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Aggression and 5HTT polymorphism in females: Study of synchronized swimming and control groups

Olga V. Sysoeva, Natalia V. Maluchenko, Marina A. Timofeeva, Galina V. Portnova, Maria A. Kulikova, Alexandr G. Tonevitsky, Alexey M. Ivanitsky

Abstract

Aggression is a heterogeneous heritable psychological trait, also influenced by environmental factors. Previous studies, mostly conducted on male population, have found some associations of the aggression with the polymorphisms of genes, regulating the activity of serotonin (5-HT) in the brain. However, psychological as well as biochemical manifestations of the aggression are different in males and females. Our study aimed to investigate the association of 5-HTT gene polymorphism with different facets of aggression (BDHI) in females. Two groups: the synchronized swimming and non-athlete control, were examined to study the possible modulation effect of sport on the association between 5-HTT gene polymorphism and aggression. It was found that in both groups the low-active 5-HTT polymorphism (SS) was associated with increased scores on Indirect Hostility scale and decreased scores on Negativism scale, compared to LL genotype. No interaction effect between sport and 5-HTT polymorphism was found. The higher percentage of LS-carriers in the synchronized swimming group compared to the control one was observed. This may be the sign of the importance of LL polymorphism of 5-HTT gene, previously associated with higher resistance to stress factors, for being an athlete, although this result has to be taken cautiously keeping in mind the stratification problem. Synchronized swimmers had lower scores on Assault, Negativism, Irritability and Verbal Hostility compared to age-matched control girls (in general and for each 5-HTT genotype separately), suggesting that they may have more matured emotional system (older control group has also lower scores on these scales).

1. Introduction

Aggression usually refers to behavior that is intended to harm or hurt others. Eight facets of aggression can be estimated with the use of Buss–Durkee Hostility Inventory (BDHI) in humans: Assault (physical violence against others); Indirect Aggression (devious hostility like gossip); Irritability (quick temper, ready to explode at slight provocation); Negativism (usually oppositional behavior against authority, refusing to cooperate); Verbal Aggression (express negative feelings in content and style, e.g., shouting); Resentment (jealousy, anger at the world over mistreatment); Suspicion (projection of hostility into others); Guilt (reflecting the degree of guilt feelings reported by the subject). Some researchers unit these facets in higher order factors, but the results are not congruent with respect to facet loadings.

It is likely, that aggression is a result of biological and environmental factors interactions. Previous twin studies have indicated that aggression is a highly heritable trait. The heritability of aggression has been shown to be between 50% and 75% depending on age and gender of the studied population (Bartels et al., 2003; Eley et al., 1999; Hudziak et al., 2003; van Beijsterveldt et al., 2003). Genes underlying aggression can be found among genes which control different neurotransmitter systems. One of the most researched neurotransmitter systems with regard to the aggression is serotonin system. It was shown that Central Nervous System (CNS) serotonergic activity correlates inversely with human aggressive behavior (Gianmanco et al., 2005). PET study revealed that serotonin transporter (5-HTT) availability was significantly reduced in the anterior cingulate cortex of individuals with impulsive aggression compared with healthy subjects (Frankle et al., 2005). The 5-HTT is responsible for the reuptake of serotonin (5-HT) from the synaptic cleft and determines the magnitude and duration of postsynaptic receptor-mediated signaling (Lesch and Merschdorf, 2000).

The polymorphism in promoter region of 5-HTT gene (SLC6A4) has demonstrated functional significance in coding high (L-allele) and low (S-allele) transporter production. According to classical studies of...
Lesch (Lesch and Merschdorf, 2000) S-allele of 5-HTT is associated with the increased scores of neuroticism (negative emotionality), anxiety, hostility and depression. There are other evidences that polymorphism of gene 5-HTT promoter region is connected with the aggressive behavior (Beitchman et al., 2006; Gerra et al., 2005; Lesch and Merschdorf, 2000; Popova, 2006). It was shown that SS genotype is more frequent among aggressive drug experimenters than among abstinent students (Gerra et al., 2005) and among aggressive children (Beitchman et al., 2006). Aggressiveness (total BDHI) and Novelty Seeking (TQ) was higher for SS compared to LL genotypes (Gerra et al., 2005). Recent study (Pezawas et al., 2005) reported that S-allele carriers had reduced grey matter volume in perigenual cingulate and amygdala, and, moreover, decreased the functional interaction between these regions during processing of fearful stimuli.

