Research article

Association between oxytocin and receptor genetic polymorphisms and aggression in a northern Chinese Han population with alcohol dependence

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HIGHLIGHTS

- Rs6133010 in the OXTR gene was associated with AD related aggression in northern Chinese Han population.
- The genotype GG of rs6133010 carriers in AD group had significant anger aggression.
- The 3-loci interaction combination of rs6133010AG, rs2254298AG and rs53576AA increases risk for AD.

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ABSTRACT

Objective: Alcohol dependence (AD) is a common chronic brain disorder precipitated by complex interactions between biological, genetic, and environmental risk factors. Aggression often occurs in the context of AD. Previous studies have shown that Oxytocin (OXT) and OXT receptor (OXTR) are involved in the regulation of aggression. The present study investigated whether variations and interactions of OXT and OXTR genes were associated with AD-related aggression in a genetically homogeneous northern Chinese Han population.

Methods: Three hundred and twenty-four male AD patients and 510 male healthy controls (HCs) were recruited. A Chinese version of the Buss-Perry Aggression Questionnaire was used as a subjective measurement of aggressive behavior. Three variations, rs2254298, rs53576, and rs6133010 were genotyped using TaqMan and ligase detection reaction for all subjects. Generalized Multifactor Dimensionality Reduction was used to detect interactions between genetic attributes and environmental attributes.

Results: The frequencies of alleles and genotypes of rs6133010 were significantly different between AD patients and HCs (p<0.001). In HCs, the effect of genotype GG of rs53576 on hostility aggression was significantly stronger than that of genotype AA and AG (p = 0.001 and p = 0.004, respectively), and the subjects with the interaction combination of rs6133010AA × rs2254298GG × rs53576AG exhibited significant effect on physical aggression (p = 0.0107).

Abbreviation: OXT, oxytocin; OXTR, oxytocin receptor; CNS, central nervous system; SNP, single nucleotide polymorphism; AD, alcohol dependence; HCs, healthy controls; GMDR, Generalized Multifactor Dimensionality Reduction; BPAQ, the Buss-Perry Aggression Questionnaire.

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1. Introduction

Alcohol dependence (AD) is a condition which involves prolonged alcohol craving caused by long-term, frequent drinking [39]. This diagnosis is used to describe an individual that is physically or psychologically dependent upon drinking alcohol. A previous study identified a link between aggression and alcohol use [22], and AD was found to be correlated with aggression [18]. Alcohol-related aggression shows large interindividual differences [4,20]. These results support the hypothesis that genetic variants may play an important effect in AD-related aggression.

Oxytocin (OXT) is synthesized in the hypothalamus [12] and plays a prosocial role in human and animal behavior [2], including maternal behavior, sexual behavior, social behavior, social recognition and social contact [36]. OXT has been correlated with brain reward system regulation [35]. A few recent studies have suggested that OXT decreases alcohol use in rats and reduces symptoms of alcohol withdrawal and craving behavior in dependent patients during abstinence [6-7,38]. Cerebrospinal fluid OXT levels were inversely correlated with life history of aggression [27]. A previous study found that OXT administration leads to a broad range of changes in aggressive response, as measured by the point subtraction aggression paradigm [1]. The central actions of OXT are mediated via oxytocin receptors (OXTRs) that are found in the regions of the mammalian brain associated with the reward system [3]. OXTRs were visualized in some regions of the amygdala and hypothalamus, the limbic and basal forebrain, olfactory nucleus and the anterior cingulate, the part of the brain that includes reward memory [5,16]. Peripheral injections of an OXTR antagonist significantly reduce male-male and female-female aggression in a highly territorial finch, providing evidence that endogenous activation of OXTR promotes resident-intruder aggression [17]. Allelic variability of OXT pathway also impacts human social functions [15]. The polymorphisms of the OXT/R genes have been correlated with the modulation of social cognitive behavior [11].

The OXT gene is located on chromosome 20p13. Three polymorphisms were chosen from dbSNP (http://www.ncbi.nlm.nih.gov/SNP/): Rs6133010 is located in the OXT promoter region [45]. The association between OXTR variants and childhood-onset aggression was found in a study with highly aggressive children [31]. The OXTR gene is located on chromosome 3p25 and has 3 exons and 3 introns [23]. Two single nucleotide polymorphisms (SNPs) located in intron 3 of the OXTR gene, rs2254298 and rs53576, were found to be related to individual differences in empathy and prosocial behaviors [9,14]. It has been reported that rs2254298 is associated with sociability, amygdala volume and differential risk for psychiatric conditions including autism, depression and anxiety disorder, depending on the quality of early environmental experiences [9]. The SNP rs53576 is involved in differences of oxytocinergic functioning [34]. The rs53576 GG genotype has been found to be associated with general social phenotypes, psychological resources, higher empathy lower stress reactivity and a prosocial temperament [44]. In addition, there is evidence that the rs53576G allele may contribute to the risk of emotional and behavioral problems by gene environment interaction [8,23].

