ANTISOCIAL BEHAVIOR: NATURE VS. NURTURE

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Abstract. Antisocial behavior (ASB) is a complex phenotype caused by the interaction between genetic and environmental factors. In past decades several genome-wide association studies (GWAS) and their meta-analyses identified up to 500 SNPs linked to externalizing pathology in Western Europeans. However, a question on their relevance to ASB in Eastern Europeans (i.e., Russians) remains open. Therefore, the present study aimed to replicate the effect of SNPs obtained from externalizing behavior GWAS meta-analysis on homicide behavior considering a possible modulating effect of social/lifestyle factors. We have selected top six SNPs ($p < 10^{-21}$) from recent GWAS meta-analysis of ASB (Karlsson Linnér *et al.*, 2021) including *CADM2* rs993137, *ZIC4* rs2279829, *REV3L* rs458806, *XKR6* rs4240671, *SORCS3* rs11596214, and *BDNF* rs6265. Subsequent genotyping was performed in the sample of homicide offenders (N = 227, 7% women) and corresponding control group (N = 254). A series of logistic regression (PLINK v.1.09) confirmed the association of *REV3L* rs458806 in the total sample (p = 0.044, OR = 1.346), while *SORCS3* rs11596214, *ZIC4* rs2279829, *XKR6* rs4240671 demonstrated their association with criminal behavior in the subgroups including smoking, low-educated offenders, individuals with psychopathologies and conflicts in families. Our findings replicated the effect of *REV3L*, *SORCS3*, *ZIC4*, and *XKR6* genetic variants on ASB in the Russian cohort under a moderating impact of social/lifestyle factors. However, the effect of social/lifestyle factors including sex, somatic diseases, and smoking on escalating antisocial behavior exceeded that of examined genetic variants.

Keywords: aggression, criminal behavior, callous-unemotional traits, replication, GWAS, sortilin receptor, *REV3L*, *XKR6*.

List of Abbreviations

ADHD – attention deficit/hyperactivity disorder
ASB – antisocial behavior
BDNF – brain derived neurotrophic factor
CADD – Combined Annotation Dependent Depletion database
CADM2 – cell adhesion molecule 2
EA – effect allele
eQTL – expression quantitative traits loci
GWAS – genome-wide association studies
GABA – gamma-aminobutyric acid
MAF – minor allele frequency
OR – odds ratio
RDB – Regulome database
SNP – single nucleotide polymorphism
SORCS3 – sortilin related VPS10 domain containing receptor 3
SSRI – selective serotonin reuptake inhibitors
REV3L – REV3 like, DNA directed polymerase zeta catalytic subunit
VPS10 – vacuolar protein sorting 10
WHO – world health organization
XKR6 – XK related 6
ZIC4 – Zic family member 4
95%CI – 95% confidence interval

Introduction

Aggression represents physical or verbal behavior, which suggests attacking another vial subject with the intent to cause harm, pain or injury. Evolutionary aggression is one of essential types of behavior, which accumulation in generations is related to biological benefits necessary for the survival of human ancestors (Baron & Richardson, 2004). Aggressive or antisocial behavior (ASB) toward someone is a serious problem for the legal system, as well as

for clinical and public safety (Fritz *et al*., 2023). According to the WHO, aggression is one of the leading causes of worldwide mortality in individuals aged 15 to 44 years (Dragovich & Borinskaya, 2019). The Ministry of Internal Affairs of the Russian Federation reported the data on more than 2.6 million crimes committed in the Russian Federation in 2022, 117.3 thousands of which were attributed to serious offences and 567.1 thousands - to homicides and intentional infliction of serious harm (http://www.crimestat.ru). Therefore, understanding the genetic basis of aggression has both biological and social significance.

The factors underlying liability to antisocial behavior include biological, psychological and socioeconomic ones (Fritz *et al*., 2023). Twin and adoption studies evidence in 50% to 80% of variance in aggression attributed to genetic component (Manchia & Fanos, 2017). During the past decades the genes belonging to regulation of monoaminergic systems (Kazantseva *et al*., 2009; Kolla & Bartolato, 2020), oxytocin and arginine vasopressin signaling (Davydova *et al*., 2020; Kazantseva *et al*., 2021), GABA receptors (Deak *et al*., 2019) and other systems have been screened under candidate gene methodology.

To date a shift toward genome-wide association studies (GWAS) instead of candidate gene approach in the analysis of complex traits has been introduced. In this regard several GWASs of various types of antisocial behavior were carried out in both children and adult cohorts. One of the first studies reported four significant genetic variants associated with childhood conduct disorder (N = 3,963) (Dick *et al*., 2011), none of which was located in the genes previously linked to molecular pathways of aggression. Subsequent GWAS of antisocial behavior in adults (N = 4,816) failed to identify associated SNPs under the level of GWAS significance ($p < 5 \times 10^{-8}$), although the most significant variant resided the *DYRK1A* gene, which was previously linked to impaired brain development and cognitive decline (Tielbeek *et al*., 2012). In addition, the studies of externalizing behavior conducted by Salvatore et al. (2015) (alcohol dependence case-control sample) and Derringer et al. (2015) (Center on Antisocial Drug Dependence) reported no evidence for a genome-wide significance of all examined variants. These failures can be partially attributed to the specificity of examined sample, which comprised of population-based cohort, a substance-dependent sample and non-homicide Finnish prisoners, and questionnaire-based measurement of antisocial behavior.

Subsequently, GWAS meta-analyses systemizing the results of GWASs of different types of antisocial behavior have given more unambiguous results. In turn, the findings obtained differ among adults (Tielbeek *et al*., 2017) and children (Pappa *et al*., 2016). The most recent and large-scale GWAS meta-analysis of antisocial behavior (Karlsson Linnér *et al*., 2021) combined the data from ~ 1,5 million individuals and was focused on the assessment of several externalizing phenotypes such as attention deficit and hyperactivity disorder (ADHD), problematic alcohol and cannabis use, number of sexual partners, risky behavior, and smoking initiation. These domains characterizing impulsivity behavior demonstrate partial genetic correlation and may be attributed to overlapping molecular mechanisms. Based on the results of abovementioned meta-analysis, the authors succeeded to establish more than 500 significant loci related to externalizing behavior. The most significant ones that were associated with all externalizing domains and present in gene regions are *CADM2* rs993137, *ZIC4* rs2279829, *REV3L* rs458806, *XKR6* rs4240671, *SORCS3* rs11596214, *BDNF* rs6265 (Karlsson Linnér *et al*., 2021).