Several lines of evidence demonstrate gender-related difference in the 5-HT-system functioning in human and animals (cf. Lesch and Merschdorf 2000). The mean rate of 5-HT synthesis in normal males was found to be 52% higher than in normal females (Nishizawa et al., 1997). Common belief is that generally females are less aggressive than males (Coie and Dodge, 1998). However, some researchers have suggested that women are not necessarily less aggressive, but that they tend to show their aggression in less overt, less physical ways (Bjorkqvist et al., 1994). It is noteworthy, that most genetic association study on aggression did not separate males and females in the analysis and involved mostly males. The genetic association studies on Neuroticism, involving large sample groups, are not conclusive in respect of the sex effect. Recent study reports the significant association of mean scores of Neuroticism with S-allele, but only for male population (Du et al., 2000). Moreover, the tendency in female group was in the opposite direction (Du et al., 2000; Gelernter et al., 1998), although other studies did not find the significant interaction effect between sex and 5-HTT gene polymorphism (Munafo et al., 2004; Willis-Owen et al., 2005). Studies of Middeldorp (Middeldorp et al., 2008, 2006) also suggest that men and women are alike in their symptom profiles for major depression and genes for depression are probably expressed in the same way in the two sexes.

Nonetheless, it is important to examine males and females separately. Our studies investigated the association between different facets of aggression with 5-HTT polymorphism in females.

Our previous study found the association of 5-HTT polymorphism with aggression in synchronized swimmers (Malichenko et al., 2007). It was found that carriers of SS genotype had higher scores on Indirect Hostility and lower on Negativism and Irritability scales compared to carriers of other 5-HTT polymorphisms. The new complex scale of “covert aggression”, characterized by dominance of indirectly or latterly expressed aggression signs, and lacking the direct aggression signs, such as disagreement and irritability, was suggested. It is calculated as the sum of normalized values of Negativism and Irritability with negative sign and Indirect Hostility with positive one. This “covert aggression” scale showed high association with 5-HTT polymorphism in synchronized swimming females.

Current study aims in investigating the differences in BDHI aggression scores between the synchronized swimmers and the control group of females, who is not involved in any professional sport. The association between the polymorphism of 5-HTT gene and the aggression was also examined on this expanded group. The possible modulation effect of sport on this association was also a point of interest. The distribution of 5-HTT gene polymorphism in the two studied groups was examined to shed the light on the possible genetic predisposition to the sport (synchronized swimming).

2. Methods

2.1. Subjects

A total of 166 healthy Caucasian female volunteers (10–26 years old) participated in the study. Sixty two of them were a qualified (from First-class athletes to Master of Sport)1 synchronized swimmers (10–18 years old), recruited from the Moscow Professional sport club for synchronized swimming, uniting promising athletes from all over Moscow. The other 64 volunteers formed an age-match control group (10–18 years old), which were recruited from Moscow schools. There were also 40 volunteers (20–26 years old) recruited from the students, mostly of biological department.

2.2. Study protocol

The volunteers were asked to fill Buss–Durkee Hostility Inventory (BDHI), adapted by Osnitsky (Osnitsky, 1994) to Russian population and the age group (10–26 years old). The questioner contained 75 items unites in 8 scales: Assault, Indirect Hostility, Irritability, Negativism, Suspicion, Resentment, Verbal Hostility and Guilt. The subjects had to make the yes/no judgment if the item corresponds to them or not.

The sportgirls filled the questioner in the middle of the day between the training sessions. Their blood was collected as a part of regular health control procedure. For the age-matched control girls the experimental procedure was a part of a lesson, introducing the biological department. The schoolgirls filled the questionnaire and gave the saliva sample. The older control group (students) came to the experimenters’ room to fill the questionnaire (one room) and to give the blood sample (another room). The filling of the questionnaires were always supervised by the investigators. All participants or their parents gave informed concern after the nature of the study was explained to them. The study was approved by the local Institutional Ethical committee.

