An interaction between a polymorphism of the serotonin transporter (5HTT) gene and the clinical picture of adolescents with combined type of ADHD (hyperkinetic disorder) and youth drinking

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Summary

Introduction: The combined type of ADHD and alcohol dependence are two different disorders. Research demonstrate that 45-55% of patients diagnosed with ADHD also suffer from comorbid substance abuse, and 11-55% of patients diagnosed with substance abuse suffer from undiagnosed ADHD. Alcohol is by far the most widely used psychoactive substance in the European culture. The serotonin transporter (5HHT) gene has been implicated as one of the candidate genes in both disorders in recent molecular genetic research.

Aim: The aim of the present study was to seek a common clinical and biological marker for hyperkinetic disorder and youth drinking.

Methods: The study was conducted between 2008 and 2012. The sample consisted of 100 combined type ADHD patients: 51 adolescents youth drinking and 100 individuals without mental disorders or addiction in a population-based group. The 5HHT gene polymorphism was examined using PCR (Polymerase Chain Reaction). Statistical analysis was conducted with STATISTICA.PL software (version 5.0.97) licensed by StatSoft, Inc. USA.

Results: A preferential trend for the “s” short allele of the investigated 5HHT gene polymorphism was observed in all the groups of adolescents compared to the population-based group of adults without alcohol dependence (p=0.01).

Conclusion: Based on the conducted study a provisional conclusion may be drawn that the presence of the short “s” allele of the 5HTT gene polymorphism may be a prognostic factor of impulsivity in ADHD and of predisposition to alcohol dependence.

Key words: ADHD, alcohol, 5-HTT polymorphism
Introduction

Symptoms which, diagnosed mainly in childhood ADHD, persist at 33-66% of adult patients, causing multiple issues related to health (recklessness, anxiety, depressive and personality disorders) and social functioning (work-related issues, marital difficulties, legal problems) [1, 2]. While hyperkinetic disorder is thought to be the most genetically conditioned mental disorder, the concordance rates in twins diagnosed with it reach 80% [3, 4]. However, the identification of specific genes involved in etiopathogenesis is difficult due to the multifactorial nature of the etiology. Genes of the serotonergic system have been implicated as the candidate genes for the disorder [4, 5].

Alcohol dependence remains a major cause of social degradation and is instrumental in the etiology of both many mental disorders and somatic diseases. AD is considered to be conditioned by both genetic and environmental factors and their comorbidity [6, 7]. The role of genetic factors in causing addiction is estimated at 40-50%. Serotonergic system genes have also been named as the candidate genes for AD [8].

A Swedish follow-up study on a sample of 50,000 people revealed that early alcohol initiation is to be considered an important predictor of subsequent work-related problems and potential exclusion from the labour market [9]. Patients with primary ADHD have shorter transition periods from adverse substance experiences to addiction [10-12]. Jester et al. analysed the trajectory from childhood aggression and inattention/hyperactivity symptoms to differences in initiation methods and ways of developing substance, including alcohol. Children aged 2 to 5 were qualified for the study and were followed over 10 years. At the time of the first examination the children were 3-5 years old. Environmental factors (familial problems, paternal alcoholism) were also considered. According to observation and CBCL (Child Behavior Checklist) and TRF (Teacher Rating Scales Report) scale scores demonstrated that hyperactive adolescents with attention deficit and aggression behaviour were most at risk of early alcohol initiation. The researchers also emphasized the relevance of genetic factors which they related to paternal alcoholism and legal problems [13].

Hallikainen et al. in their study on the 5HHT gene polymorphism found an association between the short “s” allele and a risk of early onset alcoholism with violent, impulsive behaviours [14]. Research to date has been focusing on analysing concurrent genetic and environmental factors pointing out to associations between the 5HHT gene polymorphisms and psychosocial/familial moderators [15, 16, 17, 18, 19, 20] as well as to the relevance and ways of executing impulsive behaviours. Researchers maintained that while some ADHD adolescents respond with aggression combined with behaviour disorders (which was later referred to as ‘impulsive aggression’), others make quick, inadequate decisions or are unable to wait for gratification (later known as cognitive impulsiveness) [18, 21]. The long allele of the 5HTTLPR polymorphism was associated with cognitive impulsiveness symptoms, the short allele with ADHD accompanied by mood disorders, aggression and compulsive behaviour disorders [17, 18].
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A study on 366 families that categorised 1167 genotypes demonstrated an association between the 5HTT short “s” allele and aggression in children, which was the strongest in 9-year olds. The behaviour of children was assessed by their parents and teachers using CBCL diagnostic tools [22].

