22q11 Deletion syndrome and neurotransmitter systems in unchallenged and challenged conditions

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Proton magnetic resonance spectroscopy in 22q11 deletion syndrome

Submitted

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ABSTRACT
People with velo-cardio-facial syndrome or 22q11 deletion syndrome (22q11DS) have behavioral, cognitive and psychiatric problems. Approximately 30% of affected individuals develop schizophrenia-like psychosis. Glutamate dysfunction is thought to play a crucial role in schizophrenia. However, it is unknown if and how the glutamate system is altered in 22q11DS. People with 22q11DS are vulnerable for haploinsufficiency of PRODH, a gene that codes for an enzyme converting proline into glutamate. Therefore, it can be hypothesized that glutamatergic abnormalities may be present in 22q11DS and perhaps explain their increased risk for psychosis. We employed proton magnetic resonance spectroscopy ($^1$H-MRS) to quantify glutamate and other neurometabolites in the dorsolateral prefrontal cortex (DLPFC) and hippocampus of 20 adults with 22q11DS (22q11DS SCZ+) and without (22q11DS SCZ-) schizophrenia and 23 age-matched healthy controls. Also, plasma proline levels were determined in the 22q11DS group. We found significantly increased concentrations of glutamate and myo-inositol in the hippocampal region of 22q11DS SCZ+ compared to 22q11DS SCZ-. We found no evidence for altered metabolism in the DLPFC in 22q11DS. There were no significant differences in levels of plasma proline between 22q11DS SCZ+ and 22q11DS SCZ-. There was no relationship between plasma proline and cerebral glutamate in 22q11DS. This is the first in vivo $^1$H-MRS study in 22q11DS. Although preliminary, our results suggest vulnerability of the hippocampus in the psychopathology of 22q11DS SCZ+. Altered hippocampal glutamate and myo-inositol metabolism may partially explain the psychotic symptoms and cognitive impairments seen in this group of patients.
INTRODUCTION

Velo-cardio-facial-syndrome or 22q11 deletion syndrome (22q11DS) is a fairly common genetic syndrome caused by a deletion on chromosome 22 which is accompanied by several somatic, behavioral, cognitive and psychiatric problems, and structural and functional brain abnormalities [20]. The estimated prevalence of 22q11DS in the general population is 1 in 5950 births [37]. Adults with 22q11DS face a 25 times higher risk of developing schizophrenia than the general population [37] and in people with schizophrenia an increased frequency of 22q11 deletions has been reported [23,54]. Hence, a 22q11 deletion is among the highest risk factors for the development of schizophrenia.

People with 22q11DS are vulnerable to haploinsufficiency of approximately 30 genes located on the typically deleted region of chromosome 22q11, including the gene proline dehydrogenase (PRODH) [31]. The PRODH gene encodes for the PRODH enzyme also called proline oxidase (POX). POX catalyzes the conversion of proline to Δ1-pyrroline-5-carboxylate (P5C). P5C is in turn involved in converting proline to glutamate [41]. Dysfunction or genetic variations of the PRODH gene, and consequent hyperprolinemia, have been associated with susceptibility to schizophrenia and with learning disabilities [5,25,33,45]. In fact, proline has been shown to function as modulator of glutamate neurotransmission through NMDA receptors [2,39] and dysregulation of the glutamatergic system has been widely implicated in schizophrenia [14].

The involvement of glutamate in schizophrenia is particularly related to NMDA receptor hypofunction. Glutamate is the main excitatory amino acid neurotransmitter of the brain. Glutamate binds to and activates the α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate (AMPA), kainate and N-methyl-D-aspartate (NMDA) receptors. Decreased signaling of NMDA receptors, expressed in thalamic gamma-aminobutyric acid (GABA) interneurons, can lead to disinhibition of cortical glutamate release resulting in excessive excitability and neuronal excitotoxicity [39].