2.3. Genotyping

For 5-HTTLPR genotyping, genomic DNA was extracted from venous blood and saliva samples according to standard procedures. Primers 5′-ATGCCAGCACCTAACCCCTAATGT-3′ and 5′-GACCGCAAGGTGGCCGGA-3′ were used to amplify a product that was 256 base pair (bp) product for the 14-repeat (s) allele and a 300 bp product for the 16-repeat (l) allele. Amplification reactions were performed in a total volume of 25 μL, containing approximately 100 ng of genomic template, 10 pmol of each primer, 0.2 mmol/L of each deoxynucleoside triphosphate (dNTP), 2.5 mmol/L of MgCl2, 16 mmol/L (NH4)2SO4, 0.125 mg/ml BSA, 8% glycerine, 0.001% xylencyanol and 2.5 unit of Taq polymerase. The polymerase chain reaction (PCR) cycling conditions consisted of an initial denaturation for 1 min at 94 °C, followed by 35 cycles of 94 °C for 30 s, 65 °C for 30 s, and 72 °C for 30 s. Polymerase chain reaction products were separated on a 3% agarose gel and visualized by ethidium bromide staining.

The polymerase chain reaction (PCR) based restriction fragment length polymorphism assay and real-time PCR were used in parallel. Analyses were carried out by different independent people. Fifty two DNA samples were run in duplicate. Only one mismatch was detected—that sample was excluded.

2.4. Statistical analysis

The Analysis of Covariance (ANCOVAs) with the genotype and sport status as the independent categorical factors consisting respectively of 3 levels (LL, LS, SS) and 2 levels (synchronized swimmer, control group) and age as controlling predictor variant for the complex scale of “covert aggression” and each primary scale of aggression separately were used. The “covert aggression” scores were calculated as the sum of normalized values of Negativism and

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1 According to Unified Sports Classification System of the Russian Federation the following hierarchy exists: First-class athlete equates to regional champion Candidate for Master of Sport, which equates to nationally ranked player, Master of Sports, equates to national champion.
Irritability with negative sign and Indirect Hostility with positive one. For normalizing, each scale before summation was multiplied by the coefficients 20, 9 and 13 respectively for Negativism, Irritability and Indirect Hostility, accounting for the number of questions related to these primary scales (Osnitsky, 1994). The values of the complex scale was later standardized by subtracting the theoretically possible minimum (−199, to prevent negative values of the scale), and than dividing by the number of questions, forming the scale (24). The correspondence of 5-HTT genotypes’ distributions with Hardy–Weinberg equilibrium and the difference between them for synchronized swimmers and controls were determined by \(\chi^2\) Pearson test. In addition difference test between two proportions was computed to compare the proportion of each genotype in compared groups. All the analyses were made in Statistica 5.0.

### 3. Results

Table 1 represents mean scores on 8 aggression facets for carriers of different 5-HTT polymorphism in the groups of synchronized swimmers, age-matched and older controls. The significant associations are described below.

The “covert aggression” scale showed highly significant effect of 5-HTT polymorphism, \(F(2, 159) = 6.32, p = .002\), and sport, \(F(1, 159) = 16.25, p = .0001\), but no interaction effect between these factors, \(F(2, 159) = 1.22,\)

### Table 1

Mean ± SE scores of 8 aggression facets (BDHI) and our “covert aggression” scale for each group and genotype.