Materials and methods

The protocol of the study was approved by the Bioethics Committee of the Pomeranian Medical University of Szczecin number BN-001/76/2007. Written informed consent was obtained from all subjects and their legal guardians. Diagnostic examination of 100 patients with combined type of ADHD was conducted at Private Specialist Medical Practice (92 males, 8 females, average age of 12.7). The tests of 51 patients who early experimented with alcohol were performed in randomly selected junior high schools in Zachodniopomorskie province and at the Detoxification Unit of the Nephrology Ward of a public health care institution for mother, children and young people in Szczecin (30 males, 21 females, average age of 14.3). A population-based cohort of 100 controls (90 males, 10 females, average age of 38.5) was examined at the Department of Psychiatry, Pomeranian Medical University, Szczecin to determine possible associations between some genetic polymorphisms and personality profiles of individuals without any mental disorders [23].

Questionnaire Testing Methods

All adolescents were clinically tested using standardized rating scales for ADHD screening and assessment of the disorder’s symptoms’ severity and for behaviour disorders according to ICD-10 criteria [24]. Guardians completed ADHD Diagnostic Parent Rating Scale – an experimental diagnostic tool for parents [25] and CBCL (Child Behavior Checklist) in Polish adaptation [26]. Young patients reflected on their own behaviour by filling YSR (Youth Self Report) in Polish adaptation [26] and Beck Youth Inventories of Emotional and Social Impairment in Polish adaptation [27]. Adolescents and their guardians also completed our own survey forms on early experiments with alcohol. The inclusion criteria during the study were as follows: age between 11 and 18. Patients with hyperkinetic disorder (combined type of ADHD) were diagnosed according to ICD-10 criteria [28]. Adolescents early experimenting with alcohol were 11-18 years of age and regularly consumed alcohol, i.e. at least once per month.

Laboratory methods

Genetic material was extracted from saliva using Oragene OG-500 set according to the manufacturer’s instruction. PCR (Polymerase Chain Reaction) was used to identify the 5HHT gene polymorphism (insertion/deletion variations). The following primer sequences were used: F: 5’- ggC gTT gCC gCT gAT gC; R: 5’- gAg ggA CTg AgC Tgg ACA ACC AC. Products were visualised on 3% agarose gel [29].
Statistical analysis

Statistical analysis was conducted with statistica.pl software (version 5.0.97) licensed by StatSoft, Inc. USA. A comparison of measurable values with normal distribution for three and more groups (a grouping variable has more than two values) was conducted by analysis of variance on ANOVA platform and by Laeven variance analysis of homogeneity. For variables with abnormal distribution, the Kruskal-Wallis test was used.

Discussion

Owing to negligible differences in results obtained from male and female adolescent patients, it was decided that no gender-related analysis would be presented in the paper. Results are presented in tables and as graphs.

Table 1. The number of symptoms necessary to diagnose combined type of ADHD (group A – patients with combined type of ADHD, group B – adolescents early experimenting with alcohol)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number of symptoms necessary to meet ICD-10 criteria</th>
<th>average</th>
<th>standard deviation</th>
<th>standard deviation</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>Group B n=99</td>
<td>Group B n=51</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N_AD_S</td>
<td>6-9</td>
<td>8.11111</td>
<td>5.78431</td>
<td>0.935566</td>
<td>2.48446</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>N_H_S</td>
<td>3-5</td>
<td>4.38384</td>
<td>3.09804</td>
<td>0.650072</td>
<td>1.45952</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p &lt; 0.001</td>
</tr>
<tr>
<td>N_I_S</td>
<td>1-4</td>
<td>3.59596</td>
<td>2.72549</td>
<td>0.698675</td>
<td>1.38677</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>p &lt; 0.005</td>
</tr>
</tbody>
</table>

N_AD_S (number of attention deficit symptoms); N_H_S (number of hyperactivity symptoms); N_I_S (number of impulsive symptoms)

Table 2. A comparison of average number of symptoms of combined type of ADHD between group A (patients with hyperkinetic disorder) and group C (teenagers with combined type of ADHD singled out from adolescents early experimenting with alcohol)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Number of symptoms necessary to meet ICD-10 criteria</th>
<th>average</th>
<th>standard deviation</th>
<th>standard deviation</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>Group C n=32</td>
<td>Group C n=32</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N_AD_S</td>
<td>6-9</td>
<td>8.11111</td>
<td>7.43750</td>
<td>0.935566</td>
<td>1.04534</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td>N_H_S</td>
<td>3-5</td>
<td>4.38384</td>
<td>3.96875</td>
<td>0.650072</td>
<td>0.78224</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ns</td>
</tr>
<tr>
<td>N_I_S</td>
<td>1-4</td>
<td>3.59596</td>
<td>3.34375</td>
<td>0.698675</td>
<td>1.03517</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ns</td>
</tr>
</tbody>
</table>