Notably, little studies have been performed on the roles of OXT/R genetic polymorphisms in AD-related aggression. Taken together, the association of variations and interactions of OXT/R genes with AD-related aggression warrants further investigation. Therefore, the aim of this present study was to investigate the association between these 3 loci (rs2254298, rs53576 and rs6133010) and their interactions in AD-related aggression in a Chinese Han male population.

2. Experimental procedures

2.1. Subjects

A total of 324 male AD inpatients were recruited from the Psychiatric Hospitals in northern China. All of the patients met criteria for AD according to the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV). Participants without a history of other drug abuse or dependence, with the exception of nicotine, were included.

Five hundred and ten unrelated male healthy controls (HCs) from Inner Mongolia Autonomous Region were recruited. All of the HCs lacked a history of drug abuse or dependence, with the exception of nicotine. All participants with serious liver or kidney disease, or participants or first-degree relatives of participants that have serious mental illness, were excluded. Two skillful psychiatrists conducted the diagnoses. All of the staff were trained before starting this study.

2.2. Measurement of aggression

A Chinese version of the Buss-Perry Aggression Questionnaire (BPAQ) was used to measure aggressive behavior [10]. All of the subjects described their own patterns of aggression-related behavior, emotions, and attitudes by self-reporting [46]. The revised Chinese version 30-item BPAQ provides 5-subscale scores measuring physical aggression, verbal aggression, anger, hostility and indirect aggression [1]. Informed written consent was obtained from all subjects before study participation. The BPAQ of patients was assessed in one week for the case of emotional stability. This study was approved by the Peking University Institutional Review Board.

2.3. Genotyping

A salting-out method to extract genomic DNA with 5 ml peripheral blood was used [43]. Genotyping was performed for rs2254298, rs53576 and rs6133010 in a total of 834 subjects. The 3 SNPs in the OXT/R genes were genotyped using the 5’nuclease fluorescent TaqMan™ primer (Applied Biosystems, Foster City, CA). The protocol was performed in accordance with manufacturer’s instructions. All of the laboratory procedures were carried out in a blind manner to case control status. The conditions of PCR were as follows: 50 °C for 2 min, 95 °C for 10 min, followed by 50 cycles of 95 °C for 15 s and 60 °C for 1 min Ten percent of the DNA samples were duplicated randomly and tested, and no fault genotyping was found.
Table 1
The difference of age, years of education, and aggression between the two groups.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>AD</th>
<th>HCs</th>
<th>Age</th>
<th>Years of education</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Mean ± SD</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>Age</td>
<td>45.94 ± 9.17</td>
<td>37.66 ± 9.13</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Education years</td>
<td>10.33 ± 2.79</td>
<td>11.94 ± 2.57</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sum of aggression</td>
<td>39.28 ± 16.70</td>
<td>27.59 ± 14.70</td>
<td>54.69</td>
<td>0.000</td>
</tr>
<tr>
<td>Physical aggression</td>
<td>37.58 ± 21.09</td>
<td>27.95 ± 17.86</td>
<td>57.10</td>
<td>0.000</td>
</tr>
<tr>
<td>Verbal aggression</td>
<td>38.42 ± 19.54</td>
<td>27.89 ± 17.42</td>
<td>39.26</td>
<td>0.000</td>
</tr>
<tr>
<td>Anger aggression</td>
<td>40.71 ± 23.75</td>
<td>25.42 ± 18.30</td>
<td>64.59</td>
<td>0.000</td>
</tr>
<tr>
<td>Hostility aggression</td>
<td>31.13 ± 19.45</td>
<td>20.92 ± 15.46</td>
<td>48.21</td>
<td>0.000</td>
</tr>
<tr>
<td>Self-aggression</td>
<td>32.68 ± 20.57</td>
<td>20.90 ± 16.05</td>
<td>53.52</td>
<td>0.000</td>
</tr>
</tbody>
</table>

AD: alcohol dependence. HCs: healthy controls. Age and years of education as covariance, were separately analyzed for differences using ANCOVA. p < 0.05.

Table 2
HWE for rs2254298, rs53576, and rs6133010 for the two groups.

