To: ***Acta Neuropsychiatrica***

Efficacy of intervention at traffic schools reducing impulsive action, and association with candidate gene variants

Kadi Luht[a,b], Tõnis Tokko[c], Diva Eensoo[d], Mariliis Vaht[c], Jaanus Harro[c*]

[a] *Department of Education, University of Tartu, Tartu, Estonia*

[b] *Rescue College, Estonian Academy of Security Sciences, Tallinn, Estonia*

[c] *Division of Neuropsychopharmacology, Department of Psychology, University of Tartu, Tartu, Estonia*

[d] *Department of Family Medicine and Public Health, University of Tartu, Tartu, Estonia*

* Corresponding Author: Jaanus Harro, MD, PhD, Division of Neuropsychopharmacology, Department of Psychology, University of Tartu, Ravila 14A Chemicum, 50411 Tartu, Estonia; E-mail: Jaanus.Harro@ut.ee

Abstract

Objective: Road traffic injuries are the leading cause of death among young people. Recognition of the contribution of impulsive behaviour may help novice drivers to behave more safely. Previously a brief intervention focusing on impulsive traffic behaviour conducted by psychologists in driving schools had been effective (Paaver et al., 2013; Eensoo et al., 2018). The aim of this study was an independent re-evaluation of the effect of the intervention, as conducted by driving school teachers, and assessment of the potential associations with candidate genotypes.

Methods: Driving school students (mean age 22.5, SD=7.9) were divided into intervention (n = 704) and control (n = 737) groups. Driving school teachers were trained to administer the intervention which consisted of a lecture and group work (1.5 h in total) on impulsivity. Traffic offences and crashes were monitored during three years, using police and traffic insurance fund databases. Functional polymorphisms of the dopamine transporter and serotonin transporter genes (*DAT1* VNTR and 5-HTTLPR) were assessed.

Results: The intervention significantly lowered general traffic risk and prevalence of traffic accidents. *DAT1* VNTR 9R carriers, particularly males, had higher general traffic risk in the whole sample. Female 5-HTTLPR s' allele carriers of the intervention group had the lowest general traffic risk. Intervention was most effective in female *DAT1* VNTR 10R/10R homozygotes.

Conclusions: Brief impulsivity-centred intervention appears as a promising strategy for preventing risk-taking behaviour in novice drivers and can be fully integrated to driving school curriculum.

Keywords: Impulsive Behavior; Dopamine; Serotonin; Genotype; Humans.

Significant outcomes

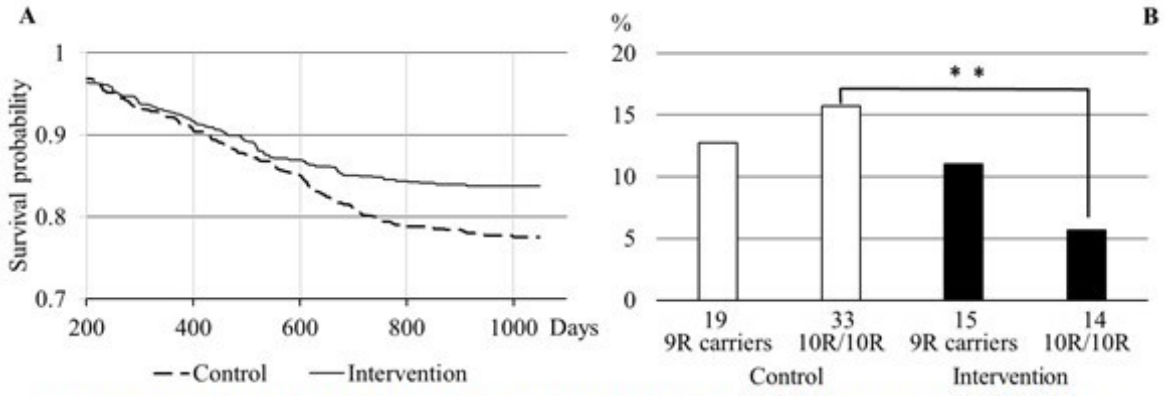
- Brief intervention in traffic schools can reduce traffic risks and prevent accidents.
- *DAT1* VNTR and 5-HTTLPR genotypes are associated with traffic behaviour.
- Efficacy of intervention may vary by genotype.

