Proton magnetic resonance spectroscopy in 22q11 deletion syndrome


Published in:
PLoS ONE

DOI:
10.1371/journal.pone.0021685

Link to publication

Citation for published version (APA):
https://doi.org/10.1371/journal.pone.0021685

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Introduction

Velo-cardio-facial syndrome or 22q11 deletion syndrome (22q11DS) is a 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 [1]. The estimated prevalence of 22q11DS in the general population is 1 in 5950 births [2]. Adults with 22q11DS face a 25 times higher risk of developing schizophrenia than the general population [3] and in people with schizophrenia an increased frequency of 22q11DS has been reported [4,5]. Hence, a 22q11 deletion is among the highest risk factors for the development of schizophrenia.

22q11DS is a genetic syndrome caused by a deletion on chromosome 22q11, including the proline dehydrogenase gene (PRODH) [6]. This gene, which encodes for the PRODH enzyme also called proline oxidase (POX), is involved in converting proline to glutamate [7]. Dysfunction or genetic variations of the PRODH gene, and consequent hyperprolinemia, have been associated with susceptibility to schizophrenia and with learning disabilities [8–13]. In fact, proline has been shown to function as modulator of glutamate neurotransmission through NMDA receptors [14,15] and dysregulation of the glutamatergic system has been widely implicated in schizophrenia.

The involvement of glutamate in schizophrenia is particularly related to NMDA receptor hypofunction. 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 produce schizophrenialike behaviors in rodents [16]; to induce positive and negative symptoms in healthy humans [17]; and to aggravate psychotic symptoms in patients with schizophrenia [18]. Glutamate also plays a role in synaptic plasticity via NMDA receptors mediating higher cognitive functions such as learning and memory. NMDA receptor dysfunction has also been implicated...
in the cognitive deficits of schizophrenia [19]. In these people, agents that enhance NMDA receptor activity have shown to improve negative symptoms and to facilitate memory consolidation [20].

The brain areas associated with NMDA receptor hypofunction in schizophrenia include the prefrontal cortex and hippocampus [21–24]. The relationship between NMDA receptor hypofunction and glutamate release is not fully understood. NMDA hypofunction in schizophrenia could be related to insufficient or excessive glutamate release which may also differ between brain regions [25]. Increased glutamate exposure and its duration could explain the psychotoxic effects in schizophrenia.

Proton Magnetic Resonance Spectroscopy (1H-MRS) is a feasible method for in vivo quantification of glutamate concentrations and other brain metabolites that, if altered, may reflect abnormal neuro-developmental features [26]. In schizophrenia an increasing number of 1H-MRS studies have been conducted. Although inconclusive, 1H-MRS findings also suggest abnormal glutamatergic neurotransmission [27–29].

To date, the glutamatergic system in 22q11DS has not been investigated. People with 22q11DS have an increased prevalence of schizophrenia and similar neuroanatomical abnormalities. 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 SCZ+ 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 levels of plasma proline and plasma glutamine in the 22q11DS group. Increased proline has been reported in 22q11DS patients [30]. In children with 22q11DS there was a relationship between increased plasma proline and decreased brain function [31]. 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 22 adults with 22q11DS (mean ± SD) (22q11DS SCZ+ n = 12, age 29.25±8.24; 22q11DS SCZ− n = 10, age 28.50±8.47) 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, 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) (2×2×2 cm) and left hippocampus (2×2×2 cm) were obtained for each subject (Figure 1). More specifically, the hippocampal voxel included areas of the hippocampus, para-hippocampal gyrus, fusiform gyrus and collateral sulcus. Iterative first order shimming was performed and water suppressed spectra were acquired using a point-resolved spatially localized spectroscopy sequence (PRESS, TE 36 ms, TR 2000 ms, 128 averages).

Table 1. Medication and dosage taken by 22q11DS patients with schizophrenia.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Dosis (mg/d)</th>
<th>Haloperidol equivalent (mg/d)*</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arbidol 5–15</td>
<td>1–7.5</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Atomoxetine</td>
<td>80</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Clozapine</td>
<td>200–300</td>
<td>4–6</td>
<td>2</td>
</tr>
<tr>
<td>Methylphenidate</td>
<td>36</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Olanzapine</td>
<td>5</td>
<td>2.5</td>
<td>1</td>
</tr>
<tr>
<td>Quetiapine</td>
<td>50</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>Risperidone</td>
<td>3–4</td>
<td>5–6</td>
<td>2</td>
</tr>
<tr>
<td>Zuclopentol</td>
<td>6</td>
<td>1.2</td>
<td>1</td>
</tr>
</tbody>
</table>


1One patient took an antipsychotic and a selective norepinephrine inhibitor.