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Gene</th>
<th>Allele</th>
<th>AD HWE p</th>
<th>HCs HWE p</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2254298</td>
<td>OXTR</td>
<td>A/G</td>
<td>0.602</td>
<td>0.132</td>
</tr>
<tr>
<td>rs53576</td>
<td></td>
<td>A/G</td>
<td>0.491</td>
<td>0.07</td>
</tr>
<tr>
<td>rs6133010</td>
<td>OXTR</td>
<td>A/G</td>
<td>1.89e-015</td>
<td>0.23</td>
</tr>
</tbody>
</table>

HWE: Hardy-Weinberg equilibrium, p < 0.05.

2.4. Statistical analysis

Hardy-Weinberg equilibrium (HWE) test, linkage disequilibrium (LD) and the frequencies of the distribution of genotypes and alleles in both AD and HCs were analyzed using SHEsis platform (http://analysis.bio-x.cn/myAnalysis.php) [41]. Generalized Multifactor Dimensionality Reduction (GMDR) is a nonparametric platform and a genetic model-free alternative to logistic regression to detect interaction between discrete genetic attributes and environmental attributes and is used to calculate the interaction of 3 loci [30]. Analysis of Covariance (ANCOVA) or t-test for continuous variables was used to test group differences. The p-values were performed for interaction models based on 1000 permutations. Statistical significance was defined at the 0.05 level after the p value was adjusted using Bonferroni's correction for multiple tests.

3. Results

3.1. General characteristics of all subjects

The ages of the AD patients and HCs ranged from 23 to 67 years and from 19 to 63 years, respectively (mean of 45.94 ± 9.17 and 37.66 ± 9.13 years, respectively). The years of education of the AD patients and HCs were from 5 to 17 and from 5 to 18, respectively (10.33 ± 2.79 and 11.94 ± 2.57 years, respectively). The ages and years of education were both significantly different for the two groups as determined by t-test (both p < 0.001). There were significant differences in aggression for the total score and five sub-scales between the two groups, with age and years of education as covariance, respectively (all p < 0.001; Table 1).

3.2. Single polymorphism analyses

The HWE calculation of the 3 SNPs for the two groups is shown in Table 2. The subjects did not show a departure from HWE for the three polymorphisms except rs6133010 in the AD group. The distributions of genotypes and alleles of the three variations are shown in Table 3, respectively. There were significant differences in the distributions of alleles and genotypes of rs6133010 for the two groups after Bonferroni's correction (both p < 0.001). There was no difference in the distributions of alleles and genotypes of rs2254298 and rs53576 between two groups (both p > 0.05). The linkage disequilibrium of rs2254298 and rs53576 between the two groups is D' = 0.409, r² = 0.030 and D' = 0.588, r² = 0.060. However, there was no haplotype of the OXTR loci was associated with AD (p > 0.05). In the HCs group, the effect of the genotype GG of rs53576 on hostility aggression was significantly stronger than that of genotype AA and AG (p = 0.001 and p = 0.004, respectively).

3.3. Gene-gene interaction analyses

Control subjects who carried the interaction combination of rs6133010AA × rs2254298GG × rs53576AG showed more physical aggression (p = 0.0107; shown in Fig. 1). The specific interaction combination for alcoholics was not identified.

4. Discussion

In the present study, we investigated the distributions of the frequencies of three loci in a group of northern Chinese Han AD patients and HCs and our data led to the following conclusions: (1) the frequency differences of the allele G and genotype GG of rs6133010 between the AD patients and HCs were significant; (2) in HCs, the effect of genotype GG of rs53576 on hostility aggression was significantly stronger than that of genotypes AA and AG, and the interaction of rs6133010AA × rs2254298GG × rs53576AG showed a significant correlation with physical aggression.

This study showed a departure from HWE for rs6133010 in the AD group, suggesting possible association with the development of AD. Rs6133010 is located on the OXT promoter region and might affect the expression level of proteins [42]. To further explore the association between aggression, AD and OXT/R genes, Ensembl [43] was used to predict the function of 3 loci. Ensembl is a genome browser for vertebrate genomes that provides gene annotation, computes multiple alignments, predicts regulatory function, and compiles disease data. Ensembl tools include BLAST, BLAT, BioMart and the Variant Effect Predictor for supported species (http://asia.ensembl.org/index.html). Ensembl was used to identify SNPs in other gene transcriptional regulatory regions [20]. Rs6133010 is located in a regulatory region that is the CCCTC-binding factor (CTCF) binding site and the G allele inactivates the expression in the cell lines. The previous study suggested the use of intranasal oxytocin as a potential treatment for drug addiction, including for cocaine, opiates, alcohol and cannabis [32]. Rs2254298 is located in an enhancer region in which the A variant inactivates the expression in the cell lines. The earlier study suggested that central administration of OXT inhibits the development of rapid tolerance to ethanol [24]. Therefore, the genotype GG of rs6133010 was associated with AD, in agreement with the previous study.