Limitations

- Reliability of stratified analyses would have benefited of larger sample than feasible in an intervention study.
- Questionnaire data were not available for all participants.

Running Head

Impulsivity and traffic accident prevention



During the three-year study period, (A) traffic offences or collisions occurred significantly less often in the intervention group ($p<0.005$); (B) this was most evident in female *DAT1* VNTR 10R homozygotes (**$p<0.01$).

Introduction

Road injuries killed 1.3 million people in 2015, being the tenth leading cause of death in the world, and the first for people in ages 15-29 (1). Road traffic collisions, injuries and mortality in traffic are strongly related to risk-taking behaviour (2-6). Focusing on information on risk and on change of attitudes has been found to produce little behavioural change (7,8). Teaching behavioural methods for controlling risky behaviour is a more effective approach to the prevention of crashes (9), as is the personal video-based analysis and feedback (10).

Decision-making in everyday life, including the traffic-related situations, is influenced by personality traits, and as to the traffic situations, particular significance can be attributed to impulsivity (11-14). We have previously shown that a brief intervention, guided by the affective neuroscience concept (15) and focusing on the acknowledgement of personal risks of impulsive traffic behaviour, can have a positive effect: The intervention group had only half as many speeding violations in the year following the intervention as compared to control (16), and the diminishing effect on drunk driving and traffic accidents was present through four years following the intervention (17).

Inter-individual differences in impulsivity and decision-making derive from genetic and developmental differences in brain function. The capacity of the brain serotonergic system and several gene variants that shape its function are strongly associated with impulse control (18-22), and measures of serotonergic activity have indeed been associated with risky traffic behaviour (23-25). At all serotonergic synapses the serotonin transporter plays a crucial role in the conduct of neurotransmission. The serotonin transporter gene promoter polymorphism (5-HTTLPR) (26) that has consequences to development in childhood (27,28) is associated with impulsivity (29,30), alcohol use (31-34), intent to drive while intoxicated (35) and actual speed limit exceeding and traffic accidents (17). The bulk of evidence suggests the 5-HTTLPR to be the “plasticity genotype”, the s-allele carriers being more

adaptive to the environment (36). The plasticity genotype concept would suggest that the s-allele carriers may be violating traffic regulations more often if these are not universally respected in the community and enforcement is not rigorous, but less often if the social norms strongly adhere with the law or if the regulations are seen personally fitting.

Besides the serotonergic system, the dopaminergic system has a major role in impulse control and risk-taking behaviour (37-41). The dopamine transporter (DAT) plays a critical role in terminating dopamine neurotransmission in the central nervous system (42), and the nine-repeat-allele (9R) of the DAT gene (*SLC6A3*) polymorphism (*DAT1* VNTR) is linked to lesser transporter activity and higher synaptic neurotransmitter levels (43-45). Van de Giessen et al. (46) and Faraone et al. (47) have shown that the *DAT1* VNTR 9R allele carriers have higher striatal DAT availability than do 10-repeat (10R) allele homozygotes and this could be associated with increased risk-taking in experimental paradigms (48). This is consistent with higher self-reported impulsivity in 9R allele carriers (49). Conclusively, the s-allele carriers of the 5-HTTLPR and 9R carriers of the *DAT1* VNTR could differ in traffic behaviour, and might be differently responsive to interventions aimed at reduction of impulsivity-related behaviours.

Aims of the study

The aim of this study was to re-evaluate the effect of a brief psychological intervention as conducted by driving school teachers who received training in applying the brief intervention technique previously successfully used by trained psychologists (16,17). We also assessed the potential association of the risk candidate genotypes (5- HTTLPR, also examined in the previous study, and newly *DAT1* VNTR) and their role as moderating factors to the eventual intervention effect.

Materials and methods

Participants

Twenty driving-schools agreed to participate in the study. After the initiation of the study, every first group formed of students applying for a passenger car driving license was assigned to the intervention condition, and every second group to the control condition. Out of 1746 subjects asked to participate in the study, in total 1441 (82.5%) (mean age 22.5 ± 7.9 years) agreed, and of these 43.3% were males. The intervention group included 321 (44%) males and 416 (56%) females, and the control group 303 (43%) males and 401 (57%) females. The study was approved by the Research Ethics Committee of the University of Tartu.