Evidence for the role of NMDA receptor hypofunction in schizophrenia comes from pharmacological studies of phencyclidine (PCP) and ketamine. These NMDA receptor antagonists have shown to 1) produce schizophrenia-like behaviors in rodents [2,43]; 2) induce positive and negative symptoms in healthy humans [1,29]; and 3) aggravate psychotic symptoms and provoke relapse in patients with schizophrenia [30,35]. Thus, impairment of glutamatergic NMDA receptors can induce psychosis.

Glutamate also plays a role in synaptic plasticity via NMDA receptors mediating higher cognitive functions such as learning and memory [46]. NMDA receptor dysfunction is implicated in the cognitive deficits of schizophrenia [32,42]. In rodents, NMDA receptor agonists enhanced memory and learning [36,40]. Also in people with schizophrenia, agents that enhance NMDA receptor activity have been shown to improve negative symptoms and facilitated memory consolidation [47,51]. Hence, NMDA hypofunction may be also underlying cognitive impairments in schizophrenia.
Chapter 7

The prefrontal cortex and hippocampus are brain areas associated with NMDA receptor hypofunction in schizophrenia [6,8,22]. A single photon emission computed tomography (SPECT) study using $^{[123]}$I-CNS-1261 tracer has reported reduced NMDA receptor binding in the hippocampus of medication-free patients with schizophrenia suggesting NMDA receptor deficiency in schizophrenia [42]. Moreover, a microdialysis study in rats showed that ketamine, a NMDA receptor antagonist, increased in vivo glutamate release in prefrontal cortex [32,36].

Taken together the above cited findings suggest a role for disturbed glutamatergic neurotransmission in schizophrenia. However, its underlying mechanisms and the relationship between NMDA receptor hypofunction and glutamate release is not fully understood. Increased glutamate exposure and its duration could explain the psychototoxic effects in schizophrenia. A reduced gene dosage of PRODH and consequent reduction of POX activity may compromise the conversion of proline to glutamate that probably results in decreased glutamate. NMDA hypofunction in schizophrenia could be related to insufficient or excessive glutamate release which may also differ between brain regions [40].

Proton Magnetic Resonance Spectroscopy (1H-MRS) is a feasible method for in vivo quantification of glutamate concentration and other brain metabolites that, if altered, may reflect abnormal neurodevelopmental features [47,51]. In schizophrenia an increasing number of 1H-MRS studies have been conducted. Although inconclusive, 1H-MRS findings also suggest abnormal glutamatergic neurotransmission [3,56,57].

To date, the glutamatergic system in 22q11DS has not been investigated. People with 22q11DS have an increased prevalence of schizophrenia and similar neuroanatomical abnormalities [49]. Hence, in this study we employed 1H-MRS to measure glutamate in the dorsolateral prefrontal cortex and hippocampus in 22q11DS patients with (22q11DS SCZ+) and without schizophrenia (22q11DS SCZ-).

We hypothesized altered glutamate concentrations in individuals with 22q11DS compared to healthy individuals and, in 22q11DS SCZ+ compared to 22q11DS SCZ-. Besides glutamate, we also analyzed other neurometabolites from 1H-MRS spectra including N-acetylaspartate, choline, myo-Inositol and creatine which reflect the status of neuronal functioning and glial cells, possibly disturbed in 22q11DS.

Furthermore, we assessed plasma proline levels in the 22q11DS group. Increased plasma proline levels have been reported in 22q11DS patients [19] and in children with 22q11DS there was a relationship between increased plasma proline and decreased brain function [59]. High levels of proline in 22q11DS, consequence of POX deficiency, may be related to glutamate dysfunction particularly in 22q11DS SCZ+. Hence, we expected that plasma proline will be increased in 22q11DS SCZ+ and that it will correlate with glutamate concentrations in the brain.
MATERIALS AND METHODS

Subjects
We included 20 adults with 22q11DS (mean ± SD) (22q11DS SCZ+ n=11, age 29.36 ± 7.19; 22q11DS SCZ- n=9, age 28.67 ± 8.97) and 23 healthy controls (HC, age 31.22 ± 9.58).