2One patient took an antidepressive and a psychostimulant drug.

doi:10.1371/journal.pone.0021685.t001
For anatomical localization transversal high-resolution structural T1-weighted volumetric images, with full head coverage, using 150 contiguous slices (1.2 mm thick, with 0.89×0.89 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 [36]. LCModel used a library of reference spectra in a basis set of model spectra (LCModel) commercial spectral-fitting package [36].

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 as estimated by LCModel. 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 AminoFac (JEOL AminoFac JLC-500/Y, 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−) because the assumption of normal distribution was not met. Following, 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+: 8m/4f, 22q11DS SCZ−: 4m/6f; $P=0.45$) and age (HC: 31.22±9.51, 22q11DS SCZ+: 29.25±8.24, 22q11DS SCZ−: 28.50±8.47; $P=0.89$).

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 $P=0.17$.

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 score was 58.95±21.85.

Metabolites

Metabolite concentrations for the DLPFC and hippocampal region are displayed in Table 2. Kruskal-Wallis H test showed no significant group differences in any of the metabolite concentrations. The mean of total PANSS score was 58.95±21.85.

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 ($n=8\ P=0.53$). The normal laboratory range for plasma proline was 77–343 μmol/l and for plasma glutamine 344–743 μmol/l.

For the 22q11DS SCZ+ group, the mean±SD for plasma proline ($n=8$) was 633.70±155.46 μmol/l and for plasma glutamine ($n=8$) was 69.67±13.82 μmol/l. The correlation between these variables was not significant ($n=8\ P=0.53$).
Figure 2. Sample of a 1H-MRS spectrum from hippocampus of a patient with 22q11DS as fit by LCModel.
doi:10.1371/journal.pone.0021685.g002

Table 2. Metabolites concentrations (mean/SD) in the DLPFC and hippocampal region in healthy controls and 22q11DS with and without psychosis.

<table>
<thead>
<tr>
<th></th>
<th>HC</th>
<th>SCZ−</th>
<th>SCZ+</th>
<th>HIP</th>
<th>HC</th>
<th>SCZ−</th>
<th>SCZ+</th>
</tr>
</thead>
<tbody>
<tr>
<td>n =</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glu</td>
<td>6.44/1.35</td>
<td>6.35/1.02</td>
<td>6.39/1.32</td>
<td>Glu*a</td>
<td>6.26/0.65</td>
<td>5.71/0.94</td>
<td>6.99/1.04</td>
</tr>
<tr>
<td>Gln</td>
<td>2.86/0.94</td>
<td>2.66/0.83</td>
<td>3.25/1.37</td>
<td>Gln</td>
<td>3.03/0.83</td>
<td>3.12/0.58</td>
<td>3.88/1.67</td>
</tr>
<tr>
<td>Glx</td>
<td>9.17/2.06</td>
<td>8.64/1.29</td>
<td>9.65/2.28</td>
<td>Glxab</td>
<td>9.29/0.94</td>
<td>8.83/1.11</td>
<td>10.87/1.66</td>
</tr>
<tr>
<td>ml</td>
<td>3.51/0.54</td>
<td>3.35/0.50</td>
<td>3.46/0.83</td>
<td>mlb</td>
<td>3.87/0.63</td>
<td>3.47/0.40</td>
<td>4.43/0.76</td>
</tr>
<tr>
<td>NAA</td>
<td>6.07/0.79</td>
<td>5.38/0.63</td>
<td>5.89/0.82</td>
<td>NAA</td>
<td>5.03/0.57</td>
<td>4.63/0.85</td>
<td>5.25/1.18</td>
</tr>
<tr>
<td>NAA+NAAG</td>
<td>6.68/0.82</td>
<td>5.96/0.92</td>
<td>6.41/1.11</td>
<td>NAA+NAAG</td>
<td>5.64/0.75</td>
<td>5.44/0.72</td>
<td>6.06/1.09</td>
</tr>
<tr>
<td>Cho</td>
<td>1.38/0.16</td>
<td>1.34/0.22</td>
<td>1.43/0.20</td>
<td>Cho</td>
<td>1.58/0.18</td>
<td>1.54/0.17</td>
<td>1.71/0.25</td>
</tr>
<tr>
<td>Cr</td>
<td>5.06/0.60</td>
<td>4.80/0.38</td>
<td>5.06/0.60</td>
<td>Cr</td>
<td>4.96/0.54</td>
<td>4.70/0.64</td>
<td>5.25/0.86</td>
</tr>
</tbody>
</table>