Procedure

The study was introduced by team members to the participants at the driving schools, collected the signed informed consent forms, and the saliva samples. The intervention „Reducing Impulsive Action in Traffic“ (16,17) consisted of a lecture (45 min) and group work (45 min) as previously described. This intervention was theoretically guided by the affective neuroscience concept (15) and aimed at acknowledgement of personal impulsive tendencies, so that subjects of intervention could build their own strategies to reduce personal risk. Lectures were carried out and the group work conducted by regular teachers of the driving schools, who had previously been trained in a tailor-made 2 European Credit Transfer and Accumulation System (ECTS) point course at the University of Tartu to carry out the intervention.

Questionnaires

After recruitment, the participants completed web-based self-report questionnaires. The Adaptive and Maladaptive Impulsivity Scale (AMIS), based on the concept of functional and dysfunctional impulsivity (50), was used to measure different facets of impulsivity: fast

decision-making, thoughtlessness, excitement seeking and disinhibition (51). Each facet was measured by 6 items on 5-point Likert scale. Subjects reported their relationship status, education and monthly income. The frequency of consuming strong and light alcoholic drinks during the previous year on a 6-point scale (none to almost every day) was reported (52). For assessing alcohol-related problems, five Diagnostic and Statistical Manual of Mental Disorders

(DSM-IV)diagnostic criteria for alcohol abuse, relating to specific life events (e.g., 'turned aggressive while drunk', 'had longer periods of alcohol use') were used. Subjects were categorized dichotomously based on whether they had ever experienced any alcohol-related problems or not.

Genotyping

Saliva samples (2 ml) were obtained from 1341 subjects (93.1% of total sample) using the SalivaGene® Collection module II (STRATEC Molecular GmbH, Berlin, Germany MACHEREYNAGEL GmbH & Co. KG, Düren, Germany). DNA was extracted from the samples using the NucleoSpin® Blood method (MACHEREY-NAGEL GmbH & Co KG) designed for extracting genomic DNA from various body fluids. Genotyping for the triallelic classification of the 5-HTTLPR polymorphism was performed according to Anchordoquy et al. (53). Genotyping was done in two stages. First all subjects were genotyped for the 5-HTTLPR VNTR polymorphism, then for single nucleotide polymorphism (SNP) rs25531 (A/G). The polymorphic region was amplified using the primers 5-HTTLPR-F: 5′-6FAM-ATG CCA GCA CCT AAC CCC TAA TGT-3′ and 5-HTTLPR-R: 5′-GGA CCG CAA GGT GGG CGG GA-3′. Then SNP rs25531 (A→G) was determined as described in detail elsewhere (54). Triallelic 5-HTTLPR genotypes were categorized into groups according to the effectiveness at the transcriptional level as follows: l_G/l_G, l_G/s, and s/s were designated as s'/s'; l_A/s and l_A/l_G as l'/s'; and l_A/l_A as l'/l'. Genotype frequencies were in the Hardy–

Weinberg equilibrium. As the original experiments have shown that the long allele of the 5-HTT gene has a more efficient promoter than the short allele, and that the l/s or s/s genotype cells (26), do not differ in this regard, we compared the s' allele carriers (s'/s' and l'/s'; n=895; 66.8%) with the l'/l' (n=444; 33.2%) homozygotes. This decision was also based on our previous study showing differences in traffic behaviour between the l'/l' homozygotes and s'-allele carriers (17). Distribution of the 5-HTTLPR genotype in control group (n=639) and intervention group (n=700) by gender is shown in Table 1. Genotype frequencies were not statistically significantly different between the groups.

The *DAT1* (SLC6A3) VNTR was genotyped following the analytical method by Anchordoquy et al. (53) as described in detail by Maksimov et al. (55). Polymorphic region were amplified using the primer rs28363170F: 5′ /56-FAM/TGT GGT GTA GGG AAC GGC CTG AG 3′ and rs28363170R: 5′ CTT CCT GGA GGT CAC GGC TCA AGG 3′ for *DAT1* 3′UTR VNTR. The VNTR repeat numbers range from 6 to 11, with 9 and 10-repeat alleles being the most common. Genotype frequencies were in the Hardy–Weinberg equilibrium. We compared the 9-repeat carriers (9R/9R and 9R/10R; n=502; 38.9%) and 10-repeat (10R/10R) homozygotes (n=810; 60.4%); subjects who had a rare VNTR genotype (10R/11R, 6R/10R) were excluded from the analysis. Distribution of the *DAT1* VNTR is shown in Table 1. Genotype frequencies were not statistically significantly different between the groups.