Individuals with 22q11DS were recruited through the Dutch 22q11DS family association and through the departments of three Dutch Clinical Genetics centers. Healthy volunteers were recruited by local advertisement. The study was conducted at the Department of Psychiatry, Academic Medical Centre Amsterdam, The Netherlands and was approved by the local Ethics Committee. All participants were capable of giving written informed consent and did so, after receiving full information on the study.

All individuals with 22q11DS were interviewed by a physician using semi-structured psychiatric interview. The 22q11DS group was subdivided into 2 groups: those who were fulfilling DSM-IV criteria for schizophrenia (22q11DS SCZ+) all taking antipsychotic medication and having duration of illness >1 year) and those who did not (22q11DS SCZ-) and were neuroleptic naive.

In addition, the Positive and Negative Symptom Scale (PANSS) [27] was used to assess positive, negative and general psychopathology in the 22q11DS SCZ+ group. The PANSS includes 30 items, subdivided in three categories: positive symptoms, negative symptoms and general psychopathology. A patient who rates “absent” (or 1) on all items would receive a total score of 30 and a subject who rates “extreme” (or 7) on all 30 items would receive a total score of 210. All patients underwent a formalized clinical interview of 35-40 minutes and the questions were in regard to the last two weeks.

For assessment of intelligence quotient (IQ) we used the shortened Dutch version of the Wechsler Adult Intelligence Scale (WAIS-III–NL) consisting of 5 subtests: vocabulary, comprehension, similarities (verbal IQ), block design, and object assembly (performance IQ) [9,60].

Also healthy volunteers were seen by a physician. They were included in the study after screening for psychiatric disorders and medical conditions affecting the brain. None of the participants had a history of substance or alcohol abuse. Urine drug screening (cocaine, tetrahydrocannabinol, opiates, amphetamines, benzodiazepines) was performed at study day and was negative in all subjects. Healthy participants were not using any medication at the time of testing.

1H-MR spectroscopy acquisition
1H-MRS data acquisition took place at the Department of Radiology (Academic Medical Centre Amsterdam, The Netherlands) using a 3 Tesla Intera MRI system (Philips, Best, The Netherlands) equipped with a 6 channel sense head coil. For estimation of metabolite concentrations, two single 8 ml voxels of interest positioned in the left dorsolateral prefrontal cortex (DLPFC) (2x2x2 cm) and left hippocampus (2x2x2 cm) were obtained for each subject (figure 1). Automated first order interactive
Shimming was performed and water suppressed spectra was acquired using a point-resolved spatially localized spectroscopy sequence (PRESS, TE 36 ms, TR 2000 ms, 128 averages).

Figure 1.
(a) Sagittal T1-weighted magnetic resonance image of the brain showing voxel (2×2×2 cm) placement for proton magnetic resonance spectroscopy (1H-MRS) in the left dorsolateral prefrontal cortex and left hippocampus. (b) Sample of a 1H-MRS spectrum from hippocampus of a patient with 22q11DS as fit by LCModel (Provencher, 1993).

For anatomical localization transversal high-resolution structural T1-weighted volumetric images, with full head coverage, using 130 contiguous slices (1.2 mm thick, with 0.89 x 0.89 mm in-plane resolution) and a TR/TE of 9.8/4.5 milliseconds (flip angle 8°, FOV 224 cm) were obtained.

1H-MRS spectra were analyzed using the Linear Combination of Model spectra (LCModel) commercial spectral-fitting package [44]. LCModel used a library of reference spectra in a basis set recorded specifically for the scanner and calibrated using the tissue water signal as an internal standard. The spectra were analyzed with a range of 3.8 ppm to 0.2 ppm (Figure 2). From the metabolites included in the LCModel basis set, we analyzed absolute levels of creatine plus phosphocreatine (Cr), glycerophosphocholine plus phosphocholine (choline), myo-inositol, N-acetylaspartate (NAA), NAA plus N-acetylaspartylglutamate (NAAG), glutamine, glutamate.

