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Genotype and haplotype frequencies of the DRD4 VNTR polymorphism in the men with no history of ADHD, convicted of violent crimes

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ABSTRACT

Purpose: The dopamine receptor 4 gene variable number of tandem repeats (*DRD4* VNTR) polymorphism is associated either with an increased risk of ADHD, or with a higher incidence rate of violent criminal behavior and aggression in the human populations. However, it cannot be excluded that the risk variants of the *DRD4* VNTR polymorphism, the 7-repeat and 5-repeat (7R and 5R) alleles might be associated with the increased occurrence of violent behavior in adults with no history of ADHD.
Methods: This study was to examine the prevalence of the certain risk variants of the *DRD4* VNTR polymorphism in the men convicted of violent crimes, with no history of ADHD (n = 161).
Results: The prevalence of the 5R and 7R *DRD4* VNTR alleles was higher in the men convicted of violent crimes, with no history of ADHD than in the general Russian Caucasian population (Novosibirsk city, n = 425).
Conclusions: This is the first evidence that both 7R and 5R, the ADHD-linked *DRD4* VNTR alleles are directly associated with the incidence of violent behavior in the men with no history of ADHD. Results support the hypothesis that proactive aggression might be a genetically-based, separate feature of personality that is independent of ADHD.

Introduction

Aggression and violent behavior imposes high social and economic burden to society, including costs of the criminal justice system and compensations for victims and their families (McCollister, French, & Fang, 2010; Tuvblad & Beaver, 2013). Several reports evidenced that the D4 dopamine receptor gene *DRD4* is widely expressed across the human brain (Mrzljak et al., 1996; Van Tol et al., 1992) and may be linked to the incidence of aggression and violent behavior in human adults (Boutwell & Beaver, 2008; Dmitrieva, Chen, Greenberger, Ogunseitan, & Ding, 2011). The *DRD4* gene has the variable number of tandem repeats (VNTR) polymorphism in the exon 3 that ranges from 2 to 11 repeats (2R, 3R, e.t.c.) (Iofrida, Palumbo, & Pellegrini, 2014; Lichter et al., 1993). The D4 dopamine receptors from the longer 7R VNTR variant were found to be less effective in binding of dopamine than the receptors from the common shorter 2R and 4R *DRD4* VNTR alleles (Van Tol et al., 1992). As a result, the 7R allele was commonly proposed to be a candidate risk allele linked either for aggressive behavior as well as for other psychiatric conditions and behavioral traits in humans and mammals (Li, Sham, Owen, & He, 2006; Wan et al., 2013).

For example, the long 7R variant of the *DRD4* VNTR polymorphism is robustly associated with the increased risk of attention deficit hyperactivity disorder (ADHD) in children (Li et al., 2006). Other studies have evidenced that the *DRD4* 7R allele might be associated with higher rates of aggression and violent behavior not only in adults (Boutwell & Beaver, 2008) but also in children and adolescents (Farbiash, Berger, Atzaba-Poria, & Auerbach, 2014). As a result, childhood ADHD was proposed to be an important transitional step from the certain "risk" genotypes toward the sequential development of antisocial and aggressive behavior across the life span (Beauchaine & McNulty, 2013; Eme, 2015).

This step-by-step model of the development of the antisocial trajectory does not exclude the alternative, complementary possibility that violent behavior could be a direct "product" of the certain *DRD4* VNTR polymorphism genotypes, with no childhood ADHD as the transitional state. No direct association between ADHD and criminality was found in a number of studies (Mordre, Groholt, Kjelsberg, Sandstad, & Myhre, 2011; Pingault et al., 2013). Caspi et al. (2008) also found that the *DRD4* VNTR polymorphism was not significantly associated with the criminal conviction in the adult men with the history of ADHD in childhood. So far, it cannot be excluded that the risk variants of the *DRD4* VNTR polymorphism might be associated with the higher

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occurrence of violent behavior in the adults with no history of ADHD or other similar disorders.

In addition, most previous studies discriminate the short 2R-5R safe variants and the long 6R–11R risk variants of the *DRD4* VNTR polymorphism (Iofrida et al., 2014). In contrast to this hypothesis, several authors have found that another allele of the *DRD4* VNTR polymorphism, the 5R variant, was associated with an increased risk of ADHD as well as with the several other 7R-variant-like behavioral traits (Li et al., 2006; Takeuchi et al., 2015). However, these valuable additional data cannot provide any understanding if the intermediate length alleles of the *DRD4* VNTR polymorphism, the 5R and the 6R variants, are associated with higher rates of aggression and violent criminal behavior in the human populations.

To investigate these questions, the current exploratory study examines the prevalence of the certain risk variants of the *DRD4* VNTR polymorphism in the men convicted of violent crimes, with no history of ADHD or similar disorders in childhood and/or adulthood. The possible relationship between the presence of the certain (5R and 7R) risk variants of the *DRD4* VNTR polymorphism and the occurrences of violent behavior was tested in the sample of the convicted men, in comparison with the sample of Caucasian male citizens of the Novosibirsk city.

Materials and methods

Compliance with ethical standards

This research was carried out in accordance with The Russian Federal Law on personal data (№152-FZ) of 27/07/2006, as well as with the International Ethical Guidelines, Declaration of Helsinki. Study was approved by the Bioethics Committee of the Institute of Cytology and Genetics (Russian Academy of Sciences, Siberian Branch) and by the Bioethic Committee of the Institute of therapy and prophylactic medicine (Russian Academy of Medical Sciences, Siberian Branch).