Table 1. Distribution of the participants by 5-HTTLPR and *DAT1* VNTR genotypes, gender and involvement in the intervention.

	Males		Females	
	Control	Intervention	Control	Intervention
	% (n)	% (n)	% (n)	% (n)
5-HTTLPR				
l'/l'	32.6 (89)	33.6 (102)	37.2 (136)	29.5 (117)
s' allele carriers	67.4 (184)	66.4 (202)	62.8 (230)	70.5 (279)
Total	100 (273)	100 (304)	100 (366)	100 (396)
DAT1 VNTR				
9R carriers	39.0 (105)	37.2 (112)	41.5 (149)	35.5 (136)
10R/10R	61.0 (164)	62.8 (189)	58.5 (210)	64.5 (247)
Total	100 (269)	100 (301)	100 (359)	100 (383)

Triallelic 5-HTTLPR genotypes were obtained for 1339 and *DAT1* VNTR for 1341 subjects; 29 subjects who had a rare VNTR genotype (10R/11R, 6R/10R) were excluded from the analysis.

Database search

Traffic offenses and crashes were monitored in the period of 01.01.2014 - 01.01.2017. Information about subjects obtaining the driving licence was derived from the Estonian Road Administration. Police and Border Guard Board database was used for collecting information about violations in traffic including drunk driving (penalties for drunk driving with an estimated blood alcohol level of 0.2‰ or more) and speed limit exceeding. Data on traffic accidents were received from the Traffic Insurance Fund database. Accidents in which the subject was at fault were classified as active and other accidents as passive. Subjects with occurrence of either recorded traffic offence or a collision were classified into the high general traffic risk group. From 278 subjects with high general traffic risk (occurrence of either a recorded traffic offence or a collision) 23 (8.3%) were also drunk drivers and 179 (64.4%) subjects with violations.

Statistical analysis

Data were analysed using IBM SPSS (version 22.0, Chicago, IL) and SAS (version 9.4 SAS Inc., Cary, NC) software. By survival analysis (Kaplan-Mayer estimates) probabilities of non-occurrence of traffic accidents and/or general traffic risk (survival probabilities) were compared between control and intervention groups. Cox regression analyses were used to investigate the effect of different variables upon the traffic accidents and on general traffic risk. By t-tests impulsivity was compared in groups by genotype. Pearson's Chi-square tests were used for comparison of distribution of traffic offences and accidents by participation in intervention, gender and/or by genotype.

Results

Effect of intervention on traffic behaviour and accidents

Control and intervention groups did not differ by gender, age, education, income, or impulsivity measures (data not shown). According to the Road Administration database the control and intervention groups did not differ significantly in any respect with regard to the obtaining of driving license. By the survival analysis, participants of the intervention group were significantly less likely in the general traffic risk group (p=0.004 for the log-rank test, DF=1, χ^2 =8.49) and, specifically, less involved in traffic accidents (p = 0.038 for the log-rank test, DF=1, χ^2=4.30) compared to controls during the three-year study period (Figure 1).