In addition, we analyzed the combination of glutamate plus glutamine (Glx). Glutamate and glutamine are closely related amino acids involved in intermediary metabolism, protein synthesis and neurotransmission. Metabolite concentrations are expressed in millimoles per liter.
Data were excluded from analysis if the voxel coordinates were not or incorrectly recorded. Spectral width (full width at half maximum, FWHM) was always lower than 0.1 p.p.m. and signal to noise ratio (SNR) greater than 11. Cramer-Rao minimum variance bounds (SD) was lower than 50% for glutamine and lower than 15% for the other metabolites.

**Plasma amino-acid analyses**

Plasma proline and plasma glutamine concentrations of the 22q11DS group were assessed by automated ion exchange chromatography with post-column ninhydrin derivatization. Plasma amino-acid analyses were performed on a JEOL AminoTac (JEOL AminoTac JLC-500/V, Tokyo, Japan) following a morning blood draw.

**Statistical analyses**

We used non-parametric Kruskal-Wallis H test to compare metabolite concentrations, age and IQ between the 3 groups (HC, 22q11DS SCZ+ and 22q11DS SCZ-). Post Hoc analyses were conducted with Mann-Whitney U tests. Correlation analyses were conducted with Spearman's rho test. Results are reported as significant when \( P < 0.05 \) (2-tailed). Statistical analyses were performed with SPSS, release 16.0.2 for Windows (SPSS Inc., Chicago, IL, USA. 2008).

**RESULTS**

**Demographics**

Patients and healthy controls did not differ with regard to sex (HC 12m/11f, 22q11DS SCZ+ 7m/4f, 22q11DS SCZ- 4m/5f \( P=0.68 \)) and age (HC 31.22 ± 9.58, 22q11DS SCZ+ 29.36 ± 7.19, 22q11DS SCZ- 28.67 ± 8.97; \( P=0.89 \)). Patients had a lower total IQ than healthy controls (HC 111.88±14.82, 22q11DS SCZ+ 69.67±13.82, 22q11DS SCZ- 81.86±7.01; \( P<0.001 \)). Also verbal IQ (HC 112.88±15.96, 22q11DS SCZ+ 75.00±11.24, 22q11DS SCZ- 85.86±9.33; \( P<0.001 \)) and performance IQ (HC 109.38±19.91, 22q11DS SCZ+ 67.89±16.60, 22q11DS SCZ- 79.43±10.53; \( P=0.002 \)) were significantly different between the groups. Post hoc analysis showed that HC compared to 22q11DS SCZ+ differed significantly for total IQ \( P=0.001 \), verbal IQ \( P=0.001 \) and performance IQ \( P<0.001 \). HC compared to 22q11DS SCZ- differed significantly for total IQ \( P=0.004 \), verbal IQ \( P=0.005 \) and performance IQ \( P=0.01 \). 22q11DS SCZ+ compared to 22q11DS SCZ- differed significantly for total IQ \( P=0.02 \) and verbal IQ \( P=0.02 \) but not performance IQ \( P=0.17 \). For the 22q11DS SCZ+ group, the mean score on the general psychopathology PANSS subscale was 30.69 ± 11.94, the negative subscale was 17.55 ± 8.21 and the positive subscale was 10.69 ± 3.81. The mean of total PANSS scores was 58.95 ± 21.85.
Metabolites

Metabolite concentrations for the DLPFC and hippocampal region are displayed in Table 1. Kruskal-Wallis H test showed no significant group differences in any of the metabolite concentrations in the DLPFC. In the hippocampal region, significant group differences were found in concentrations of glutamate (P=0.04) and myo-Inositol (P=0.04). Post hoc analysis indicated that these metabolite concentrations were significantly higher in 22q11DS SCZ+ compared to 22q11DS SCZ- patients (glutamate P=0.02 and myo-Inositol P=0.02). Hippocampal Glx was higher in 22q11DS SCZ+ compared to HC (P=0.05).