<table>
<thead>
<tr>
<th>Groups (age)</th>
<th>Control (10–18, 13 ± 0.3) N = 64</th>
<th>Control (20–26, 23 ± 0.3) N = 40</th>
<th>Swimmers (10–18, 13 ± 0.3) N = 62</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HTT polymorphism</td>
<td>LL 31% LS 59% SS 10%</td>
<td>LL 35% LS 45% SS 20%</td>
<td>LL 53% LS 32% SS 15%</td>
</tr>
<tr>
<td>Age</td>
<td>13 ± 0.5 14 ± 1.1</td>
<td>23 ± 0.4 24 ± 0.7</td>
<td>13 ± 0.4 13 ± 0.7</td>
</tr>
<tr>
<td>Assault</td>
<td>5.5 ± 0.4 3.5 ± 0.6</td>
<td>4.8 ± 0.4 5.8 ± 0.6</td>
<td>4.8 ± 0.3 4.2 ± 0.3</td>
</tr>
<tr>
<td>Indirect Hostility</td>
<td>5.1 ± 0.3 5.5 ± 0.7</td>
<td>4.8 ± 0.4 5.8 ± 0.6</td>
<td>4.9 ± 0.3 4.5 ± 0.4</td>
</tr>
<tr>
<td>Irritability</td>
<td>5.7 ± 0.5 5.7 ± 0.8</td>
<td>4.4 ± 0.6 6.0 ± 0.4</td>
<td>4.7 ± 0.4 5.8 ± 0.6</td>
</tr>
<tr>
<td>Negativism</td>
<td>3.2 ± 0.3 1.7 ± 0.5</td>
<td>3.4 ± 0.4 3.4 ± 0.5</td>
<td>3.4 ± 0.3 3.4 ± 0.4</td>
</tr>
<tr>
<td>Resentment</td>
<td>4.2 ± 0.4 4.3 ± 0.7</td>
<td>3.4 ± 0.4 3.4 ± 0.5</td>
<td>3.4 ± 0.3 3.4 ± 0.4</td>
</tr>
<tr>
<td>Suspicion</td>
<td>5.3 ± 0.5 4.2 ± 0.7</td>
<td>3.4 ± 0.5 3.4 ± 0.5</td>
<td>4.9 ± 0.3 4.9 ± 0.6</td>
</tr>
<tr>
<td>Verbal Hostility</td>
<td>8.1 ± 0.5 7.7 ± 0.9</td>
<td>5.2 ± 0.7 6.0 ± 0.6</td>
<td>6.9 ± 0.4 6.6 ± 0.6</td>
</tr>
<tr>
<td>Guilt</td>
<td>6.5 ± 0.5 5.3 ± 1.2</td>
<td>6.2 ± 0.4 5.4 ± 0.8</td>
<td>6.4 ± 0.3 6.7 ± 0.3</td>
</tr>
<tr>
<td>“Covert aggression”</td>
<td>6.2 ± 0.3 7.8 ± 0.6</td>
<td>6.9 ± 0.4 7.3 ± 0.6</td>
<td>6.9 ± 0.2 7.4 ± 0.3</td>
</tr>
</tbody>
</table>

Fig. 1. Association of the Indirect Hostility and Negativism scales of BDHI and complex “covert aggression” scale with 5-HTT genotypes for swimmers and control group. The mean scores and standard errors (SE) are represented as bars and whiskers for each genotype and group. Effect of 5-HTT polymorphism was significant for all these scales, although the effect of sport was significant only for Negativism and “covert aggression” scales. No interaction effect between sport and 5-HTT polymorphism was observed.
p = .3 (Fig. 1). There was also significant effect of age, F(1, 159) = 12.59, p = .001. However, when the older control group (>20 years old) was excluded from the analysis, the effect of 5-HTT, F(2, 119) = 8.63, p = .0003, and sport, F(1, 119) = 10.81, p = .001, was still significant, but the age effect disappeared, F(1, 119) = 0.81, p = .4. The absence of interaction effect was confirmed, F(2, 119) = .52, p = .6).

The ANCOVA of each of the BDHI facets separately revealed the significant effect of 5-HTT polymorphism on Indirect Hostility, F(2, 159) = 3.38, p = .036, and Negativism, F(2, 159) = 4.38, p = .014, (Fig. 1). In addition, the effect of sport, F(1, 159) = 13.71, p = .0003 and age, F(1, 159) = 11.21, p = .001, was present for the Negativism scale. The effect of age disappeared when older control group were excluded from the analysis, F(1, 119) = .10, p = .7. However, the effect of 5-HTT polymorphisms and sport persisted, F(2, 119) = 6.12, p = .003 and F(1, 119) = 8.27, p = .005, respectively).

The effect of age was significant for Resentment and Suspicion scales both in total and young (less than 20 years old) groups (p < .001), and for Assault (p < .001) and Verbal Hostility scales (p = .05) only in total group. The aggression scores on the above mentioned aggression facets decreased with age.

