PAX-6 gene promoter polymorphism and other factors involved in brain atrophy in alcohol dependent patients.

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Summary
Aim. To find a correlation between PAX-6 promoter polymorphism and other parameters pertaining to addictions like e.g. manner of drinking, proneness to faster cerebral and cerebellar atrophy.

Material and method. In a group of 68 alcohol dependent subjects the history of alcohol dependence was assessed using a composite interview, PAX–6 gene promoter polymorphism was studied, and the occurrence of the processes of cerebral and cerebellar atrophy was evaluated using a CT scan.

Results. A negative correlation between the quantity of (AC)m(AG)n repeats in PAX-6 gene promoter B and the cerebral and cerebellar atrophy was found. The occurrence of atrophy was correlated with a decrease in alcohol tolerance and the presence of withdrawal symptoms complicated by alcohol delirium.

Conclusions. Genetic factors, especially a polymorphism in the region of PAX-6 gene promoter B, can be considered as one of the essential causes of a tendency of brain atrophy in patients with alcohol dependence. Brain atrophies lead to complicated deep withdrawal syndromes and are related to a decreased alcohol tolerance.

alcohol / cerebral and cerebellar atrophy / PAX – 6 gene

INTRODUCTION

Besides social and psychological consequences, alcohol dependency also results in somatic effects [1]. The concept of alcohol induced brain damage contains, among other things cognitive function disorders, Wernicke-Korsakoff syndrome, alcoholic cerebellar degeneration and alcoholic dementia [2]. Neurotoxic effects of alcohol, thiamine and vitamin PP deficiency, liver damage, head injuries, prenatal exposition to alcohol effects – all these play an important part in the process [3, 4, 5]. Skullerud et al. found cerebellar atrophy in 37% of alcoholics who underwent autopsy, although brain damage appeared more often in people living a vagabond life [6]. It was ascertained that brain damage is more intense in people addicted to alcohol with Wernicke-Korsakoff syndrome in comparison to people addicted to alcohol with liver cirrhosis or those without any of these complications. The
degeneration of neurones in the superficial layers of the cerebral cortex occurred in equal intensity in all the three above mentioned groups [4]. The manner of drinking also affects brain damage. In rats a lower brain mass was observed if they were given alcohol at the time of brain development in a pattern that its momentary concentration was higher in those rats even if they drank less alcohol than in the rats in which momentary alcohol concentrations were lower [7].

To understand the essence of alcoholism thoroughly, it is essential to find the genetic basis of the particular neurobiological and psychobiological components of dependence. On the grounds of nuclear structure, localisation, and a potential switching the activating processes on and off, it was ascertained that PAX genes are the factors controlling the development of a foetus, and they especially participate in proper regionalisation of the brain. Their expression starts from the eighth day after fertilisation [8]. They participate in the origination of precursor lines of neuronal cells and in their further specialisation. As hybridisation in situ demonstrated, in adults the regional distribution of PAX gene transcripts remains unaltered and influences neuroplasticity, neurodegenerative and neuroreparative processes [9, 10, 11].

In the study the PAX-6 gene, which contains 14 axons and is located in the human chromosome 11p13, and is probably connected to alcohol dependency, was chosen [12]. There are loci for the genes of the D4 receptor and tyrosine hydroxylase in PAX-6. Moreover, PAX-6 plays an important part in the development of the limbic system and in the process of migration and differentiation of those cerebral cortical neurones which originated lately [13]. PAX-6 influences development of dopaminergic neurones, i.e. the systems playing a vital role in the development of dependence through the award effect [10]. Nearly all of the PAX-6 mutations cause a loss of alleles’ functions resulting in e.g. absence of the iris in humans, and in the phenotype of the Small eye in mice [9]. Substitutions of nucleotides in a region of axons cause origination of 80% of nonsensical codons (80% of the codons become nonsensical) [9]. The transcription of PAX-6 is regulated by 2 separate promoters named A and B, although the transcription in the mature brain is controlled mostly by promoter B. Moreover, a sequence of repetitions of polymorphic dinucleotides having a structure (AC)m (AG)n is localised approximately 1 kb in the opposite direction from the place of transcription initiation, relating to promoter B [14]. The existence of approximately 80% of alleles with 24-28 repetitions has been found. The activity of the promoter with the variant having 29 or more repetitions is 4 to 9 times higher than the activity of the allele having 26 repetitions (‘short alleles’). The amount of mRNA was two times bigger in people with long alleles than in those with short alleles [15].