Table 1

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>DLPFC</th>
<th></th>
<th></th>
<th>Hip</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td>SCZ-</td>
<td>SCZ+</td>
<td>HC</td>
<td>SCZ-</td>
<td>SCZ+</td>
</tr>
<tr>
<td>n=</td>
<td>23</td>
<td>6</td>
<td>10</td>
<td>n=</td>
<td>16</td>
<td>7</td>
</tr>
<tr>
<td>Glu</td>
<td>6.44/1.35</td>
<td>6.35/1.12</td>
<td>6.63/1.12</td>
<td>Glu**</td>
<td>6.26/0.65</td>
<td>5.71/0.94</td>
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<tr>
<td>Gln</td>
<td>2.86/0.94</td>
<td>2.81/0.83</td>
<td>3.37/1.39</td>
<td>Gln</td>
<td>3.03/0.83</td>
<td>3.12/0.58</td>
</tr>
<tr>
<td>Glx</td>
<td>9.17/2.06</td>
<td>8.70/1.40</td>
<td>10.00/2.06</td>
<td>Glx*</td>
<td>9.29/0.94</td>
<td>8.83/1.11</td>
</tr>
<tr>
<td>mI</td>
<td>3.51/0.54</td>
<td>3.26/0.49</td>
<td>3.50/0.87</td>
<td>mI**</td>
<td>3.87/0.63</td>
<td>3.47/0.40</td>
</tr>
<tr>
<td>NAA</td>
<td>6.07/0.79</td>
<td>5.35/0.68</td>
<td>5.97/0.81</td>
<td>NAA</td>
<td>5.03/0.57</td>
<td>4.63/0.85</td>
</tr>
<tr>
<td>NAA+NAAG</td>
<td>6.68/0.82</td>
<td>6.00/1.01</td>
<td>6.48/1.14</td>
<td>NAA+NAAG</td>
<td>5.64/0.75</td>
<td>5.44/0.72</td>
</tr>
<tr>
<td>Cho</td>
<td>1.38/0.16</td>
<td>1.37/0.22</td>
<td>1.46/0.18</td>
<td>Cho</td>
<td>1.58/0.18</td>
<td>1.54/0.17</td>
</tr>
<tr>
<td>Cr</td>
<td>5.06/0.60</td>
<td>4.70/0.31</td>
<td>5.11/0.81</td>
<td>Cr</td>
<td>4.96/0.54</td>
<td>4.70/0.84</td>
</tr>
</tbody>
</table>

HC: Healthy controls, SCZ-: 22q11DS without psychosis, SCZ+: 22q11DS with psychosis
Glu: glutamate, Gln: glutamine, Glx: Glu+Gln, mI: myo-Inositol, NAA+N-acetylaspartate, NAA+NAAG: NAA+N-acetylaspartylglutamate
Metabolite concentrations are expressed in millimoles per liter
**P<0.05 for SCZ- vs. SCZ+, *P=0.05 for SCZ+ vs. SCZ-

Plasma Proline and Plasma Glutamine

For the whole 22q11DS group, the mean±SD for plasma proline was n=13, 354±128.88 μmol/l and for plasma glutamine n=8, 540.62±68.14 μmol/l. The correlation between these variables was not significant (n=8, r=0.26, P=0.53). The normal laboratory range for plasma proline was 77-343 μmol/l and for plasma glutamine 344-743 μmol/l.

