Is There a Dysregulation of Backdoor Pathway of Androgen Synthesis in Autistic Disorders?

By Benedikt Gasser, Johann Kurz, Bernhard Dick & Markus Mohaupt

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Material & Methods: Urine samples were collected from 20 boys originally diagnosed with Asperger syndrome, 21 boys with Kanner syndrome, 8 with Atypical autism as well as 5 girls with Asperger syndrome, 10 girls with Kanner Syndrome and one with Atypical autism and a control group for gas chromatography mass spectrometry based steroid hormone analysis. As Etiocholanolone (E) originates almost exclusively from the classic pathway and Androsterone (A) may be derived additionally from the backdoor pathway analyses of A/E ratios in affected autistic boys and girls were used to identify a potential dysregulation of backdoor pathway of androgen synthesis.

Results: In Kanner boys Androsterone and Eticholanolone showed increased concentrations of around fifty percent (p < 0.01). In addition, in boys with Asperger Syndrome an increase of Androsterone (p < 0.01) and Eticholanolone (p < 0.01) was detected.

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Discussion: Increased values of Androsterone and Etiocholanolone during puberty are in line with findings from others of a hyperandrogenemia in autism. However, A/E ratios indicative for a dysregulation of backdoor pathway of androgen synthesis were not altered. In consequence, other previously developed theories such as an increase of cholesterol references as precursor of steroid hormones gain additional support.

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I. Introduction

In the younger past a relatively encompassing view came up that not only androgens but steroid hormones in general (glucocorticoids, mineralocorticoids, androgens) and its precursors are involved in autistic disorders. [1,2] Increased concentrations of cortisol [3] and of androgens [4,5,6,7,8,9] were several times described and positive correlations between testosterone and severity of autism was implied. [10] Furthermore, genes involved in androgen metabolism such as CYP11B1, CYP17A1, and CYP19A1 (aromatase) relevant for androgen synthesis showed a significant association with autism. [11, 12, 13, 14] Extreme male theory of autism [15] was developed, whereby especially prenatal increased androgens were favored as relevant [9] and later cholesterol hypothesis postulating a general dysregulation of cholesterol homeostasis deepened the premise of a altered steroid hormones in autism. [1, 2]

II. Material & Methods

a) Participants

Twenty boys diagnosed with Asperger syndrome (BMI 18.2±3.2; average age 15.3±2.9 years), and 21 boys diagnosed with Kanner’s syndrome (BMI 20.4 ± 5.9; average age 13.6 ± 3.6 years), and 8 boys...
with Atypical Autism (n=8) (BMI 18.1 ± 2.9; average age 14.1 ± 3.7) and a cohort of 49 unaffected healthy control boys (BMI 17.6 ± 3.6; average age 13.7 ± 5.9).

As well as ten girls with Kanner’s syndrome (BMI 17.3 ± 3.4 average age 18.3 ± 4.8) and five with Asperger Syndrome (n=5)(BMI 16.5 ± 2.0 average age 13.0 ± 1.9) and one girl with atypical autism (BMI 16.8 age 19) and a cohort of 16 control girls (BMI 17.7 ± 5.4 average age 15.4 ± 3.1) for a gas chromatography mass spectrometry based steroid hormone metabolite analysis.

b) Study Design

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or with comparable ethical standards. The study was approved by the governmental ethics board of Graz, Austria, and registered at ClinicalTrials.gov. Involvement in the study was voluntary and not compensated. After study procedures had been fully explained, the parents of the participants read and signed informed consent forms. Autistic and control boys were recruited from the area of Leipzig (Austria). Enrolment took place from mid-2009 to mid-2012. All participants were Caucasians. Participants were excluded if they had aneurological and psychiatric disorder other than autism and comorbid disorders, a history of liver diseases, renal or endocrine disorders, a current infection, or fever. Mental retardation or behavioral disorders were exclusion criteria only for the control group but were allowed as comorbid conditions in the autistic group. The diagnosis was given in the first years of life of the children according to the diagnostic criteria of the DSM-IV and was cross-checked by experienced clinicians (medical doctors and/or psychologists) during enrolment of the study. Key criteria such as the extent of language impairment were used in order to distinguish between Kanner’s syndrome and Asperger syndrome. [35] A further tool used to increase the accuracy of diagnosis was the Marburg questionnaire for Asperger syndrome (MBAS).

