GENETICS

Effect of Functional Catechol-O-Methyltransferase Val158Met Polymorphism on Physical Aggression

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Genetic and psychological analysis of the relationships between catechol-O-methyltransferase Val158Met polymorphism and various types of aggressiveness was performed in 114 women. Dispersion analysis revealed significant association of ValVal genotype with elevated physical aggression.

Key Words: *catechol-O-methyltransferase; Val158Met polymorphism; physical aggressiveness*

Heritability of some psychological traits in humans is about 30-60% [1]. Aggressiveness, similarly to other emotional traits, depends on the integral effects of genes of the brain neurotransmitter system, including catecholamines (dopamine, norepinephrine, *etc*.) and indolamines (serotonin and others).

Catechol-O-methyltransferase (COMT) participates in dopamine degradation in human prefrontal cortex [9]. COMT gene is located in chromosome 22q11. A point mutation located in the 4th exon (codon 158) leads to a decrease in enzyme activity by 40% [2]. In individuals with this mutation, adenine in position 472 is substituted for guanine, which leads to substitution of valine (Val) for methionine (Met). The frequency of Met allele in different populations ranges from 0.01 to 0.62 (~0.5 for Russian population) [10].

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There are contradictory reports on the relationship between COMT gene and aggressive behavior in humans. Some authors revealed a correlation of mutant allele Met with increased aggressiveness; significant correlations were demonstrated for men [14]. Other investigators reported that higher aggression is associated with Val allele [8]. Experiments on animals also produced contradictory results. Experimental increase in dopamine concentration in rat brain (injection of 6-hydroxydopamine) sharply reduced the number of confrontations between the animals [11]. COMT-knockout mice are characterized by increased aggressive behavior [5]. Brain content of dopamine should increase in the absence of the key enzyme of catecholamine catabolism, similarly to the case of administration of dopamine analog. It should be noted, that increased aggressiveness was observed only in male COMT-knockout mice. Thus, difficulties in the study of this mutation are explained by sex-related differences in polymorphism associations with psychological traits.

The aim of the present work was to study association of COMT genotypes with signs of aggressive behavior in women.

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MATERIALS AND METHODS

Genetic analysis was performed in 114 young women (17±5 years). All participants signed informed consent for the use of their DNA and results of psychological tests for the study. All genetic and psychological studies were approved by Ethical Committee of Institute of Sport and Physical Education.

Venous blood samples were used. COMT gene polymorphism was analyzed by PCR-based restriction fragment length polymorphism. DNA was isolated from 100 μl whole blood using DNA-Sorb B kits (Institute of Epidemiology). PCR with primers specific for Val158Met polymorphism was carried out as described elsewhere [7].

The reaction was performed in a volume of 25 μl. The standard reaction mixture contained 67 mM Tris-HCl, pH 8.4, 16 mM ammonium sulfate, 2.5 mM $MgCl_2$, 0.125 mg/ml BSA, 8% glycerol, 0.001% xylene cyanol, 2.5 U Taq-DNA-polymerase (Institute of Epidemiology), 0.2 mM each deoxynucleoside triphosphate, and 4 pmol each primer. We used hot-start PCR technique (nucleotides and primers were initially separated from Taq-polymerase with a wax layer).

For PCR amplification we used primers flanking the polymorphic site of the COMT gene (COMT-Forward 5'-TCG TGG ACG CCG TGA TTC AGG-3' and COMT-Reverse 5'-AGG TCT GAC AAC GGG TCA GGC-3').

The expected length of the amplification product was 217 b.p. for both alleles. PCR was carried out according to the following protocol: 5 min at 94°C (1 cycle), 30 sec at 94°C, 30 sec at 55°C, and 1 min at 72°C (35 cycles), 10 min at 72°C (1 cycle). The samples were stored at 10°C.

Restriction nuclease NlaIII (amount equivalent to 1U) was added to 7.5 μl reaction mixture and amplification was performed overnight at 37°C.

Val allele (wild type) is cleaved into fragments of 136 and 81 b.p., while mutant Met allele is cleaved into fragments of 96, 81, and 40 b.p.

The products were separated by electrophoresis in 10% acrylamide gel. The gel was prepared on TBE buffer (0.1 M Tris, 0.1 M boric acid, 2 mM EDTA, pH 8.3) using original 19:1 acrylamide:bisacrylamide mixture. Electrophoresis was performed at 100 V for 1.5 h. The gel was stained with 0.00001% ethidium bromide for 15 min. The results were detected on a TCP 15M transilluminator (Vilber lourmat) using a DNA Analyzer video detection system.