The aim of the present study was to find a correlation between PAX-6 promoter polymorphism and other parameters pertaining to addictions like e.g. manner of drinking, proneness to faster cerebral and cerebellar atrophy.

MATERIAL AND METHOD

The course of the study obtained an approval of the Ethical Commission in Rudolf Virchow Clinical Hospital of Freie Universitat in Berlin. The participants expressed their informed consent after they received information in writing about the course of the study and its aim.

68 alcohol addicted patients, German nationality, among them 59 males (at the age of 41.2 ± 9.5) and 9 females (aged 38.3 ± 8.9) participated in the study. They fulfilled the ICD-10 criteria of alcohol dependency. The participants were recruited from the patients of the Psychiatric Clinic of Freie Universitat in Berlin. The histories of addiction were assessed using a composite interview according to the Composite International Diagnostic Interview (CIDI), the intensity of withdrawal symptoms by the CIWA-R scale. In addition, internal medicine and psychiatric examinations were conducted. Dependence on substances other than alcohol or nicotine and a serious psychiatric illness diagnosed in the interview excluded the patients from the study. Moreover, the groups of people having alcohol withdrawal syndrome complicated by convulsions or delirium were separated.

The people qualified for the study were subjected to a CT scan of the cerebrum and cerebellum which was analysed by two independent radiologists from the Radiology Clinic of Ben...
Genetic and other factors involved in brain atrophy in alcohol dependent patients

DNA preparation

PCR was proceeded in a total volume of 25 µl containing 60 ng of genomic DNA, 10 pM of every particular primer, 200 µM of every dNTP, 2 µCi (α-32P)-dCTP (10 mCi/ml, 3000 Ci/mmol), 1.5 mM MgCl2 75 mM Tris – HCl; pH 9.0 20 mM (NH4)2SO4, 0.01 % Tween 20 and 0.5 U of Taq DNA polymerase (Eurogentec).

The primers:
P6repU (sense): 5'-CTC CGT GGA CTG AGA AGA C-3'
P6repD (antisense): 5'-GGA TGA CCA ATG CTG GGA – 3'

PCR conditions

Temperature of 95ºC for 3 min + [94ºC for 40s (denaturation) + 56ºC for 30s (annealing) + 72ºC for 1 min (elongation)] by 35 cycles, final elongation for 5 min at 72ºC. 8µl of terminating solution (consisted of 97% deionised formamid; 10 mM EDTA: pH 7.5; 0.03% bromphenol blue, 0.003% xylencyanol FF) were added to 2µl of PCR product. The mixture was heated up to 90ºC for 3 min, then cooled in ice and placed on 6% polyacrilomadic gel where fragments of alleles marked by radioactive phosphor 32P were separated. The electrophoresis was performed for 2.5 h by 65 W, and afterwards the dry gel was stored in a cassette containing a film and kept for 12 to 48 h in a refrigerator at the temperature of -70 ºC.

Statistical analysis

Spearman’s rang test and Mann-Whitney’s test were applied in order to evaluate the correlation between PAX-6 gene promoter polymorphism and the parameters characterising the group of alcohol dependent people with a tendency to brain atrophy.

RESULTS

In the obtained results the Hardy-Weinberg equation was satisfied. The frequency of patients’ alleles and genotypes is demonstrated in a study published by the present authors in Addiction Biology [17].

In Table 1, the analysis of the correlation between cerebral atrophy and the quantity of repetitions in PAX-6 gene promoter is shown. Table 2 shows the correlation between changes in various brain regions and clinical features such as a decrease of alcohol toleration, the quantity of alcohol drunk in 24-hours, history of head injuries, alcohol epilepsy, alcohol withdrawal, pal-