Although several genetic companies provide individual reports on genetic predisposition to certain complex traits, they are mainly based on GWAS findings revealed for Western European populations including data from UK Biobank. However, many of reported genetic loci from European GWASs have to be verified in other ethnic groups, for instance, in Russians. To date no studies replicating the effect of top GWAS SNPs on manifesting antisocial behavior in Russian cohort have been published. It should be also mentioned that certain social/lifestyle factors may also predispose to criminal antiso-

cial behavior. To be more precise, such possible predictors include previous suicidal attempts (Swann *et al*., 2020), substance use (Tielbeek *et al*., 2018), education level, childhood maltreatment (Jung *et al.*, 2018), and mental dysfunctions in family (Li *et al*., 2017). A possible effect of social/lifestyle factors on manifesting aggression can be explained by their regulation of epigenetic remodeling (Mustafin *et al*., 2019; Borinskaya *et al*., 2021).

In this regard, the present study aimed to replicate the effect of SNPs obtained from externalizing behavior GWAS meta-analysis on manifesting severe criminal behavior considering a possible modulating effect of social/lifestyle factors.

Materials and Methods

Study sample

The sample of the present study was enlarged from previously reported one (Kazantseva *et al*., 2021) and consisted of 227 homicide offenders (7% women, mean age 41.5 ± ± 14.5 years), who convinced their severe crimes (murders) in the Republic of Bashkortostan. Criminal offenders belonged to three main ethnic groups residing Bashkortostan (48% Russians, 34.8% Tatars and 17.2% Bashkirs). They were subjected to a forensic psychiatric examination in a criminal case in the Republican Clinical Psychiatric Hospital (Ufa, Russia). All the offenders were recognized as sane persons by the Court. In order to assess the possible influence of social/lifestyle factors on manifesting antisocial behavior, the information on specificity of rearing, education level, tobacco smoking, alcohol addiction, somatic diseases, familial history of psychopathologies, suicidal attempts, and present conflicts in family was obtained.

A group of 254 mentally healthy adults served as control groups and were similar to the group of homicide offenders by sex (12% women), age (mean age 37.10 ± 18.38 years), and ethnic background (46% Russians, 35.8% Tatars and 18.2% Bashkirs). Control individuals were absent of familial history of psychopathologies and were non-registered in the psychiatric database.

The study was approved by the Biological Ethics Committee at the Institute of Biochemistry and Genetics–Subdivision of the Ufa Federal Research Centre of the Russian Academy of Sciences (Ufa, Russia) (protocol code 15, date of approval, October 12, 2017). A written informed consent was obtained from all participants after they were acquainted with the procedures. All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

SNPs selection and genotyping

DNA isolation was carried out using phenol-chloroform extraction approach from the peripheral blood leukocytes, which was followed by concentrations and DNA quality measurement (NanoDrop 1000 spectrophotometer, Thermo Fisher Scientific, USA). We performed a search for the most significant SNPs ($p < 10^{-21}$), which have been extracted from one of recent GWAS meta-analysis of externalizing behavior and self-regulation Karlsson Linnér *et al*., 2021). We have selected the following top six SNPs: *XKR6* rs4240671, *SORCS3* rs11596214, *BDNF* rs6265, *CADM2* rs993137, *ZIC4* rs2279829, and *REV3L* rs458806 based on the following criteria: 1) the lowest statistical significance level in abovementioned GWAS; 2) minor allele frequency (MAF) above 0.05 in European populations; 3) SNP location in the gene and neighboring regions; 4) selection of one SNP per gene; 5) SNP effect was significant for all examined externalizing phenotypes including ADHD, alcohol, cannabis and tobacco addiction, risky behavior and the number of sexual partners; 6) reported regulatory effect of SNP according to the RDB (Regulome Database, https://regulomedb.org/regulome-search) and CADD (Combined Annotation Dependent Depletion, https://cadd.gs.washington.edu) databases. Lower RDB score indicates higher possible regulatory effect of SNP on gene expression. The CADD database reflects the highest destructive effect of SNP on protein translation (higher CADD score coincides with more detrimental effect).

Genotyping of genetic loci was carried out using real-time PCR with KASP kits (Maxim Medical LLC, LGC Genomics, UK) and end-point fluorescence analysis on CFX96 DNA Analyzer (BioRad, USA).

Statistical analysis

At the primary stage we examined for the correspondence of the observed allele and genotype frequencies to the expected Hardy-Weinberg equilibrium. A comparison of genotype frequencies distribution among different ethnic groups was based on a calculation of Pearson χ^2 criterion (adjusted for Yates continuity) and a significance level. For the replication purposes we performed a series of logistic regression analyses under additive models with SNPs as independent variables and the status (homicide offenders vs. control group) as the outcome (PLINK v.1.09) controlling for sex and age. To determine a possible impact of abovementioned social/lifestyle factors on escalating antisocial behavior, logistic regression analyses were initiated. Subsequently, since the majority of examined social factors significantly affected individual sensitivity to manifesting ASB, we performed association analysis in the subgroups stratified by social factor. To determine a possible combined effect of genetic and social predictors, we performed a series of logistic regressions, which initially included all examined SNPs and significant environmental factors as predictors. Subsequently, a procedure of backward elimination was used to select the best predicting model based on the best Akaike information criterion (AIC) and p-value in R (v.4.3.0). A level of statistical significance was set at $p < 0.05$.