Participants and procedures

The sample groups were recruited in 2002–2014. The sample of convicted of violent, non-impulsive crimes against the person was composed of 161 male participants of age 19–64 (Fatal offenses – 85; non-fatal wounding or causing grievous bodily harm – 76) that were sentenced to a term in a Russian federal penitentiary. The history of the criminal convictions was obtained from the Russian Federal Crime Register. All studied individuals were Caucasians (mainly Russians), raised in two-parent families with medium to high level of income, with no history of ADHD, any conduct disorder or similar disease. Psychiatric interviews were conducted by a trained psychiatrist.

Informed consent was obtained from all participants after a detailed explanation of the study was provided by a psychiatrist. No money were promised to any potential participant or paid for participating in the research. Participation or refusal to participate did not affect the prisoner in any way, and the prison authorities were not informed of the decision of the prisoner. The prisoners who chose not to participate in the study did not differ significantly from those who participated in terms of demographic measures, such as age, educational level, occupational status, crime of conviction, and duration of sentence.

Preliminary, the participants were interviewed with the Structured Clinical Interview for DSM-IV-Disorders (SCID). Non-Caucasian ethnicity, intellectual disability, inability to read or speak Russian, a history of serious systemic or dermatologic conditions, a history of any central nervous system illness or insult were the main exclusion criteria. An additional exclusion criterion important for this study was the absence of emotional trauma in childhood or/and early adolescence. The including of each participant into the sample of convicted was confirmed by an independent psychiatrist using the SCID interview. Before the study, a total of 360 convicts were screened, of which, 45 (12.5%) refused to participate in the study and 154 (42.8%) were excluded using the aforementioned criteria.

An additional recurrent interview conducted by a trained psychiatrist was used to classify the subjects with the certain genotypes as proactive, reactive-only and nonaggressive. The classification of the subjects as proactive, reactive-only and nonaggressive was validated by an independent specialist.

The general population sample was obtained from 425 Novosibirsk male citizens (Caucasians, the Russian population) from The World Health Organization MONICA (Multinational monitoring of trends and determinants in cardiovascular disease) project. The control group was developed by random selection of the individuals from the voting list of the population of one of the Novosibirsk districts.

DRD4 genotyping

DNA was extracted from the whole blood sample by phenol and chloroform deproteinization, and stored under refrigeration (−80 °C) before the genotyping stage. The genotyping of the *DRD4* 48-bp VNTR polymorphism in the exon 3 with the variable length (96–384 bp) was performed as described previously (Nanko et al., 1993). The PCR reaction mix (total volume of 25 μl) consisted of 100 μM for each dNTP, 1.5 mM $MgCl_2$, 10% DMSO, 0.01% Tween-20, 20 mM $(NH_4)_2SO_4$, 75 mM Tris–HCl (pH 9.0), 1.25 units Taq (SibEnzyme, Russia), 0.5 μg of the genomic DNA template and of 0.4 μM forward primer 5′-AGGT GGCACGTCGCGCCAAGCTGCA-3′, 0.4 μM reverse primer (5′-TCTGCGGT GGAGTCTGGGGTGGGAG-3′). The PCR conditions were 95 °C (4 min), 35 cycles of 95 °C (1 min), 65 °C (1 min), 72 °C (1 min) and a final elongation, 65 °C (10 min). The number of repeats was determined by electrophoresis through a 4% polyacrylamide gel and ethidium bromide staining. The genotyping was performed in the Institute of therapy and prophylactic medicine SB RAMS, the control genotyping was performed in the Institute of Cytology and Genetics SB RAS. An accuracy of the genotyping was determined by the duplicate analysis of 72 samples selected randomly. The error rate was less than 0.005.

Haplotype computational estimations

The genotype and allele frequencies (the portion of individuals with a particular genotype and the portion of chromosomes with a particular allele, respectively) of the variants of the *DRD4* VNTR polymorphism in each study group were analyzed using contingency tables and the Arlequin software 3.5.2.2 (Excoffier & Lischer, 2010).

The conventional Expectation-Maximization (EM) and Excoffier-Laval-Balding (ELB) algorithms were used to determine the most probable haplotype constitution of each sample (Excoffier, Laval, & Balding, 2003; Excoffier & Slatkin, 1995). The EM algorithm was applied together with the following parameters: an Epsilon value for stopping iterations – $1*10^{-12}$; a number of starting points for algorithm – $1*10^{+3}$; a maximum number of iterations – $1*10^{+4}$; estimating standard deviation (SD) through the bootstrap test with the number of bootstraps to perform at $1*10^{+4}$ and the number of starting points at 99. The ELB algorithm was applied together with the following parameters: a Dirichlet prior alpha – 0.01; an epsilon value – 0.01; a gamma parameter preventing adaptive windows to grow too much – 0; a sampling interval – 500; burn-in steps – 100 000; recombination steps – 0. The Markov chain approximation in the ELB algorithm was used with $1*10^{+7}$ steps and $1*10^{+7}$ dememorization steps definition. The independent runs showed the same results by all methods, until mentioned otherwise.

Statistical analysis

Hardy–Weinberg equilibrium was tested by the Markov-chain random walk, Fisher's exact test similar algorithm (Guo & Thompson, 1992). When appropriate, the genotypic and allelic frequencies were