There were no significant differences between 22q11DS SCZ- and 22q11DS SCZ+ for plasma proline (22q11DS SCZ-, n=6, 376.37±145.64 μmol/l, 22q11DS SCZ+, n=5, 316.20±100.56 μmol/l, P=0.56) or plasma glutamine (22q11DS SCZ-, n=4, 555.25±79.47 μmol/l, 22q11DS SCZ+, n=5, 540.80±63.70 μmol/l, P=0.78). There was no significant correlation between plasma proline and plasma glutamine.
The correlation between DLPCF glutamate and plasma proline for the whole 22q11DS group was not significant (n=11 p=0.26 P=0.43). Also, there was no significant correlation between proline and DLPCF glutamate for the 22q11DS SCZ- (n=5 p=0.30 P=0.62) and 22q11DS SCZ+ group (n=6 p=0.37 P=0.47). The correlation between hippocampal glutamate and plasma proline for the whole 22q11DS group was not significant (n=10 p=0.21 P=0.56). There was no significant correlation between proline and hippocampal glutamate for the 22q11DS SCZ- (n=6 p=0.03 P=0.96) and 22q11DS SCZ+ group (n=4 p=0.40 P=0.80).

**DISCUSSION**

In this first in vivo ¹H-MRS study in 22q11DS we measured absolute metabolite concentrations of the DLPCF and hippocampal region in adults with and without schizophrenia and in healthy controls. Our main findings are increased hippocampal glutamate and myo-inositol concentrations in 22q11DS SCZ+. DLPCF metabolites did not differ significantly across the groups.

¹H-MRS studies of the hippocampus in schizophrenia have shown ambivalent results concerning glutamate; there were no alterations of glutamate concentrations in subjects experiencing prodromal symptoms of schizophrenia [53], in first episode schizophrenia [24] or in chronic schizophrenia [28,34]. Other studies reported increased hippocampal glutamate in patients with schizophrenia [58] or a tendency towards increased glutamate in a group of medicated first episode patients [16].

In the present ¹H-MRS study we found increased concentrations of glutamate in the hippocampal region of 22q11DS SCZ+ compared to 22q11DS SCZ-. Also, hippocampal Glx was increased in 22q11DS SCZ+ compared to healthy controls. NMDA receptor antagonist, which mimics NMDA hypofunction, has been shown to increase glutamate release [32,36]. Moreover, excessive release of glutamate and consequent overstimulation of postsynaptic receptors might have an influence on the cognitive and psychotic symptoms associated with the NMDA hypofunction in schizophrenia [40]. In line with these observations and in agreement with previous research in schizophrenia, our finding of increased hippocampal glutamate in 22q11DS SCZ+ suggests that glutamate disturbance may be underlying psychotic symptoms in 22q11DS SCZ+. The 22q11DS SCZ+ had overall lower IQ than 22q11DS SCZ-. Increased hippocampal glutamate could also explain the cognitive impairment in 22q11DS SCZ+ since this brain area is involved in learning and memory functions. Although speculative, increased hippocampal glutamate in 22q11DS SCZ+ might also indicate NMDA receptor hypofunction in this group.

Glutamate neurotransmission may in part be influenced by proline. Increased concentrations of proline associated with hyperprolinemia type II (proline levels 10–15 fold above normal and excretion of Δ¹- pyrroline-5-carboxylate in urine) have been shown to potentiate glutamate transmission via NMDA receptors and to inhibit synaptic release of glutamate in the hippocampus [12,13]. Hyperprolinemia
type I (plasma proline levels with a range of 3–10-fold above normal) which results from inherited deficiency of POX enzyme has been observed in patients with 22q11DS [19,45]. In our study, half of the 22q11DS patients had increased proline levels in the range of hyperprolinemia type I. Contrary to our expectation of increased proline in 22q11DS SCZ+, that could be related to glutamate disturbance, we found similar plasma proline in 22q11DS SCZ+ and 22q11DS SCZ-. Increased proline levels may depend on genetic variation of the PRODH allele [5] or on interaction with other genes. For instance, a study of hyperprolinemia in 22q11DS showed an association between hyperprolinemia and psychosis in 22q11DS patients only when Met, the low activity allele of the COMT gene, was taken into account [45]. We found no correlation between plasma proline, plasma glutamine and cerebral glutamate concentrations in the whole 22q11DS group or in 22q11DS SCZ- vs. 22q11DS SCZ+. Thus, although we found increased hippocampal glutamate concentrations in 22q11DS SCZ+ its underlying mechanisms remain unclear.