Results

Distribution of allele and genotype frequencies of all examined SNPs in control groups corresponded to that expected by the Hardy-Weinberg equilibrium (Table 1). Subsequent analysis revealed the absence of differences in allele and genotype frequencies between ethnic groups comprising the sample ($p > 0.05$).

Primarily, we checked for the possible impact of social/lifestyle factors on escalating criminal behavior. A significant influence of sex ($\beta = 1.13$, $p < 0.001$), level of education ($\beta = -2.04$, $p < 0.001$), present tobacco smoking ($\beta = 1.10$, $p < 0.001$), familial history of mental diseases ($\beta = 1.37$, $p < 0.001$), alcohol addiction ($\beta = 6.17$, $p < 0.001$), and severe somatic diseases ($\beta = 1.53$, $p < 0.001$) on manifesting exaggerated aggression were revealed (Table 2). Namely, criminal behavior was significantly more frequent in men, individuals with low education level, with nicotine and alcohol addiction, somatic diseases, with accumulated psychopathologies in families, and in youngest children in families. Therefore, we performed logistic regression analyses in the total sample and in the subgroups split by social parameter.

In the total sample we failed to replicate the effect of examined genetic loci on escalating antisocial behavior except for *REV3L* rs458806 (for allele C: $\beta = 2.02$, $p = 0.044$) (Table 2). The same direction of association was observed in the following subgroups of criminal offenders with proactive aggression ($\beta = 2.11$, $p = 0.035$), non-smoking individuals ($\beta = 2.07$, $p = 0.038$), with somatic diseases ($\beta = 2.79$, $p = 0.039$), with family conflicts ($\beta = 2.02$, $p = 0.005$). In this regard, *REV3L C*-allele carriers had higher probability of demonstrating ASB (OR = 1.346, 95%CI 1.01 – 1.79 – in the total sample; OR = = 1.376, 95%CI 1.02 – 1.85 – in criminal offenders with proactive aggression; OR = 1.568, 95%CI 1.03 – 2.40 – in non-smoking individuals; OR = 1.409, 95%CI 1.02 – 1.95 – in individuals with somatic diseases; OR = 1.677, 95%CI 1.17 – 2.41 – in persons with conflicts in family).

Subgroups analysis revealed that higher risk of manifesting criminal behavior was associated with *SORCS3* rs11596214 G-allele, which was prominent for smoking offenders ($\beta = 2.06$, $p = 0.040$, OR = 1.357, 95%CI 1.02 – 1.82), persons with accumulated mental disorders in family ($\beta = 2.56$, $p = 0.010$, OR = 1.608, 95%CI 1.02 – 2.32), and somatically healthy offenders ($\beta = 2.27$, $p = 0.023$, OR = 1.515, 95%CI 1.05 – 2.17) (Table 2). The association of *ZIC4* rs2279829 C-allele with higher probability to develop ASB was observed in low-educated criminal offenders ($\beta = 2.29$, $p = 0.022$, OR = = 1.610, 95%CI 1.08 – 2.44). Finally, we observed a link between increased risk of devel-

Table 1

Genotype frequencies and the Hardy-Weinberg equilibrium in antisocial behavior and control groups

Gene	SNP	Minor/major allele	Group	Genotype frequency			P_{HWE}
CADM2	rs993137	C/T	AB	0.037	0.428	0.535	0.047
			Control	0.093	0.418	0.489	0.878
ZIC4	rs2279829	T/C	AB	0.036	0.324	0.64	1
			Control	0.041	0.376	0.583	0.368
REV3L	rs458806	C/T	AB	0.1	0.42	0.48	0.875
			Control	0.07	0.37	0.56	0.731
XKR6	rs4240671	A/G	AB	0.18	0.51	0.31	0.587
			Control	0.22	0.47	0.31	0.459
SORCS3	rs11596214	A/G	AB	0.15	0.51	0.34	0.489
			Control	0.22	0.49	0.29	0.707
BDNF	rs6265	C/T	AB	0.04	0.24	0.72	0.125
			Control	0.03	0.23	0.74	0.141

Note: P_{HWE} – P-value for Hardy-Weinberg equilibrium.

oping ASB and *XKR6* rs4240671 G-allele only in individuals with permanent conflict situation in their families ($\beta = 2.18$, $p = 0.030$, OR = = 1.471, 95%CI 1.04 – 2.08) (Table 2).

In order to estimate a combined effect of both genetic and social factors on severe ASB, we used a backward selection procedure to find a model with the most significant predictors. The final model ($p < 0.001$, $r^2 = 0.29$) included sex ($\beta = -2.31$, $p = 1 \times 10^{-4}$), severe somatic diseases ($\beta = 2.3$, $p = 5.0 \times 10^{-5}$), and tobacco smoking ($\beta = 2.38$, $p = 2.9 \times 10^{-6}$), while genetic variants were excluded.

Discussion

The present study is the pioneer one, which examined the effect of previously determined genetic loci in the *XKR6, SORCS3, BDNF, CADM2, ZIC4,* and *REV3L* genes on escalating severe forms of antisocial behavior (homicide) in Russian cohort within GWAS replication framework. The main finding is that social/lifestyle factors have more prominent impact on predisposing ASB compared to analyzed genetic variants. We revealed that a combined effect of such social predictors as sex, severe somatic diseases, and tobacco smoking explained up to 29% of variance in ASB. Hazardous impact of such environmental factors on developing aggression and externalizing behavior has been well-established (Fairchild *et a*l., 2019).