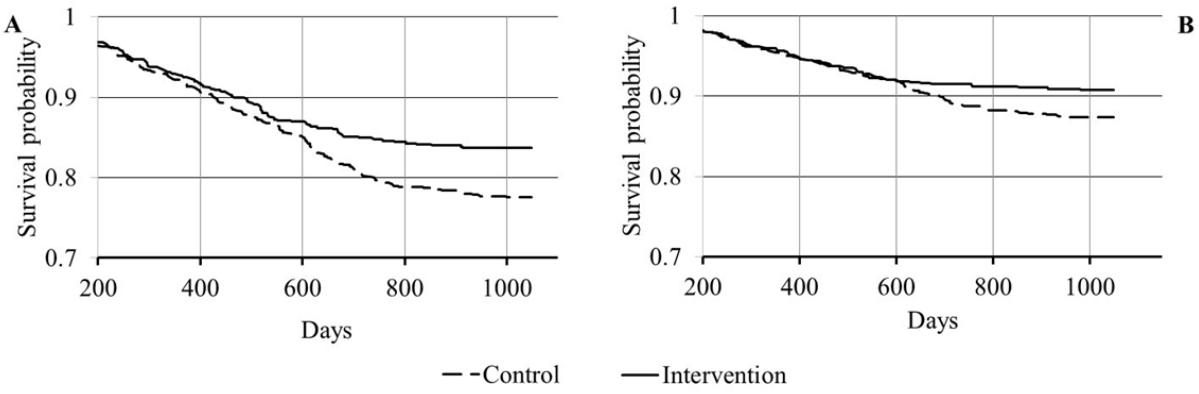


Figure 1. Occurrence of indicators of high general traffic risk (A, occurrence of either recorded traffic offence or a collision) and of traffic accidents (B) during the 3-year study period.

Table 2 presents the occurrence of traffic accidents and violations by intervention and gender. Both male and female intervention groups had significantly lower general traffic risk than control group, and intervention reduced the occurrence of both active and passive accidents in females.

Table 2. Traffic accidents and violations by gender and participation in the intervention.

	Males		Females		Total	
	Control	Intervention	Control	Intervention	Control	Intervention
	% (n)	% (n)	% (n)	% (n)	% (n)	% (n)
Traffic accidents	17.2 (52)	15.3 (49)	9.2 (37)	**4.6 (19)**	12.6 (89)	**9.2 (68)**
Passive traffic accidents	6.3 (19)	6.5 (21)	3.7 (15)	**1.7 (7)**	4.8 (34)	3.8 (28)
Active traffic accidents	12.5 (38)	10.6 (34)	6.0 (24)	**3.4 (14)**	8.8 (62)	6.5 (48)
All violations in traffic	24.4 (74)	19.6 (63)	5.7 (23)	4.6 (19)	13.8 (97)	11.1 (82)
Drunk driving	4.0 (12)	3.1 (10)	0.0 (0)	0.2 (1)	1.7 (12)	1.5 (11)
Speed limit exceeding	14.2 (43)	10.9 (35)	2.7 (11)	2.4 (10)	7.7 (54)	6.1 (45)
High general traffic risk[a]	34.3 (104)	**27.1 (87)**	13.5 (54)	**7.9 (33)**	22.4 (158)	**16.3 (120)**

Bold values represent statistical significance $p < 0.05$, significant difference compared to respective control group.

[a] Occurrence of either a recorded traffic offence or a collision.

Predicting traffic accidents and the general traffic risk

For further regression analyses we selected to two summary measures affected by the intervention, traffic accidents and high general traffic risk, as the remaining measures in Table 2 are contained in one or another. Using Cox regression, traffic accidents and high general traffic risk were first independently predicted by selected variables one by one (Table 3). For traffic accidents, gender and participation in the intervention were significant predictors. High general traffic risk was also predicted by gender and participation in the intervention, but additionally by the *DAT1* VNTR genotype, alcohol use, occurrence of alcohol-related problems, excitement seeking, fast decision-making and educational level. For clarifying how these significant predictors together influence the occurrence of traffic accidents and high general traffic risk, additional models were composed. If all single statistically significant predictors were included in a common model predicting accidents, the effect of the intervention remained significant (n=1441; -2 LOG L without covariate = 2225.82; -2 LOG L with gender as a covariate = 2190.44). While significant predictors for high general traffic risk from univariate analysis were included in the multivariate model, significance of the effect of intervention decreased (n=824; -2 LOG L without covariates = 1846.49; -2 LOG L with covariates = 1771.32).

Table 3. Cox regression models predicting participation in the traffic accident and high general traffic risk.