In addition to increased hippocampal glutamate, we found higher concentrations of myo-Inositol in 22q11DS SCZ+ compared to 22q11DS SCZ-. Increased concentrations of myo-Inositol have previously been reported in mild cognitive impairment and Alzheimer disease [10,50]. Also in Down syndrome increased hippocampal myo-Inositol has been associated with reduced cognitive ability [4]. Myo-Inositol is primarily found in glial cells and changes in myo-Inositol levels may reflect abnormalities in membrane metabolism, in intracellular signaling mechanisms, neuronal development and survival [24]. Elevated myo-Inositol concentrations may also indicate increased number of glial cells which is a marker of neuronal degeneration. Reduction of hippocampal volume and functioning is reported in schizophrenia [18,21] and also in 22q11DS patients [15,16,26]. Hence, changes in myo-Inositol may explain part of the hippocampal brain abnormalities and learning disabilities seen in 22q11DS SCZ+.

We found no significant variation in neurometabolites concentration between the whole 22q11DS patient group and the healthy control group. This might be explained by group differences in the proportion of gray matter/white matter within the DLPFC and hippocampal voxels. Also, we found no evidence for altered glutamate in the DLPFC of 22q11DS patients (22q11DS SCZ+ vs. 22q11DS SCZ-) vs. healthy controls. In patients with chronic schizophrenia, 1H-MRS studies of the frontal cortex have shown increased [11,48,58] and reduced glutamate concentrations [34,38,55,56]. Perhaps, brain dysfunction related to psychosis in 22q11DS involves particularly regions of the temporal lobe [17,26]. Furthermore, it is also possible that abnormalities in glutamatergic function in this brain region may exist at the level of NMDA receptor or in second messenger signaling without alterations in glutamate concentration.

Strengths of this study include the evaluation of neuronal integrity in 22q11DS according to psychiatric status of 22q11DS SCZ- and 22q11DS SCZ+ and in comparison to age matched healthy controls. Also, all MRS spectra were carefully inspected and were included only if fulfilling the quality criteria of LCmodel. We have to acknowledge some limitations of the study; we were not able to determine...
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tissue contributions to measured metabolites. The use of unsegmented voxels (i.e., assessment of metabolite concentrations without addressing the impact of different tissue included in the voxel of interest) may increase the standard error of measurement and diminish the power to detect significant differences. Moreover, the effect of medication can be a potentially confounding factor in the $^1$H-MRS studies [7]. Whereas the 22q11DS SCZ- patients were not medicated, all 22q11DS SCZ+ patients were treated with antipsychotics. It is therefore possible that medication effects may have resulted in changes in energy metabolism in the frontal lobes, thereby explaining no differences between the 22q11DS SCZ- and 22q11DS SCZ+ and 22q11DS SCZ+ and HC.

Due to similar chemical components glutamate and glutamine overlap significantly in the $^1$H resonance spectrum. The use of higher field strengths and implemented spectroscopy analysis technique it is possible to improve glutamate quantification [52]. Discrepancies across earlier $^1$H-MRS studies that proposed to investigate glutamate in psychosis could have resulted from differences in brain regions of interest, patient population and stage of disease or issues of spectroscopy measurements.

In conclusion, our findings suggest vulnerability of the hippocampus in the psychopathology of 22q11DS SCZ+. Although the generalizability of the results is restricted by the relatively small sample size, altered glutamate and myo-Inositol metabolism may partially explain the psychotic symptoms and cognitive impairments seen in this group of patients. Future $^1$H-MRS studies with larger sample sizes including other prefrontal and temporal brain regions will help to clarify brain metabolism and integrity in 22q11DS.
REFERENCES


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