With respect to examined genetic variants, although their impact on developing ASB was diminished compared to social predictors, we replicated the effect of *REV3L* rs458806 on manifesting antisocial behavior in Russian cohort in the total sample and in subgroups. Notably, the most significant result of *REV3L* rs458806 association with ASB was shown in individuals reporting permanent conflicts in their families. Our findings on *REV3L* C-allele effect on ASB is congruent with large-scale meta-analysis (Karlsson Linnér *et al*., 2021), which also reported a link between this allele and various externalizing phenotypes ($p = 1.3 \times \times 10^{-29}$). The *REV3L* gene encodes a catalytic subunit of DNA polymerase zeta, which is involved in DNA synthesis and DNA protection from damage in mitochondria. Previously, mitochondrial dysfunction was shown to be caused by stressful life events and childhood maltreatment (Picard &McEwen, 2018; Enikeeva *et al*, 2022). In this regard, a possible involvement of the *REV3L* gene in regulation of emotions, behavioral self-inhibition and self-control explains our findings. It should be men-

Table 2

Logistic regression in the total sample and in the subgroups examining the effect of SNPs on manifesting antisocial behavior

Parameter	**β (p-value)**	**Group**	***CADM2*** **rs993137**	***ZIC4*** **rs2279829**	***REV3L*** **rs458806**	***XKR6*** **rs4240671**	***SORCS3*** **rs11596214**	***BDNF*** **rs6265**
			C/T*	**C/T***	**C/T***	**G/A***	**G/A***	**T/C***
Direction of effect in GWAS meta-analysis**			**C↑**	**C↑**	**C↑**	**G↑**	**G↑**	**C↑**
Total sample (N = 227)			0.081 (0.764)	0.247 (1.211)	**0.044 (1.346)**	0.528 (1.085)	0.065 (1.277)	0.647 (1.080)
Sex (men/women)	**1.13 (< 0.001)**	Men (N = 212)	0.119 (0.774)	0.377 (1.166)	0.118 (1.284)	0.766 (1.043)	0.186 (1.203)	0.549 (1.114)
		Women (N = 15)	–	–	–	–	–	–
Education (high/low)	**-2.04 (< 0.001)**	High (N = 92)	0.313 (0.818)	0.468 (0.858)	0.264 (1.247)	0.580 (1.098)	0.095 (1.342)	0.817 (0.947)
		Low (N = 135)	0.081 (0.727)	**0.022 (1.610)**	0.056 (1.380)	0.699 (1.164)	0.209 (1.211)	0.430 (1.168)
Aggression type (proactive/ reactive)	23.48 (0.985)	Proactive (N = 211)	0.132 (0.789)	0.270 (1.205)	**0.035 (1.376)**	0.408 (1.117)	0.082 (1.269)	0.782 (1.05)
		Reactive (N = 16)	–	–	–	–	–	–
Smoking (yes/no)	**1.10 (< 0.001)**	Yes (N = 160)	0.198 (0. 804)	0.285 (1.218)	0.173 (1.247)	0.805 (1.035)	**0.040 (1.357)**	0.497 (1.134)
		No (N = 67)	0.160 (0.719)	0.573 (1.152)	**0.038 (1.568)**	0.317 (1.218)	0.570 (1.117)	0.960 (0.987)
Familial psychopathology (yes/no)	**1.37 (< 0.001)**	Yes (N = 88)	0.059 (0.663)	0.288 (1.274)	0.095 (1.395)	0.769 (0.951)	**0.010 (1.608)**	0.658 (1.108)
		No (N = 139)	0.348 (0.849)	0.409 (1.174)	0.155 (1.272)	0.281 (1.179)	0.354 (1.153)	0.601 (1.108)
Alcohol addiction (yes/no)	**6.17 (< 0.001)**	Yes (N = 150)	0.094 (0.745)	0.078 (1.406)	0.113 (1.298)	0.963 (0.993)	0.080 (1.305)	0.742 (0.938)
		No (N = 77)	0.335 (0.813)	0.789 (0.941)	0.071 (1.452)	0.170 (1.290)	0.251 (1.239)	0.170 (1.380)

End of table 2

Parameter	β (p-value)	Group	*CADM2* rs993137	*ZIC4* rs2279829	*REV3L* rs458806	*XKR6* rs4240671	*SORCS3* rs11596214	*BDNF* rs6265
			C/T*	**C/T***	**C/T***	**G/A***	**G/A***	**T/C***
Direction of effect in GWAS meta-analysis**			**C↑**	**C↑**	**C↑**	**G↑**	**G↑**	**C↑**
Somatic diseases (yes/no)	**1.53 (< 0.001)**	Yes (N = 136)	0.239 (0.808)	0.181 (1.302)	**0.039 (1.409)**	0.849 (1.029)	0.393 (1.140)	0.199 (1.276)
		No (N = 91)	0.104 (0.715)	0.573 (1.135)	0.321 (1.221)	0.314 (1.196)	**0.023 (1.515)**	0.254 (0.740)
Suicidal attempts (yes/no)	18.02 (0.976)	Yes (N = 43)	0.157 (0.669)	0.674 (1.131)	0.124 (1.479)	0.954 (1.013)	0.313 (1.266)	0.464 (0.779)
		No (N = 176)	0.156 (0.795)	0.230 (1.237)	0.110 (1.284)	0.463 (1.106)	0.064 (1.302)	0.369 (0.899)
Conflicts in family (yes/no)	17.37 (0.993)	No (N = 122)	0.150 (0.771)	0.328 (1.219)	0.590 (1.101)	0.371 (0.872)	0.148 (1.255)	0.553 (1.127)
		Yes (N = 91)	0.230 (0.782)	0.230 (1.307)	**0.005 (1.677)**	**0.030 (1.471)**	0.100 (1.340)	0.855 (1.042)

Note: N represents number of subjects with antisocial behavior. P-values (ORs) for additive logistic regression model are shown. Statistically significant p-values ($p < 0.05$) are shown in bold. Dashes stand for low sample size groups, which could cause type I and II errors. *Effect/other alleles of examined SNPs are indicated. **Arrows indicate a direction of association with ASB. For the parameter a probability to manifest antisocial behavior in the examined group (given in brackets) compared to reference is given.

tioned that stronger effect of the *REV3L* gene variant on ASB was observed under unfavorable social conditions such as the presence of somatic diseases and family conflicts. The same direction of association determined in non-smoking individuals and unobserved in smoking ones can be explained by a bidirectional influence of nicotine on emotional states at different stages of addiction. A periodic smoking can be used to diminish negative emotional symptoms, while permanent long-term smoking can promote their increase (Weltens *et al*., 2021). Although no other studies determined the effect of rs458806 on antisocial behavior, several SNPs in the *REV3L* gene have been linked to risky behavior (Baselmans *et al*.,2021) and smoking initiation (Brazel *et al*., 2018; Cai *et al*., 2020; Saunders *et al*., 2022; Pasman *et al*., 2021; Erzurumluoglu *et a*l., 2020).