Table 1. The analysis of interdependence between the atrophy in cerebrum and the quantity of repetitions (from 24 to 36 dinucleotide repetitions) in promoter B of PAX-6 gene in 68 patients. Correlation coefficients are presented in the table.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PAX6-1</th>
<th>PAX6-2</th>
<th>PAX6 carr</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral atrophy (u: n = 63)</td>
<td>-0.396</td>
<td>-0.333</td>
<td>-0.425</td>
</tr>
<tr>
<td></td>
<td>p = 0.001*</td>
<td>p = 0.005*</td>
<td>p &lt; 0.001*</td>
</tr>
<tr>
<td>Atrophy in frontal region (u: n = 59)</td>
<td>0.028</td>
<td>-0.066</td>
<td>-0.129</td>
</tr>
<tr>
<td></td>
<td>p = 0.821</td>
<td>p = 0.595</td>
<td>p = 0.293</td>
</tr>
<tr>
<td>Atrophy in occipital region (u: n = 13)</td>
<td>0.092</td>
<td>0.02</td>
<td>0.039</td>
</tr>
<tr>
<td></td>
<td>p = 0.455</td>
<td>p = 0.871</td>
<td>p = 0.75</td>
</tr>
<tr>
<td>Atrophy in parietal region (u: n = 59)</td>
<td>0.036</td>
<td>-0.005</td>
<td>-0.052</td>
</tr>
<tr>
<td></td>
<td>p = 0.773</td>
<td>p = 0.966</td>
<td>p = 0.675</td>
</tr>
<tr>
<td>Atrophy in temporal region (u: n = 44)</td>
<td>0.047</td>
<td>-0.062</td>
<td>-0.062</td>
</tr>
<tr>
<td></td>
<td>p = 0.704</td>
<td>p = 0.614</td>
<td>p = 0.614</td>
</tr>
<tr>
<td>Cerebellar atrophy (u: n = 27)</td>
<td>-0.329</td>
<td>-0.429</td>
<td>-0.550</td>
</tr>
<tr>
<td></td>
<td>p = 0.006*</td>
<td>p &lt; 0.001*</td>
<td>p &lt; 0.001*</td>
</tr>
</tbody>
</table>

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In the studied group of 68 ethanol dependent people, an atrophy in various brain parts was found in 92.6 % of the patients. The percentage is higher than in the studies of Skullerud and et. [6], where a different method of evaluation was applied and moreover his studies referred to another population.

The obtained results imply that there is an inversely proportional correlation between the quantity of repeats in the PAX-6 promoter gene and the presence of cerebral and cerebellar atrophy. It can be related to the influence this gene has on neuroplastic processes (degeneration and regeneration) in the central nervous system. The expression of a gene containing at least 29 dinucleotide repeats (AC)m (AG)n is approximately 2-3 times bigger in humans, as it was demonstrated in the studies conducted by Okladnova et al. [15, 18]. This may explain a probably higher regenerative ability of the brain in addicted people having a version of the gene that contains longer, especially ≥ 29 of (AC)m (AG)n repeats in the promoter B of the gene PAX-6 [15].

In our own research we found no correlation between the polymorphism of PAX-6 gene promoter and alcohol dependence syndrome (ADS), even in precisely defined subgroups of patients with alcohol dependence, like those having hereditary transmission of alcoholism, early (< 26 y.o.) onset, withdrawal syndrome complications in their history – alcohol seizures or alcohol de-
lirium or in those with ADS and dissocial personality [17, 18].

In spite of our expectations, no correlation was found between numerous factors which according to data from the literature or intuitive anticipation should have an influence on cerebral atrophy processes [5, 6]. Certainly, the data obtained from the interview should be treated cautiously. However, it should be emphasised that in the studied groups of patients with ADS the correlation between brain atrophy and the clinical factors characterising dependency has not been found; only the most important factors were considered in this study.

The data shown in Table 2 should be treated as information about the effects but not about the causes of atrophy. These effects are very important because they include a decrease in alcohol toleration and the presence of withdrawal symptoms including alcohol delirium.

The correlation between alcohol delirium presence and the atrophy in a given brain region seems to be very evident. Following on from this conclusion atrophy should be treated as one of the risk factors for the appearance of alcohol delirium.

CONCLUSIONS

Genetic factors, especially a polymorphism in the region of PAX-6 gene promoter B, can be considered as one of the essential causes of a tendency of brain atrophy in patients with alcohol dependence.

Brain atrophies lead to complicated deep withdrawal syndromes and are related to a decreased alcohol tolerance.

REFERENCES