Aggression was diagnosed using a Bass-Darky inventory adapted by A. K. Osnitskii allowing evaluation of 8 primary characteristics: physical, indirect, and verbal aggression, irritability, negativism, resentment, jealousy, and feelings of guilt.

The relationship between the genotype and aggression (characteristics and their combinations) were studied by dispersion analysis, where COMT genotypes were considered as categorical independent factors. Then, the method of multiple paired comparisons was applied for identification of the factor determining the observed differences.

RESULTS

The distribution of allele frequencies in the examined sample little differed from the data for European population [10]. Dispersion analysis revealed significant association of genotypes with physical aggression (Fig. 1). No significant associations with other scales were found. It was found that physical aggression practically linearly decreased in the following order: ValVal (wild type)>MetVal>MetMet (mutant type) $F_{(2,111)}=4.6$ ($p=0.01$). Physical aggression, a type of aggression based on physical strength directed against other individuals, is an uncivilized and primitive form of aggression. Our findings suggest that mutant homozygotes (MetMet) are least aggressive, while wild type homozygotes (ValVal) exhibited maximum aggression.

In carriers of Val allele, COMT is in a highly active state, which leads to a decrease in intracellular dopamine concentration, and hence weaker activation of neurons in the prefrontal cortex [15]. Studies of the brain function and structure revealed association of Val allele with better processing of adverse visual stimuli. In examinees with ValVal genotype, activation of the prefrontal cortex after presentation of adverse visual stimuli decreased

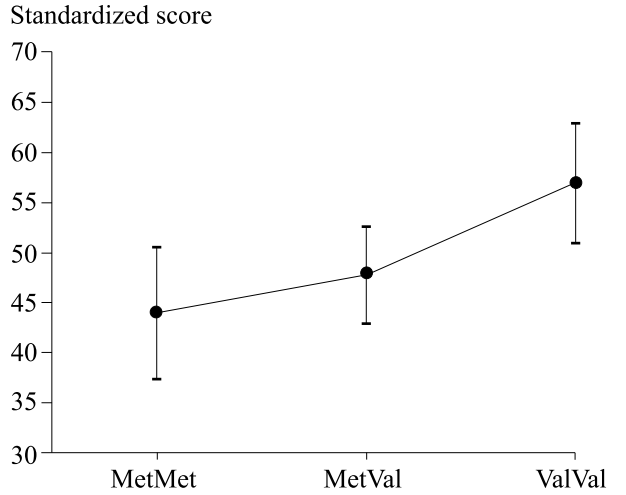

Fig. 1. Association of COMT genotypes (Val158Met polymorphism) with physical aggression in women.

more rapidly than in carriers of Met allele. Carriers of ValVal genotype are characterized by lower anxiety and pain sensitivity [3], which can contribute to increased physical aggression. Carriers of Met allele determining low activity of the enzyme are characterized by increased activation of prefrontal cortex maintaining excitation of the limbic system. Hence, Met allele is associated with higher sensitivity to adverse stimuli [13]. In women, Met-Met genotype is associated with high harm avoidance score determined by Cloninger inventory [4]. These individuals are characterized by fear of the danger and unknown, anxiety, and timidity. The combination of these traits is opposite to physical aggression.

Our findings confirm published data that increased physical aggression against other people is associated with ValVal genotype, but in previous studies significant association was demonstrated only for men, which can be explained by low percent of women in the studied sample (25%) [8]. We showed that similar relationship is observed in women. There are published reports that carriers of ValVal genotype are characterized by higher extraversion compared to carries of Met allele [12], which agrees with our findings, because physical aggression is a manifestation of high emotional excitement typical of extroverts.

Evaluation of Val158Met polymorphism in apes revealed no mutant allele Met [10], hence this mutation is relatively new and probably first appeared in humans. Alleles Val and Met are equally distributed in Caucasian population, which confirms high functional significance of this polymorphism fixed in the polupation. Mutant homozygotes demonstrate better memory and cognitive capacities compared to wild-type homozygotes [6]. Therefore, this mutation is an evolutional factor in cognitive function development, where the important role is played by the prefrontal cortex. During human evolution, natural selection was aimed at cognitive capacities and more complex organization of the brain, but not at physical strength and aggression. Therefore, it seem justified that mutation promoting improvement of cognitive functions and reducing physical aggression as the evolutionary archaic defense characteristic was fixed.

Thus, our study confirms association between physical aggression and Val158Met mutation of the COMT gene, in particular, association of wild-type ValVal homozygote with increased physical aggression in women.

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