The examined rs458806 represents eQTL of the *REV3L* gene in cerebellum and has predicted regulatory impact on gene expression according to RDB and CADD databases. However, a direction of functional significance effect of this SNP remains undetermined. In turn, reduced expression of the *REV3L* gene in the brain was linked to rs462779 A-allele, which was associated with a decreased risk of smoking initiation (Erzurumluoglu *et al*., 2020) and is proxy to rs458806 T-allele based on LDLink (https://ldlink.nci.nih.gov, accessed on 31 August 2023, r^2=1). According to these results, we suggest that escalating antisocial behavior can be attributed to enhanced expression of the *REV3L* gene associated with *REV3L* C-allele. However, functional studies are required to confirm this suggestion.

Another association of *SORCS3* rs11596214 and ASB in individuals from Russia was replicated only under a moderating effect of tobacco smoking and mental impairment in the family. Sortilin-related receptor SORCS3 belongs to the VPS10P domain receptor family, which is responsible for trafficking of proteins between intracellular vesicles and the plasma membrane, and controls neuronal viability and function (Briederhoff *et al*., 2013). Interestingly, the *SORCS3* gene is highly expressed in hippocampus – brain region involved in memory storage and retrieval. Moreover, *SORCS3*-deficient mice were characterized by impaired emotional behavior and accelerated extinction of fear memory caused by modified glutamate receptor trafficking (Briederhoff et al., 2013). In this regard the role of SORCS3 receptor in behavioral disinhibition and antisocial behavior in humans is non-surprising. In line with abovementioned study, a recent research also determined that diminished expression of the *SORCS3* gene, which negatively affected synaptic function, was characteristic for impaired mental states (Kamran *et al*., 2023).

In accordance with our findings, previous GWAS studies also implicated *SORCS3* rs11596214 G-allele in higher risk of antisocial phenotypes (Karlsson Linnér *et al*., 2021), increased depression risk (Thorp *et al*., 2021), and lower Neuroticism (Hill *et al*., 2020). Recent GWAS also depicted SNPs in the *SORCS3* gene as linked to ADHD, a disorder frequently genetically overlapping antisocial behavior (Demontis *et al*., 2023). Nevertheless, molecular mechanisms underlying SORCS3-linked predisposition to escalating aggression have to be clarified.

The *XKR6* gene, which is involved in apoptotic process, was implicated in the development of criminal behavior in the present study. Although functional studies reporting a link between its up/downregulation at the molecular level are absent to date, its partial deletion within the 8p23.1 microdeletion syndrome is also accompanied by emotional problems, intellectual disability, and epilepsy (Akcakaya *et al*., 2017). Absence seizures accompanying epilepsy phenotype are attributed to frontal lobe dysfunction (Hughes, 2009), a region also significant for inhibitory control and, thus, for impulsive and aggressive behavior. In turn, 8p23.1 duplication of 3.68 Mb region including the *XKR6* gene manifested as fertility problems with speech delay in childhood (El Karaaoui *et al*., 2023) and hyperactivity (Barber et al., 2013). Our findings also demonstrated a relation between the *XKR6* gene variant and externalizing behavior, which was evident only in the case of present stressful conditions in the family. The last ones are known to be key me-

diating factors in community violence (Fairchild *et al*., 2019). It should be noted that the direction of effect of *XKR6* rs4240671 corresponded to that determined in previous meta-analysis (Karlsson Linnér *et al*., 2021). Published findings also indicate the possible involvement of the *XKR6* genetic variants in smoking initiation (Liu *et al*., 2019), risky (Karlsson Linnér *et al*., 2019) and aggressive behavior (Baselmans *et al*., 2021). In addition, a large-scale multi-trait GWAS meta-analysis (Amare *et al*., 2018) identified genetic variant in the *XKR6* gene as one of top eight linked to SSRI efficacy in major depression and neuroticism.

Another gene, which was also related to social violence in our study, was the *ZIC4* gene encoding a zinc finger protein of the cerebellum, which regulates the development of brain motor control region and is involved in neurogenesis (Alagöz *et al*., 2022). A deletion of the *ZIC1* and *ZIC4* genes in a heterozygous state causes so called Dandy-Walker malformation, which represents a structural birth defect of the human cerebellum (Aruga & Millen, 2018). Moreover, the *ZIC4* gene is related to the cortical evolution and its coding sequence was positively selected in mammals (Alagoz *et al*., 2022). Therefore, a relation of the *ZIC4* gene to emotional regulation and our findings on the association of the *ZIC4* gene variant with aggressive behavior seem to be probable. It should be mentioned that higher risk of manifesting criminal behavior was prominent for low-educated *ZIC4* rs2279829 C-allele carriers, which is congruent with the effect of this allele on various domains of ASB in GWAS meta-analysis (Karlsson Linnér *et al*., 2021). A moderating role of educational attainment in manifesting aggression coincides with the existence of negative genetic correlation between these traits (r = -0.52) (Tielbeek *et al*., 2017).

Unfortunately, we failed to replicate the association of brain-derived neurotrophic factor (*BDNF*) and *CADM2* genetic variants in escalating homicide in Russian cohort. Nevertheless, previous research evidence in the role of neurotrophic factors in regulation of emotions and conduct behavior (Kretschmer *et al*., 2014, 2015). In turn, *CADM2* encodes a member of the synaptic cell adhesion molecules and its differences in gene expression alter neuronal connectivity. The results of a phenome-wide association study conducted in UK Biobank on a large number of psycho-behavioral traits, revealed the effect of *CADM2* genetic variants on such traits as irritability, nervous/worrier/anxious feelings, addictive behavior, abuse traits, sex-related risky behavior, and self-control that usually demonstrate causal relationships with antisocial behavior (Pasman *et al*., 2022; Morris *et al*., 2019).