N_AD_S (number of attention deficit symptoms); N_H_S (number of hyperactivity symptoms); N_I_S (number of impulsive symptoms)

Although the number of symptoms required to diagnose hyperkinetic disorder was statistically significant (Table 1), 63% of adolescents youth drinking fulfilled
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the criteria of combined type of ADHD (Graph 1). Given this kind of disorder distribution, a third group of adolescents was defined for further analysis – group C (teenagers with combined type of ADHD singled out from adolescents early experimenting with alcohol). Table 2 presents a comparison of the number of symptoms required to diagnose hyperkinetic disorder in groups A and C. The results are not statistically significant.

Graph 1. Disorder distribution in investigated adolescents

Whereas 12-13-year-old adolescents in Poland are typically pupils in the 6th form of a primary school, adolescents who are 14-15 years of age attend the 2nd form of a junior high school (middle school). According to ESPAD (The European School Survey Project on Alcohol and Other Drugs) survey data collected from young people until the 2nd form of a junior high school, adolescent alcohol initiation in Poland typically occurs in the 2nd form of a junior high school (middle school). Our study showed that 5% of ADHD patients confirmed they had consumed alcohol. Since the average age of adolescents with hyperkinetic disorder was lower, it is possible they had not gone through alcohol initiation yet [30, 31].

Researchers suggest that adolescents with hyperkinetic disorder start alcohol experiments by approximately 1.5 years earlier than their peers in the control group [32]. Attention deficit disorder symptoms and hyperactivity are the most significant predictors of subsequent alcohol-related crime [33]. Patients with primary ADHD have shorter transition periods from adverse substance experiences to addiction [10-12]. Analysis of results obtained in the present study demonstrates that intensive preventive measures geared at stopping adolescents with combined type of ADHD from conducting alcohol experiments should be undertaken by the 5th form of primary school at the latest.
Table 3. A comparison of average severity of (combined type) ADHD symptoms between group A (patients with combined type of ADHD), group B (adolescents early experimenting with alcohol) and group C (teenagers with combined type of ADHD singled out from adolescents early experimenting with alcohol)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Maximum severity of combined type ADHD symptoms</th>
<th>average</th>
<th>average</th>
<th>average</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>S_AD_S</td>
<td>27</td>
<td>22.54545</td>
<td>16.01961</td>
<td>19.59375</td>
<td>0.00001</td>
</tr>
<tr>
<td>N_H_S</td>
<td>15</td>
<td>11.84849</td>
<td>8.39216</td>
<td>10.59375</td>
<td>0.0031</td>
</tr>
<tr>
<td>S_I_S</td>
<td>12</td>
<td>10.17172</td>
<td>7.64706</td>
<td>9.40625</td>
<td>ns</td>
</tr>
</tbody>
</table>

S_AD_S (severity of attention deficit symptoms); N_H_S (number of hyperactivity symptoms); S_I_S (severity of impulsive symptoms)

Graph 2. A comparison of impulsive symptom severity compared to maximum scores in group A (patients with combined type of ADHD), group B (adolescents early experimenting with alcohol) and group C (teenagers with combined type of ADHD singled out from adolescents early experimenting with alcohol)

Meta-analysis of different effects of genetic and environmental factors on ADHD symptoms (attention deficit disorders, hyperactivity/impulsiveness disorders) revealed that both dimensions, i.e. attention deficit disorders as well as hyperactivity/impulsiveness disorders, are hereditary (71% – attention deficit disorders; 73% – hyperactivity/impulsiveness disorders). Interestingly, they probably follow different mechanisms of hereditary pathways [17]. In Sonuga-Brake’s model of delay aversion, attention deficit is associated with executive function deficit disorders (anatomically associated with the prefrontal cortex). Hyperactivity is more connected with the problems
of motivation and a way to respond to the prize (anatomically related to striatum and nucleus accumbens which are thought to be critical components of the reward system) [17, 21].

