT, Wechsler Individual Achievement Test; WISC, Wechsler Intelligence Scale for Children.

$a$

$p < .05$

$b$

Moderate and large effect sizes.
Figure 3. Scatter plots demonstrating the correlation between Global Assessment of Functioning (GAF) and N-acetylaspartate (NAA) in children with chromosome 22q11.2 deletion syndrome (22q11DS) and control subjects: NAA has a strong correlation with GAF in the 22q11DS group and this is not present in the control subjects (group 1 = 22q11DS; group 2 = control subjects). w.w., wet weight.

Discussion

To our knowledge, this is the first study of 1H spectroscopy findings in children with 22q11DS. In contradiction to our hypothesis, mean absolute NAA level in the left DLPFC in our cohort of 22q11DS patients was higher than in the control group, with decreasing NAA levels with age in the control group but not in the 22q11DS group. Although the results were not significant once corrections for multiple comparisons were applied, the evidence is suggestive that the developmental trajectory of NAA levels may be different in the two groups.

NAA, which is the second most abundant free amino acid in the brain next to glutamate, is synthesized in neuronal mitochondria from acetyl coenzyme A (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), is primarily localized in white matter with highest activity in oligodendrocytes (51). This is consistent with cell culture studies showing that NAA is localized in both neurons, oligodendrocytes, and in oligodendrocyte-type-2 astrocyte progenitor cells (52 and 53) and provides evidence of intercompartmental cycling of NAA between neurons and oligodendrocytes (51,52 and 54). Thus, NAA may be a marker of functioning neuroaxonal tissue that includes functional aspects of the formation and/or maintenance of myelin (54). However, in the context of brain development, NAA levels do show dynamic age-related changes. Early in postnatal brain development, cortical NAA levels are low; NAA dramatically increases in early postnatal development (23 and 24) and reaches a plateau in later childhood/early adolescence followed by decreasing levels in late adolescence and early adulthood (i.e., similar to an inverted-U trajectory) (25, 26, 27 and 30). Specifically, the brain regions showing greatest NAA elevations during development include the cortical gray matter, cerebellum, and the thalamus (30). This provides additional evidence that
NAA does not merely reflect the number of neurons but is a marker of functioning neurons. Additionally, in the context of development, the timing of the inverted-U trajectory of NAA is consistent with the temporal maturation of the prefrontal cortex (29, 30 and 55). Therefore, the nonprogressive decreasing NAA levels in 22q11DS compared with control subjects suggest a developmental deviation in 22q11DS. The decreasing myo-inositol with age in 22q11DS contrasted by age-invariant changes in control subjects suggests there is decreasing glial content in 22q11DS, which may or may not be related to the lack of synaptic pruning. Interestingly, low levels of myo-inositol have been reported in individuals with long-standing schizophrenia, thought to be related to reduced glial activity (56). Further investigation is warranted.

Our finding of higher NAA levels within the DLPFC could be a sensitive indicator of neuropathology in 22q11DS, since NAA is an indicator of neuronal integrity, whereas volumetric studies are an indirect indicator of underlying neuronal pathology, with gray matter consisting of a complex combination of neurons with dendrites and synapses, as well as glia and vasculature (17). Further evidence for altered neuronal development in 22q11DS is provided by altered cortical development in children with the condition (57 and 58). Although the exact cause of the higher NAA in our cohort of children with 22q11DS is unclear, several mechanisms could exist. One possibility is that there is an increase in gray matter neuronal volume within the voxel that we placed in the DLPFC. However, although frontal lobe gray matter volumes are relatively preserved in children with 22q11DS (14, 15 and 59), an actual increase in gray matter in this area has not been reported. An alternate explanation is that the volumetric gray matter reductions within the DLPFC, which we reported previously in children with 22q11DS, could involve only nonneuronal tissue, with preservation of neuronal tissue or even an excess of it, effectively altering the ratio of neuronal to nonneuronal tissue.

We performed voxel segmentation analyses and there were no group differences in the gray matter tissue percentages, and tissue fraction values were then used in the calculation of absolute metabolite concentration levels. Thus, our results reflect gray matter neurometabolite levels and thus would not be reflective of white matter, although white matter volumetric abnormalities are common in 22q11DS (60 and 61). We did not correlate gray matter volumes in the DLPFC with the NAA levels, since the voxel we placed would not capture all of the DLPFC, and additionally, the gray matter composition of the voxel was approximately 50% and thus valid inferences could not be made about the volume of the DLPFC and the NAA levels. The only other study of brain 1H MRS in individuals with 22q11DS was in a small group of adults, comparing those with schizophrenia with those without schizophrenia and control subjects. There were no significant changes in neurometabolites in the DLPFC between the groups, but interestingly, the authors noticed a trend toward higher levels of neurometabolites in the 22q11DS group without schizophrenia compared with the healthy control subjects and 22q11DS individuals with schizophrenia (32). It was postulated that neurometabolites may be higher in individuals with 22q11DS before psychosis and that in the transition toward psychosis the levels of neurometabolites would decrease. Ongoing longitudinal studies on our cohort would enable further examination of this hypothesis.

Our finding of an increased DLFPC NAA in 22q11DS being related to ADHD and decreased GAF is intriguing. A relative increase in neuronal versus nonneuronal tissue due to neuronal dysmaturation in children with 22q11DS could be associated with elevated NAA and lower
global functioning and higher rates of ADHD. Studies on ADHD in the general population show reduced NAA in prefrontal cortex and an elevated NAA/PCr + Cr ratio ([31], [62], [63] and [64]), but the reports are inconsistent, with some studies reporting no abnormalities in prefrontal cortex NAA in association with ADHD (31). The elevation of NAA that we found is more likely to be due to neuronal dysmaturation than a compensatory mechanism to lower neurocognition in children with 22q11DS, since the elevated NAA is associated with lower functionality and ADHD and not with IQ. Delayed cortical maturation is thought to be related to the impulsivity and inattention seen in ADHD in the general population ([65] and [66]). Our preliminary report needs to be confirmed in larger cohorts, as well as in a longitudinal manner.

In comparing the findings of our study with those seen in spectroscopy studies of individuals with schizophrenia and those at high risk for the disorder in the general population, we did not see a reduction in NAA within the DLFPC in children with 22q11DS, who are considered to be at high risk for schizophrenia in later life. Although this was contrary to our expectation, the elevated NAA in our study may be related to abnormalities in brain maturation in 22q11DS. Further longitudinal studies in children with 22q11DS and high-risk individuals in the general population are needed for clarity on the relationship between neurometabolites in the DLPFC and the psychosis risk.

The advantages of our study were that we only had children who were nonpsychotic and thus the effects of psychosis on psychological function and NAA levels were not confounds; our subjects were not recruited from one referral source, such as the pediatric cardiology or child psychiatry clinic, and thus are representative of the wide spectrum of manifestations seen in 22q11DS.

In conclusion, children with 22q11DS demonstrate elevated levels of NAA in conjunction with higher rates of ADHD and lower global function. The increase in NAA in 22q11DS children may be reflective of relatively preserved neuronal tissue in the DLPFC due to delayed neuronal maturation and synaptic pruning. The relationship between ADHD and global functioning with the NAA levels underscores the importance of alterations of neurometabolites that are not directly related to the deleted genes, but rather a consequence of altered neuronal maturation/structure, in mediating the psychological abnormalities in these children.

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Address correspondence to Vandana Shashi, M.D., Duke University Medical Center, Division of Medical Genetics, Durham, NC 27710

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