Conclusion

The present study is a pioneer one that sought to replicate the association of genetic variants in the *XKR6, SORCS3, BDNF, CADM2, ZIC4*, and *REV3L* genes with severe antisocial behavior (homicide) in Russian cohort. The main finding of our study is replicated association of *REV3L* rs458806 in the total sample, while *SORCS3* rs11596214, *ZIC4* rs2279829, and *XKR6* rs4240671 were associated with an increased risk of escalating criminal behavior only in the subgroups. Revealed findings indicate that these associations are replicable under unfavorable social/lifestyle conditions such as tobacco smoking, low education level, the presence of psychopathologies and conflict situations in families. It should be noted that we have observed the same direction of association as in GWAS meta-analysis of other types of externalizing behavior (Karlsson Linnér *et al*., 2021), thus confirming the existence of overlapping mechanisms underlying the development of different antisocial phenotypes. Recently, we have also demonstrated a specific pattern of replicated associations with cognitive traits in Russian cohort (Kazantseva *et al*., 2023) that indicates that GWAS data transfer from UK Biobank to Russian populations should be carried out with caution. Although a collection of study sample of criminal homicide offenders is rather complicated, future studies involving larger sample size from Russia assessing all top SNPs or GWAS are required.

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References

AKCAKAYA N.H., CAPAN Ö.Y., SCHULZ H., SANDER T., CAGLAYAN S.H. et al. (2017): De novo 8p23.1 deletion in a patient with absence epilepsy. *Epileptic Disord.* **19**(2), 217–221. DOI: 10.1684/epd.2017.0906.

ALAGÖZ G., MOLZ B., EISING E., SCHIJVEN D., FRANCKS C. et al. (2022): Using neuroimaging genomics to investigate the evolution of human brain structure. *Proc. Nat. Acad. Sci. USA* **119**(40), 2200638119. DOI: 10.1073/pnas.2200638119.

AMARE A.T., SCHUBERT K.O., TEKOLA-AYELE F., HSU Y.H., SANGKUHL K. et al. (2018): Association of the Polygenic Scores for Personality Traits and Response to Selective Serotonin Reuptake Inhibitors in Patients with Major Depressive Disorder. *Front. Psychiatry* **9**, 65. DOI: 10.3389/fpsyt.2018.00065.

ARUGA J. & MILLEN K.J. (2018): *ZIC1* Function in Normal Cerebellar Development and Human Developmental Pathology. *Adv. Exp. Med. Biol.* **1046**, 249–268. DOI: 10.1007/978-981-10-7311-3_13.

BARBER J.C., ROSENFELD J.A., FOULDS N., LAIRD S., BATEMAN M.S. et al. (2013): 8p23.1 duplication syndrome; common, confirmed, and novel features in six further patients. *Am. J. Med. Genet. A.* **161A**(3), 487–500. DOI: 10.1002/ajmg.a.35767.

BARON R. A. & RICHARDSON D. R. (2004): *Human aggression.* Springer Science & Business Media, 420 pp.

BASELMANS B., HAMMERSCHLAG A.R., NOORDIJK S., IP H., VAN DER ZEE M. et al. (2021): The Genetic and Neural Substrates of Externalizing Behavior. *Biol. Psychiatry Glob. Open Sci.* **2**(4), 389–399. DOI: 10.1016/j.bpsgos.2021.09.007.

BORINSKAYA S.A., RUBANOVICH A.V., YANKOVSKY N.K., LARIN A.K., GENEROZOV E.V. et al. (2021): Epigenome-wide association study of CPg methylation in aggressive behavior. *Russian Journal of Genetics* **57**(12), 1454–1460. DOI: 10.1134/S1022795421120048.

BRAZEL D.M., JIANG Y., HUGHEY J.M., TURCOT V., ZHAN X. et al. (2019): Exome Chip Meta-analysis Fine Maps Causal Variants and Elucidates the Genetic Architecture of Rare Coding Variants in Smoking and Alcohol Use. *Biol. Psychiatry* **85**(11), 946–955. DOI: 10.1016/j.biopsych.2018.11.024.

BREIDERHOFF T., CHRISTIANSEN G.B., PALLESEN L.T., VAEGTER C., NYKJAER A. et al. (2013): Sortilin-related receptor *SORCS3* is a postsynaptic modulator of synaptic depression and fear extinction. *PLoS One* **8**(9), 75006. DOI: 10.1371/journal.pone.0075006.

CAI N., REVEZ J.A., ADAMS M.J., ANDLAUER T.F.M., BREEN G. et al. (2020): Minimal phenotyping yields genome-wide association signals of low specificity for major depression. *Nat. Genet.* **52**(4), 437–447. DOI: 10.1038/s41588-020-0594-5.

CRIMESTAT. Portal of legal statistics of the Prosecutor General's Office of the Russian Federation: crime rates in Russia. Russia: the Prosecutor General's Office of the Russian Federation, 2023. http://www.crimestat.ru. 2023.

DAVYDOVA Y.D., KAZANTSEVA A.V., ENIKEEVA R.F., GILYAZOVA I.R., KHUSNUTDINOVA E.K. et al. (2020): The role of oxytocin receptor (*OXTR*) gene polymorphisms in the development of aggressive behavior in healthy individuals. *Russian Journal of Genetics* **56**(9), 1129–1138. DOI: 10.1134/S1022795420090057.

DEAK J.D., GIZER I.R., OTTO J.M., BIZON C. & WILHELMSEN K.C. (2019): Effects of Common and Rare Chromosome 4 GABAergic Gene Variation on Alcohol Use and Antisocial Behavior. *J. Stud. Alcohol Drugs* **80**(6), 585–593. DOI: 10.15288/jsad.2019.80.585.

DEMONTIS D., WALTERS G.B., ATHANASIADIS G., WALTERS R., THERRIEN K. et al. (2023): Genome-wide analyses of ADHD identify 27 risk loci, refine the genetic architecture and implicate several cognitive domains. *Nat. Genet.* **55**, 198–208. DOI: 10.1038/s41588-022-01285-8.