c) Methods

Analysis of urinary steroids was conducted via gas chromatography–mass spectrometry. Urine samples were taken between 7 a.m. and 9 a.m. in the morning after breakfast. Urine sample preparation comprised pre-extraction, enzymatic hydrolysis, extraction from the hydrolysis mixture, derivatization, and gelfiltration were conducted as described by us and others. [36, 37, 38] The recovery standard was prepared by adding 2.5 µg of medroxyprogesterone to 1.5 mL of urine. The sample was extracted on a Sep-Pak C18 column (Waters Corp., Milford, MA, USA), dried, reconstituted in a 0.1 M acetate buffer, pH 4.6, and hydrolysed with a powdered Helix pomatia enzyme (12.5 mg; Sigma Chemical Co., St. Louis, MI, USA) and 12.5 µL of β-glucuronidase/ arylsulfatase liquid enzyme (Roche Diagnostics, Rotkreuz, Switzerland). The resulting free steroids were extracted on a Sep-Pak C18 cartridge. A mixture of internal standards (2.5 µg each of 5α-androstan-3α, 17α-diol, stigmasterol, and cholesterol butyrate, and 0.15 µg of 3αβ-tetrahydroaldosterone) was added to this extract, and the sample was derivatised to form the methyloxime-trimethylsilyl ethers. Analyses were performed on a Hewlett-Packard gas chromatograph 6890 (Hewlett Packard, Palo Alto, CA, USA) with a mass selective detector 5973 by selective ion monitoring (SIM). One characteristic ion was chosen for each compound measured. The derivatised samples were analysed during a temperature-programmed run (210–265 °C) over a 35 min period. The calibration standard consisted of a steroid mixture containing known quantities of all steroid metabolites to be measured. Responses and retention times were recorded regularly. In each case, the ion peak was quantified against the internal stigmasterol standard. All results were adjusted for creatinine in urine to check renal function.

d) Statistical Analysis

Descriptive statistics for Androsterone and Ethicholanolone while calculating Mean, SEM, Median and range were calculated separately for boys with Kanner Syndrome, Asperger Syndrome, Atypical Autism and for the healthy control cohort of boys respectively girls. Two sided-heteroscedastic t-tests were performed to analyze differences in metabolites and A/E ratios between Asperger respectively Kanner respectively Atypical Autism and the healthy controls. Due to only one girl originally diagnosed with Atypical Autism no t-tests could be conducted for this subgroup. Linear regression while calculating coefficient of determination between age respectively BMI and A/E ratios were conducted. Calculations were made with GraphPad Prism (GraphPad Software, Inc., La Jolla, CA, USA) and Microsoft Excel (Microsoft Inc., Redmond, WA, USA).

III. Results

Tab. 1 shows the values of Androsterone, which was significantly increased in affected boys with Asperger respectively Kanner Syndrome (p < 0.01) and Ethicholanolone, which was in addition significantly increased in Asperger (p < 0.01) and Kanner Syndrome (p < 0.01). In contrast to boys, no significant increased concentrations can be detected for girls. Focusing on ratios only for Kanner Syndrome in girls a significant increase can be detected (p < 0.01), however for boys the A/E ratios are not significantly different.

Concerning the sub-samples it is to mention that for each subgroup BMI and age was not
significantly different between affected and healthy controls on $\alpha = 0.01$ level. Therefore, potential explanation power of BMI and age was elucidated while calculating several linear regression models. The additionally calculated models for the group of Kanner boys respectively Asperger boys revealed some explanation power of age for A/E ratios in Kanner (A/E = 0.1077 * age + 0.3908 / $R^2 = 0.2751$) and for Asperger (A/E = 0.1029 * age + 0.1241 / $R^2 = 0.2416$) in contrast to the control cohort (A/E = -0.008 * age + 1.7185 $R^2 = 0.0018$) with a very low coefficient of determination. However, in contrast to age BMI does not seem to explain variance of A/E ratios with low coefficients of determination for all affected boys (A/E = 0.0104 * BMI + 1.9277 / $R^2 = 0.0026$) and slightly higher for the healthy boys (A/E = 0.0389 * BMI + 0.9398 / $R^2 = 0.054$). To sum up, age but not BMI seems to have relevance for A/E ratios.