The examination of patients early experimenting with alcohol seems to implicate early alcohol initiation with impulsiveness, poor emotion regulation skill, tendency to externalised behaviour and potential development of dissocial personality. Neurobiological research shows that those at risk of early alcohol initiation, e.g. due to family history of alcohol abuse, have reduced volume of prefrontal cortex, which is also involved in mediating emotional behavior [34].

In the present study, the number of impulsive symptoms necessary to fulfil diagnostic criteria was statistically different only between group A (patients diagnosed with combined type of ADHD) and B (adolescents early experimenting with alcohol) p=0.005. Groups A and C (teenagers with combined type of ADHD singled out from adolescents early experimenting with alcohol) did not statistically differed in this respect (Tables 1 and 2). High impulsivity scores of 75% (relative to the possibly highest score in a symptom rating scale) were recorded in all the sample. In groups A, B and C the severity of impulsivity was at a similar level and no statistically significant differences were found (Table 3). Detailed analysis revealed the highest severity of impulsive symptoms in group A, compared to group B. A comparison of group A vs group C did not find any significant differences (Graph 2). It is fair to state that group B adolescents experimenting with alcohol who did not meet diagnostic criteria for hyperkinetic disorder displayed lower impulsivity than ADHD teenagers and those who met (combined type) ADHD criteria singled out from adolescents who early experimented with alcohol (group C).

Table 4. Frequency distribution of genotypes and alleles of the 5HTT gene in group A (patients with combined type of ADHD), group B (adolescents early experimenting with alcohol) and group C (teenagers with combined type of ADHD singled out from adolescents early experimenting with alcohol) compared to population-based group (PBG)

<table>
<thead>
<tr>
<th>Group</th>
<th>Genotypes</th>
<th>Alleles</th>
<th>p</th>
<th>Genotypes</th>
<th>Alleles</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>p</td>
<td></td>
<td>n</td>
<td>p</td>
<td></td>
</tr>
<tr>
<td></td>
<td>l/l (%)</td>
<td></td>
<td></td>
<td>L (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>l/s (%)</td>
<td></td>
<td></td>
<td>s (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>s/s (%)</td>
<td></td>
<td></td>
<td>ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PBG</td>
<td>100</td>
<td>48 (48)</td>
<td>0.043</td>
<td>200</td>
<td>139 (69.5)</td>
<td>0.01</td>
</tr>
<tr>
<td>A+B+C</td>
<td>175</td>
<td>56 (32)</td>
<td>0.00475</td>
<td>300</td>
<td>206 (58.9)</td>
<td>47 (48)</td>
</tr>
<tr>
<td>A</td>
<td>96</td>
<td>39 (40.6)</td>
<td>ns</td>
<td>192</td>
<td>124 (64.6)</td>
<td>68 (35.4)</td>
</tr>
<tr>
<td>B</td>
<td>49</td>
<td>10 (20.4)</td>
<td>0.00475</td>
<td>98</td>
<td>51 (52)</td>
<td>47 (48)</td>
</tr>
<tr>
<td>C</td>
<td>30</td>
<td>7 (23.3)</td>
<td>0.035</td>
<td>60</td>
<td>31 (51.7)</td>
<td>29 (48.30)</td>
</tr>
</tbody>
</table>
Associations between a polymorphism of the serotonin transporter (5HTT) gene and impulsive behaviour and early experiments with alcohol by adolescents were discussed in the introduction. It is worth mentioning a study conducted by Retz who found that in a population of young offenders the 5HTT gene polymorphism was associated with ADHD [35]. Regression analysis for Retz study revealed an association between ADHD that survived into adulthood and HTTLPR l/l genotype. Additionally, individuals with the short “s” allele were more susceptible to environmental factors [19]. A difference of polymorphic variants of the 5HTT gene was observed in the present study between the population-based group and the whole sample for the “s” allele in s/s and l/s combinations. This distribution of the polymorphism may suggest its pleiotropic character. The short “s” allele of the 5HTT gene is clinically associated with increased severity of impulsive and risk-seeking behaviour. The severity of impulsive behaviour was substantially high throughout all the groups of our sample. l/s and s/s genotypes occurred significantly more frequently in all the sample. Genotypes with the short “s” allele in both l/s and s/s forms were more frequent in groups B and C. It was particularly noticeable in group B of adolescents early experimenting with alcohol. ADHD patients had a distribution of “s” and “l” alleles similar to that of the controls (Table 4).

Conclusions

Based on the conducted study, a tentative conclusion can be drawn that the presence of the short “s” allele of the 5HTT gene polymorphism may be a prognostic predictor of impulsive behaviour in ADHD and may be implicated in susceptibility to alcohol dependence.
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References


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