DERRINGER J., CORLEY R.P., HABERSTICK B.C., YOUNG S.E., DEMMITT B.A. et al. (2015): Genome-Wide Association Study of Behavioral Disinhibition in a Selected Adolescent Sample. *Behavior Genetics* **45**(4), 375–381. DOI: 10.1007/s10519-015-9705-y.

DICK D.M., ALIEV F., KRUEGER R.F., EDWARDS A., AGRAWAL A. et al. (2011): Genome-wide association study of conduct disorder symptomatology. *Molecular Psychiatry* **16**(8), 800–808. DOI: 10.1038/mp.2010.73.

DRAGOVICH A.Y. & BORINSKAYA S.A. (2019): Genetic and genomic basis of aggressive behavior. *Russian Journal of Genetics* **55** (12), 1381–1396. DOI: 10.1134/S0016675819090054.

EL KARAAOUI A., GHAZEERI G. & ASSAF N. (2023): Insight into the 8p23.1 duplication syndrome: Case report of a young women with infertility. *Heliyon* **9**(4), 15515. DOI: 10.1016/j.heliyon.2023.e15515.

ENIKEEVA R.F., KAZANTSEVA A.V., DAVYDOVA YU.D., MUSTAFIN R.N., LOBASKOVA M.M. et al. (2022): Individual differences in the number of mitochondrial DNA copies: the effect of socio-demographic factors. *Yakut Med. J.* **2**(78), 13–16. DOI: 10.25789/YMJ.2022.78.03.

ERZURUMLUOGLU A.M., LIU M., JACKSON V.E., BARNES D.R., DATTA G. et al. (2020): Meta-analysis of up to 622,409 individuals identifies 40 novel smoking behaviour associated genetic loci. *Mol. Psychiatry* **25**(10), 2392–2409. DOI: 10.1038/s41380-018-0313-0.

FAIRCHILD G., HAWES D.J., FRICK P.J., COPELAND W.E., ODGERS C.L. et al. (2019): Conduct disorder. *Nat. Rev. Dis. Primers* **5**(1), 43. DOI: 10.1038/s41572-019-0095-y.

FRITZ M., SORAVIA S.M., DUDECK M., MALLI L. & FAKHOURY M. (2023): Neurobiology of Aggression-Review of Recent Findings and Relationship with Alcohol and Trauma. *Biology (Basel)* **12**(3), 469. DOI: 10.3390/biology12030469.

HILL W.D., WEISS A., LIEWALD D.C., DAVIES G., PORTEOUS D.J. et al. (2020): Genetic contributions to two special factors of neuroticism are associated with affluence, higher intelligence, better health, and longer life. *Mol. Psychiatry* **25**(11), 3034–3052. DOI: 10.1038/s41380-019-0387-3.

HUGHES J.R. (2009): Absence seizures: a review of recent reports with new concepts. *Epilepsy Behav*. **15**(4), 404–412. DOI: 10.1016/j.yebeh.2009.06.007.

JUNG H., HERRENKOHL T.I., SKINNER M.L. & ROUSSON A.N. (2021): Does Educational Success Mitigate the Effect of Child Maltreatment on Later Offending Patterns? *J. Interpers. Violence* **36**(3-4), 1833–1855. DOI: 10.1177/0886260518756113.

KAMRAN M., LAIGHNEACH A., BIBI F., DONOHOE G., AHMED N. et al. (2023): Independent Associated SNPs at *SORCS3* and Its Protein Interactors for Multiple Brain-Related Disorders and Traits. *Genes (Basel)* **14**(2), 482. DOI: 10.3390/genes14020482.

KARLSSON LINNÉR R., BIROLI P., KONG E., MEDDENS S.F.W., WEDOW R. et al. (2019): Genome-wide association analyses of risk tolerance and risky behaviors in over 1 million individuals identify hundreds of loci and shared genetic influences. *Nat. Genet.* **51**, 245–257 (2019). DOI: 10.1038/s41588-018-0309-3.

KARLSSON LINNÉR R., MALLARD T.T., BARR P.B., SANCHEZ-ROIGE S., MADOLE J.W. et al. (2021): Multivariate analysis of 1.5 million people identifies genetic associations with traits related to self-regulation and addiction. *Nat. Neurosci.* **24**(10), 1367–1376. DOI: 10.1038/s41593-021-00908-3.

KAZANTSEVA A.V., GAYSINA D.A., MALYKH S.B. & KHUSNUTDINOVA E.K. (2009): Role of dopamine transporter gene (*DAT1*) polymorphisms in personality traits variation. *Russian Journal of Genetics* **45**(8), 974–980. DOI: 10.1134/S1022795409080122.

KAZANTSEVA A., GAYSINA D., KUTLUMBETOVA Y., KANZAFAROVA R., MALYKH S. et al. (2015): Brain derived neurotrophic factor gene (BDNF) and personality traits: the modifying effect of season of birth and sex. *Prog. Neuropsychopharmacol. Biol. Psychiatry* **56**, 58–65. DOI: 10.1016/j.pnpbp.2014.08.001.

KAZANTSEVA A.V., DAVYDOVA YU.D., ENIKEEVA R.F., VALINUROV R.G., GAREEVA A.E. et al. (2021): The association study of polymorphic variants of hypothalamic-pituitary-adrenal system genes (*AVPR1B, OXTR*) and aggressive behavior manifestation: a focus on social environment. *Research Results in Biomedicine* **7**(3), 232–244. DOI: 10.18413/2658-6533-2021-7-3-0-3.

KAZANTSEVA A.V., DAVYDOVA YU. D., ENIKEEVA R.F., TAKHIROVA Z.R., MUSTAFIN R.N. et al. (2023): Replication study of gwas-associated variants in the *TUFM, SH2B1, ZNF638, NEGR1, ATP2A1, EXOC4*, and *CSE1L* genes and cognitive abilities. *Russian Journal of Genetics* **59**(9), 1059–1069. DOI: 10.31857/S0016675823090060.