HC: Healthy controls SCZ−: 22q11DS without psychosis SCZ+: 22q11DS with psychosis.
Metabolite concentrations are expressed in millimoles per liter.
aP < 0.05 for SCZ− vs. SCZ+.
bP = 0.05 for HC vs. SCZ+.
doi:10.1371/journal.pone.0021685.t002
Increased hippocampal glutamate and myo-inositol may be underlying psychotic symptoms in 22q11DS patients. The finding of increased glutamate and myo-inositol may be tightly related to each other in the psychopathology of schizophrenia [29,39,52,53]. Perhaps, brain dysfunction associated with psychosis is therefore warranted. In schizophrenia patients with 22q11DS in the at risk mental state (ARMS) [56,57], the transition to psychosis instead of the development of schizophrenia may depend on genetic variation of the PRODH allele [8] 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 [12]. However, 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 [43,44]. Also in Down syndrome increased hippocampal myo-inositol has been associated with reduced cognitive ability [45]. Changes in myo-inositol levels may reflect abnormalities in membrane metabolism, in intracellular signaling mechanisms, neuronal development and survival [46]. Hence, increased myo-inositol may explain part of the hippocampal brain abnormalities and learning disabilities seen in 22q11DS SCZ+.

An interesting observation is that most of the metabolite alterations in glutamate concentration. The correlation between increased proline levels (10–15 fold above normal) which results from inherited deficiency of POX enzyme [12,30]. In the present study half of the 22q11DS patients had elevated proline levels. Contrary to our expectation, we found similar proline levels in 22q11DS SCZ+ and 22q11DS SCZ−. Increased proline levels may depend on genetic variation of the PRODH allele [8] 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 [12]. 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 [43,44]. Also in Down syndrome increased hippocampal myo-inositol has been associated with reduced cognitive ability [45]. Changes in myo-inositol levels may reflect abnormalities in membrane metabolism, in intracellular signaling mechanisms, neuronal development and survival [46]. Hence, increased myo-inositol may explain part of the hippocampal brain abnormalities and learning disabilities seen in 22q11DS SCZ+.

The finding of increased glutamate and myo-inositol may be tightly related to each other in the psychopathology of schizophrenia [29,39,52,53]. Perhaps, brain dysfunction associated with psychosis is therefore warranted. In schizophrenia patients with 22q11DS in the at risk mental state (ARMS) [56,57], the transition to psychosis instead of the development of schizophrenia may depend on genetic variation of the PRODH allele [8] 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 [12]. 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 [43,44]. Also in Down syndrome increased hippocampal myo-inositol has been associated with reduced cognitive ability [45]. Changes in myo-inositol levels may reflect abnormalities in membrane metabolism, in intracellular signaling mechanisms, neuronal development and survival [46]. Hence, increased myo-inositol may explain part of the hippocampal brain abnormalities and learning disabilities seen in 22q11DS SCZ+.
unlike in the hippocampus, we found a significant positive correlation between dosage of medication and glutamine concentration and a trend towards positive correlation between dosage of medication and Glx concentration in 22q11DS SCZ+ patients. This may also indicate that antipsychotics modulates neuronal metabolism in a regionally specific fashion.

Due to similar chemical components glutamate and glutamine overlap significantly in the ¹H resonance spectrum. The use of higher field strengths and implemented spectroscopy analysis technique makes it possible to improve glutamate quantification. Discrepancies across earlier ¹H-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 ¹H-MRS studies with larger sample sizes including other prefrontal and temporal brain regions will help to clarify brain metabolism and integrity in 22q11DS.

Author Contributions

Conceived and designed the experiments: TVa NS FdSA. Performed the experiments: FdSA EB. Analyzed the data: FdSA CL PP AN EB. Contributed reagents/materials/analysis tools: JV AN CL. Wrote the paper: FdSA EB TVa NS LiH DL.