A previous study demonstrated that participants carrying the rs53576 AA/AG genotype showed lower trust behavior than the GG genotype carriers; and the AA/AG genotype carriers had lower dispositional empathy than the GG genotype group [25]. Another meta-analysis on the association of rs53576 with sociality
revealed that G allele homozygotes were generally more sociable than A allele carriers, but these results did not include Chinese data [28]. However, the genotypic frequencies of AA and GG are basically opposite for Chinese and Europeans/Caucasians based on dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/snref.cgi?rs=53576). This may help explain our result that the effect of genotype GG of rs53576 on hostility aggression was significantly stronger than that of genotype AA and AG.

The previous results of the association between OXT/R and aggression are not consistent. OXT was repeatedly found to be associated with different types of aggression in rodent models [40]. Low OXT level in cerebrospinal fluid may be associated with a higher level of expressed aggressive behaviors during the lifetime in humans [21]. However, the real function of rs6133010 AA might increase OXT level as predicted by the online platform EnsemblHSA (http://asia.ensembl.org/index.html), and rs6133010 might play a role in regulating aggression. The carriers who were homozygous for rs2254298G exhibited the smallest amygdala volume [19] and were over-represented for depression [13]. Both the frequencies of genotype GG and allele G of rs2254298 have been associated with major depression [33]. In addition, a lower amygdala volume in men is associated with childhood aggression and future violence [37]; participants carrying the rs53576 AA/AG genotype showed lower trust behavior than did the GG genotype carriers [25], which might indicate that the interaction of rs6133010AA × rs2254298GG × rs53576AG had a significant effect on physical aggression in the HCs group.

Some limitations of the present study should be noted. First, the age and the years of education were not well-matched between the HCs and AD subjects. These discrepancies may cause the observed differences of aggression. Generally for male drinkers, daily alcohol intake increases with age but declines in those with more education [29]. Second, only one polymorphism of OXT gene was genotyped, which might not comprehensively evaluate the genetic information. More variations of these two genes, including tags and functional variations for the northern Chinese population should be genotyped to obtain more information.

To our knowledge, this present study is the first report of OXT genetic polymorphism showing that the G allele of rs6133010 might correlate with susceptibility for AD, and provided clear evidence that the genotype AA of rs6133010 in normal people is related to aggression in the northern Han Chinese population. These results implied that the polymorphisms of OXT and receptor genes might play a key role in AD and HCs related aggression in northern Chinese. More subjects and loci of OXT/R must be recruited and genotyped to confirm the results for further studies.

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**Table 3**
The frequencies of genotypic and allelic distributions of rs2254298, rs53576 and rs6133010 in the two groups.

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Subjects</th>
<th>Genotype frequency (%)</th>
<th>p</th>
<th>Allele frequency (%)</th>
<th>p</th>
<th>OR(95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>AA</td>
<td>AG</td>
<td>GG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs2254298</td>
<td>AD</td>
<td>26(0.883)</td>
<td>135(0.431)</td>
<td>152(0.486)</td>
<td>0.844</td>
<td>187(0.299)</td>
</tr>
<tr>
<td></td>
<td>HCs</td>
<td>40(0.797)</td>
<td>230(0.452)</td>
<td>239(0.470)</td>
<td></td>
<td>310(0.305)</td>
</tr>
<tr>
<td>rs53576</td>
<td>AD</td>
<td>136(0.487)</td>
<td>121(0.434)</td>
<td>22(0.079)</td>
<td></td>
<td>393(0.704)</td>
</tr>
<tr>
<td></td>
<td>HCs</td>
<td>270(0.530)</td>
<td>188(0.369)</td>
<td>51(0.100)</td>
<td>0.180</td>
<td>728(0.715)</td>
</tr>
<tr>
<td>rs6133010</td>
<td>AD</td>
<td>191(0.620)</td>
<td>49(0.159)</td>
<td>68(0.221)</td>
<td></td>
<td>431(0.700)</td>
</tr>
<tr>
<td></td>
<td>HCs</td>
<td>396(0.818)</td>
<td>81(0.167)</td>
<td>7(0.014)</td>
<td>0.00e+000</td>
<td>873(0.902)</td>
</tr>
</tbody>
</table>

AD: alcohol dependence. HCs: healthy controls.

* p < 0.05.

![Fig. 1](image-url)