KOLLA N.J. & BORTOLATO M. (2020): The role of monoamine oxidase A in the neurobiology of aggressive, antisocial, and violent behavior: A tale of mice and men. *Prog. Neurobiol.* **194**, 101875. DOI: 10.1016/j.pneurobio.2020.101875.

KRETSCHMER T., VITARO F. & BARKER E.D. (2014): The Association Between Peer and own Aggression is Moderated by the *BDNF* Val-met Polymorphism. *J. Res. Adolesc.* **24**(1), 177–185. DOI: 10.1111/jora.12050.

LI P., BECKER J.B., HEITZEG M.M., MCCLELLAN M.L., REED B.G. et al. (2017): Gender differences in the transmission of risk for antisocial behavior problems across generations. *PLoS One* **12**(5), 0177288. DOI: 10.1371/journal.pone.0177288.

LIU M., JIANG Y., WEDOW R., LI Y., BRAZEL D.M. et al. (2019): Association studies of up to 1.2 million individuals yield new insights into the genetic etiology of tobacco and alcohol use. *Nat. Genet.* **51**, 237–244. DOI: 10.1038/s41588-018-0307-5.

MANCHIA M. & FANOS V. (2017): Targeting aggression in severe mental illness: The predictive role of genetic, epigenetic, and metabolomic markers. *Progress in Neuro-Psychopharmacology and Biological Psychiatry* **77**, 32–41. DOI: 10.1016/j.pnpbp.2017.03.024.

MORRIS J., BAILEY M.E.S., BALDASSARRE D., CULLEN B., DE FAIRE U. et al. (2019): Genetic variation in *CADM2* as a link between psychological traits and obesity. *Sci. Rep.* **9**(1), 7339. DOI: 10.1038/s41598-019-43861-9.

MUSTAFIN R.N., KHUSNUTDINOVA E.K., KAZANTSEVA A.V., ENIKEEVA R.F., DAVYDOVA Y.D. et al. (2019): Epigenetics of aggressive behavior. *Russian Journal of Genetics* **55**(9), 1051–1060. DOI: 10.1134/S1022795419090096.

PAPPA I., ST POURCAIN B., BENKE K., CAVADINO A., HAKULINEN C. et al. (2016): A genome-wide approach to children's aggressive behavior: The EAGLE consortium. *Am. J. Med. Gene.t B. Neuropsychiatr. Genet*. **171**(5), 562–572. DOI: 10.1002/ajmg.b.32333.

PASMAN J.A., DEMANGE P.A., GULOKSUZ S., WILLEMSEN A.H.M., ABDELLAOUI A. et al. (2021): Genetic Risk for Smoking: Disentangling Interplay Between Genes and Socioeconomic Status. *Behav. Genet.* **52**(2), 92–107. DOI: 10.1007/s10519-021-10094-4.

PASMAN J.A., CHEN Z., SMIT D.J.A., VINK J.M., VAN DEN OEVER M.C. et al. (2022): The *CADM2* Gene and Behavior: A Phenome-Wide Scan in UK-Biobank. *Behav. Genet.* **52**(4-5), 306–314. DOI: 10.1007/s10519-022-10109-8.

PICARD M. & MCEWEN B. S. (2018): Psychological Stress and Mitochondria: A Systematic Review. *Psychosomatic medicine* **2**(80), 141. DOI: 10.1097/PSY.0000000000000545.

SALVATORE J.E., EDWARDS A.C., MCCLINTICK J.N., BIGDELI T.B., ADKINS A. et al. (2015): Genome-wide association data suggest *ABCB1* and immune-related gene sets may be involved in adult antisocial behavior. *Translational Psychiatry* **5**, 558. DOI: 10.1038/tp.2015.36.

SAUNDERS G.R.B., WANG X., CHEN F., JANG S.K., LIU M. et al. (2022): Genetic diversity fuels gene discovery for tobacco and alcohol use. *Nature* **612**, 720–724. DOI: 10.1038/s41586-022-05477-4.

SWANN A.C., LIJFFIJT M., O'BRIEN B. & MATHEW S.J. (2020): Impulsivity and Suicidal Behavior. in Recent Advances in Research on Impulsivity and Impulsive Behaviors. *Springer International Publishing* **47**, 179–195. DOI: 10.1007/7854_2020_144.

THORP J.G., CAMPOS A.I., GROTZINGER A.D., GERRING Z.F., AN J. et al. (2021): Symptom-level modelling unravels the shared genetic architecture of anxiety and depression. *Nat. Hum. Behav.* **5**(10), 1432–1442. DOI: 10.1038/s41562-021-01094-9.

TIELBEEK J.J., MEDLAND S.E., BENYAMIN B., BYRNE E.M., HEATH A.C. et al. (2012): Unraveling the genetic etiology of adult antisocial behavior: a genome-wide association study. *PLoS One* **7**(10), 45086. DOI: 10.1371/journal.pone.0045086.

TIELBEEK J.J., JOHANSSON A., POLDERMAN T.J.C., RAUTIAINEN M.R., JANSEN P. et al. (2017): Genome-Wide Association Studies of a Broad Spectrum of Antisocial Behavior. *JAMA Psychiatry* **74**(12), 1242-1250. DOI: 10.1001/jamapsychiatry.2017.3069.

TIELBEEK J.J., VINK J.M., POLDERMAN T.J.C., POPMA A., POSTHUMA D. et al. (2018): Genetic correlation of antisocial behaviour with alcohol, nicotine, and cannabis use. *Drug Alcohol Depend* **1**, 187:296-299. DOI: 10.1016/j.drugalcdep.2018.03.020.

WELTENS I., BAK M., VERHAGEN S., VANDENBERK E., DOMEN P. et al (2021): Aggression on the psychiatric ward: Prevalence and risk factors. A systematic review of the literature. *PLoS One* **16**(10), 0258346. DOI: 10.1371/journal.pone.0258346.