The effect of sport was significant for Assault, F(1, 159) = 5.18, p = .02, Irritability, F(1, 159) = 6.7194, p = .01, Negativism, F(1, 159) = 13.706, p = .0003, and Verbal Hostility, F(1, 159) = 5.47, p = .02. In all these cases synchronized swimmers showed lower scores than control group (Fig. 2).

No scale showed significant interaction between 5-HTT polymorphisms and sport as factors.

The distribution of SS, LS, and LL genotype frequencies in the control and synchronized swimmers groups were 13%, 54%, and 33%, and 15%, 32%, and 53%, respectively (Table 1). The genotype distribution did not significantly deviate from that expected according to the Hardy–Weinberg equilibrium (for synchronized swimmers χ² = 3.18, df = 2, p = .05, for the control group χ² = 1.49, df = 2, p = .2). But the proportion of LL-carriers in the group of synchronized swimmers was significantly higher then that in the control group (p = .01, proportion comparison) and the opposite was true for LS-carriers (p < .001). Their genotype distributions were different according to Pearson χ² test (χ² = 8.04, df = 2, p = .017). Noteworthy, genotype distribution in our control group were similar with those reported previously (Munafo et al., 2004; Sjoberg et al., 2006).

4. Discussion

Previously, 5-HTT gene was mostly associated with neuroticism and anxiety (Lesch and Merschdorf, 2000), although some studies did not replicate this finding (Willis-Owen et al., 2005). Our recent study showed that SS-carriers of 5-HTT gene are very accurate in current time estimation, probably because of their high anxiety scores (Portnova et al., 2007). The SS-genotype of 5-HTT was associated with impulsive aggression and suicidality, but in the study, which involved only male subjects (Gorraud et al., 2000). It was also shown that SS genotype is more frequent among aggressive drug experimenters than among abstinent students (about equal proportion of males and females) (Geria et al., 2005). The higher Aggressiveness (total BDHI) and Novelty Seeking (TQI) scores for SS- compared to LL-carriers was also reported in that study. The higher percentage of SS-carriers was found among aggressive children (mostly males) if compared to adult control group (Beitchman et al., 2006).

Our previous work (Maluchenko et al., 2007) showed that in synchronized swimmers group Indirect Hostility scores are higher, but Negativism and Irritability scores are smaller for carriers of SS genotype compared to carriers of LL and LS genotypes. Moreover, the complex scale consisted of positive values of Indirect Hostility and negative values of Negativism and Irritability showed even more
significant correlation with 5-HTT polymorphism. This indicates that these three facets of aggression may form complex scale interpreted by us as "covert aggression", which is 5-HTT dependent.

This result was confirmed in the current study (Table 1, Figs. 1 and 2). The new "covert aggression" scale, as well as, Indirect Hostility and Negativism scales was associated with 5-HTT gene polymorphism similarly for the control group of students and for synchronized swimmers.

Irritability scale was not associated with 5-HTT gene in control group (Table 1). This could be due to the complex gene–environment interaction influencing this form of aggression. Cates and colleagues (Cates et al., 1993) in twin study of adult women showed that environmental influences on Irritability is high and much stronger then those for Indirect Hostility. Probably the sport decrease the Irritability specifically in SS-carries by given them more option to discharge their aggression in more convenient way. But this inference has to be taken cautiously keeping in mind low number of subjects with SS genotype. The ANCOVA analysis did not reveal any significant interaction effect between sport and 5-HTT polymorphism.

Nonetheless, the clear result of our study is the association of Indirect Hostility and Negativism scales of BDHI with polymorphism of 5-HTT gene. It is noteworthy, that Indirect Hostility increased, but Negativism decreased in SS-compared to LL-carriers. This reciprocal association can be explained as a sign of preference of covert way of aggression expression for SS genotype: SS-compared to LL-carriers do not show their negative reactions directly, agreed with authorities (lower scores on Negativism), but express their aggression indirectly by banging the doors, making mean jokes and so on (higher scores in Indirect Hostility). This association was not reported previously, except our recent work (Malichenko et al., 2007). Two possible reasons for this can be suggested.