IV. Discussion

The aim of this study was to analyze Androsterone and Eticholanolone respectively ratios of Androsterone to Eticholanolone indicative for a dysregulation of backdoor pathway in a cohort of autistic boys and girls originally diagnosed with Kanner Syndrome, Asperger Syndrome or Atypical Autism. We suggested an altered activity of backdoor pathway persisting or reinitiated during puberty in affected autistic individuals. However, ratios of Androsterone to Eticholanolone show no significant difference between affected and healthy controls. Based on these data it is implied that during puberty no stimulation of backdoor pathway seems to be present, but unfortunately data does not allow to imply that there were never alterations for example in earlier sensitive stages of development. It is currently accepted that steroid profile changes prenatal to postnatal and androgen biosynthesis during minipuberty favors the backdoor pathway over the classic pathway with several pathologic associations (21-Hydroxylase Deficiency, Fragile-X-Syndrome or an altered sexual development. [16, 20, 21, 22, 23, 24, 25] Despite limited by a relatively small sample size especially for girls a significant increase for boys but not girls of the two androgens Androsterone and Eticholanolone during puberty persists. The increased androgens during this sensitive period of development might additionally promote autistic regression. These suggestions could be combined with newer theories such as a general dysregulation of steroid hormones respectively precursors (Cholesterol hypothesis) or with extreme male theory of autism [1, 2, 15]

To sum up, despite significantly increased Androsterone and Eticholanolone metabolites in affected autistic boys and girls, no significant difference in the ratio of Androsterone to Eticholanolone in boys and girls can be detected indicative of no special alterations of activity of the backdoor pathway of androgen synthesis. In conclusion, other theories such as Cholesterol hypothesis of autism gain additional support.

References Références Referencias


Figure legends

**Table 1:** Etiocholanolone (ratio of Androsterone (mmol/l) to urine creatinine (µmol/l)) and A/E ratios for autistic boys and controls in the upper part and for girls in the lower part for Kanner, Asperger and atypical Autism.

<table>
<thead>
<tr>
<th>Boys</th>
<th>Androsterone</th>
<th>Etiocholanolone</th>
<th>A/E</th>
<th>Median</th>
<th>range</th>
<th>p-value ratios subsamples versus controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kanner (n=21)</td>
<td>89.3 ± 17.4</td>
<td>59.8 ± 13.4</td>
<td>1.64</td>
<td>1.6</td>
<td>4.07</td>
<td>0.93</td>
</tr>
<tr>
<td>Asperger (n=20)</td>
<td>91.3 ± 18.2</td>
<td>60 ± 13.1</td>
<td>1.83</td>
<td>1.3</td>
<td>2.97</td>
<td>0.49</td>
</tr>
<tr>
<td>Atypical Autism (n=8)</td>
<td>77.6 ± 27.4</td>
<td>46.8 ± 16.6</td>
<td>1.81</td>
<td>1.69</td>
<td>1.58</td>
<td>0.52</td>
</tr>
<tr>
<td>Controls (n=49)</td>
<td>49.5 ± 6.3</td>
<td>28.8 ± 3.7</td>
<td>1.66</td>
<td>1.57</td>
<td>2.82</td>
<td></td>
</tr>
<tr>
<td>Girls</td>
<td>Androsterone</td>
<td>Etiocholanolone</td>
<td>A/E</td>
<td>Median</td>
<td></td>
<td>p-value ratios</td>
</tr>
<tr>
<td>Kanner (n=10)</td>
<td>91.1 ± 29.9</td>
<td>74.7 ± 23.3</td>
<td>1.27</td>
<td>1.16</td>
<td>1.2</td>
<td>0.28</td>
</tr>
<tr>
<td>Asperger (n=5)</td>
<td>32.8 ± 15.3</td>
<td>24.1 ± 4.8</td>
<td>1.29</td>
<td>1.25</td>
<td>2.16</td>
<td>0.02</td>
</tr>
<tr>
<td>Atypical Autism (n=1)</td>
<td>210.8</td>
<td>172.3</td>
<td>1.22</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls (n=16)</td>
<td>52.9 ± 6.8</td>
<td>35.8 ± 5.9</td>
<td>1.74</td>
<td>1.8</td>
<td>2.76</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1: Androsterone and Etiocholanolone were significantly increased for all subgroups Kanner, Asperger and atypical Autism in pubertal autistic boys compared to healthy controls, whereas in girls only for the ratio of A/E in Asperger versus healthy controls can be detected.