In our study each facet of aggression was analyzed separately, while most genetic association studies analyzed higher order factors and, therefore, lost the information about different aspects of aggression, which may be under the influence of different physiological mechanisms. The other specificity of our study is that it involved only females, although previous works did not separate men and women in the analysis. The girls and women were shown to be eager to express their aggression in indirect way, using social manipulation compared to boys and men (Bjorkqvist et al., 1994). Therefore, our finding can be specific only for female population.

Our analysis did not found any interaction effect between 5-HTTLPR polymorphism and sport on the aggression scores. This means that effect of 5-HTTLPR polymorphism expressed similar in synchronized swimmers and control group. Nonetheless, the significantly lower scores on the Assault, Irritability, Negativism and Verbal Hostility facets were found for swimmers if compared to control group. This association was also hold if compared with only age-matched control group and within each genotype separately (except for SS-genotype on Assault scale). Moreover, the scores on these aggression facets were also lower for older control groups compared to the younger control group, age-matched to swimmers (Table 1, Fig. 2). This is in concordance with the hypothesis that sport discharges aggression, although the synchronized swimming may not be considered as an aggressive sport. It may be interpreted as a sign of more matured emotional system, as far as our data showed that the scores in these aggression facets also decreased with age. The life of young sportgirls differs significantly from that of their classmates and more resembles adult life with long hours of training, competitions, lacking time for resting and gaming. Although alternative explanations are possible due to stratification problem. For example, the family who send their children to professional sport and accept their high physical load can be different from the ordinary ones. The difference in family situation may lead to the difference in these facets of aggression. Moreover, the lower aggressiveness may be an advantage for such a cooperative sport as synchronized swimming, where work with partner is very important. Further studies may investigate these issues.

Our study also showed the higher percentage of LL- and lower percentage of LS-genotype among swimmers compared to control groups. This result is not surprising because the LL-carriers are shown to be more resistance to stress or trauma (Caspi et al., 2003), for the female male–female sample. This protective affect of LL genotype may be very important for the athletes, whose life is full of stress factors. The data from the English national-level swimmers (13 males and 18 females) (Golby and Sheard, 2006), showing trend for association between LL-genotype and higher levels of positive psychological development, are in line with this interpretation. In our study carriers of LL-genotype was characterized as having lower scores on the "covert aggression", but compared with carriers of SS-genotype. Difference between LL- and LS-genotypes was mostly not significant. There was no difference in the presence of SS-genotype between synchronized swimmers and control groups, indicating that predisposition for the higher Indirect Hostility and lower Negativism scores has no significant influence on being a synchronized swimmer. People with SS-genotype, being less resistant to stress, compared to those with LL-genotype, may had some other characteristics, which help them to achieve high results in sport (synchronized swimming). These results may be considered as preliminary taken into account the possible heterogeneity of the studied groups.

5. Conclusions

Our study investigated influence of sport (synchronized swimming) and polymorphisms of 5-HTT gene on the aggression in the female Caucasian population. No interaction effect between sport and 5-HTT polymorphism on any aggression facets (BDHI) were found.

The significant effect of 5-HTT polymorphism was found for Indirect Hostility and Negativism scales (BDHI), as well as for our complex "covert aggression" scale consisting of Negativism, Irritability (negative values) and Indirect Hostility (positive values) facets. SS genotype of 5-HTT gene was associated with increased scores of Indirect Hostility and decreased scores in Negativisms compared to LL genotype. SS-carriers had higher scores on "covert" aggression. These aggression facets behave similarly for groups of synchronized swimmers and control girls.

The significant effect of sport was found for the Assault, Irritability, Negativism and Verbal Hostility scales. Professional synchronized swimmers had lower scores on Assault, Irritability, Negativism and Verbal Hostility compared to age-matched control girls (in general and compared within each genotype), probably indicating that the sportgirls’ control have more matured emotional system (these aggression facets also decreased with age).

The higher percentage of LL-carriers and lower of LS-carriers in synchronized swimmer group compared to control one was observed. LL polymorphism of 5-HTT gene was previously associated with higher resistance to stress factors. Our result indicates that this characteristic may be important for being an athlete.

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