	Univariate analysis		Multivariate analysis	
	Traffic accident	General traffic risk	Traffic accident	General traffic risk
	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
1. Gender, male vs. female	**2.47 (1.78-3.42)**	**3.24 (2.51-4.17)**	**2.47 (1.78-3.43)**	**2.93 (2.05-4.20)**
2. Intervention, yes vs. no	**0.72 (0.52-0.98)**	**0.70 (0.56-0.89)**	**0.72 (0.52-0.98)**	0.72 (0.52-1.01)
3. 5-HTTLPR, s' carriers vs. l'/l'	0.97 (0.69-1.37)	0.91 (0.71-1.18)	-	-
4. *DAT1* VNTR, 9R carriers vs. 10R/10R	1.04 (0.72-1.44)	**1.28 (1.01-1.64)**	-	1.36 (0.97-1.89)
5. Alcohol related problems, yes vs. no	1.47 (0.90-2.39)	**1.86 (1.31-2.65)**	-	1.23 (0.81-1.87)
6. Frequency of using strong alcoholic beverages	1.14 (0.91-1.43)	**1.43 (1.20-1.70)**	-	1.11 (0.87-1.42)
7. Frequency of using light alcoholic beverages	1.12 (0.92-1.36)	**1.23 (1.06-1.43)**	-	1.09 (0.90-1.33)
8. Excitement seeking	1.05 (0.99-1.09)	**1.07 (1.04-1.10)**	-	1.03 (1.00-1.08)

9. Fast decision-making	1.02 (0.98-1.06)	**1.07 (1.04-1.11)**	-	1.01 (0.96-1.05)
10. Thoughtlessness	1.01 (0.97-1.05)	1.02 (0.99-1.05)	-	-
11. Disinhibition	0.96 (0.92-1.01)	0.99 (0.95-1.02)	-	-
12. Age	1.00 (0.98-1.02)	0.99 (0.98-1.01)	-	-
13. Education, high vs. low	0.80 (0.40-1.58)	**0.48 (0.25-0.91)**	-	0.70 (0.35-1.40)
14. Relationship status, couple vs. single	0.96 (0.63-1.46)	0.80 (0.57-1.11)	-	-
15. Income	1.03 (0.91-1.17)	1.01 (0.92-1.12)	-	-

Bold values represent statistical significance $p < 0.05$. HR – hazard ratio with 95 percent confidence intervals (CI).

5-HTTLPR genotype, traffic behaviour, and the intervention effect

5-HTTLPR s'-allele carriers had significantly lower mean score in fast decision making than l'/l' homozygotes (17.6, SD=4.5 vs. 18.1, SD = 4.0; p=0.008). This difference was more evident in females (16.8, SD=4.4 vs. 17.8, SD = 3.9; p=0.042). No other significant association between aspects of impulsivity and the genotype was found in this sample.

5-HTTLPR genotype had no significant predicting effect on general traffic risk and traffic accidents, neither in the total sample (Table 3) nor if stratified by gender (data not shown). However, the lowest proportion of traffic accidents or general traffic risk were observed in female s' allele carriers after intervention: for the general traffic risk (χ^2=(3)7.91; p=0.048) and for traffic accidents (χ^2=(3)8.70; p=0.034) (Table 4).

Table 4. Proportion (%) of subjects with traffic offences and accidents in subgroups by, gender, intervention, and 5-HTTLPR.

	Males				Females			
	Control		Intervention		Control		Intervention	
	l'/l'	s' carriers	l'/l'	s' carriers	l'/l'	s' carriers	l'/l'	s' carriers
Traffic accidents	16.9	18.5	11.8	16.8	11.0	9.1	6.8	**3.9**
Passive accidents	5.6	7.1	4.9	7.4	4.4	3.9	0.9	2.2
Active accidents	13.5	12.5	7.8	11.9	7.4	5.7	6.0	2.5
All violations in traffic	28.1	21.7	19.6	19.8	8.1	4.8	3.4	5.0
Drunk driving	4.5	3.3	2.9	3.0	0	0	0	0.4
Speed limit exceeding	15.7	12.5	11.8	10.4	2.2	3.5	3.4	2.2
High general traffic risk[a]	37.1	33.7	25.5	28.2	15.4	13.5	9.4	**7.5**

Bold values represent statistical significance $p < 0.05$. [a] Occurrence of either recorded traffic offence or a collision.

DAT1 VNTR genotype, traffic behaviour, and the intervention effect

The *DAT1* VNTR genotype was associated with traffic behaviour, but differently in males and females: While male 9R allele carriers had more frequently been driving drunk (odds ratio (OR) = 2.89; 95% CI = 1.12-7.47), and belonged more frequently to the high general traffic risk group (OR = 1.46; 95% CI = 1.02-2.10), in females the genotype was not significantly associated with traffic risk behaviour and there was rather a tendency for the 10R homozygotes to have higher traffic risk (Figure 2). The intervention effect was independent of genotype in males, but in females largely on account of the 10R/10R genotype.

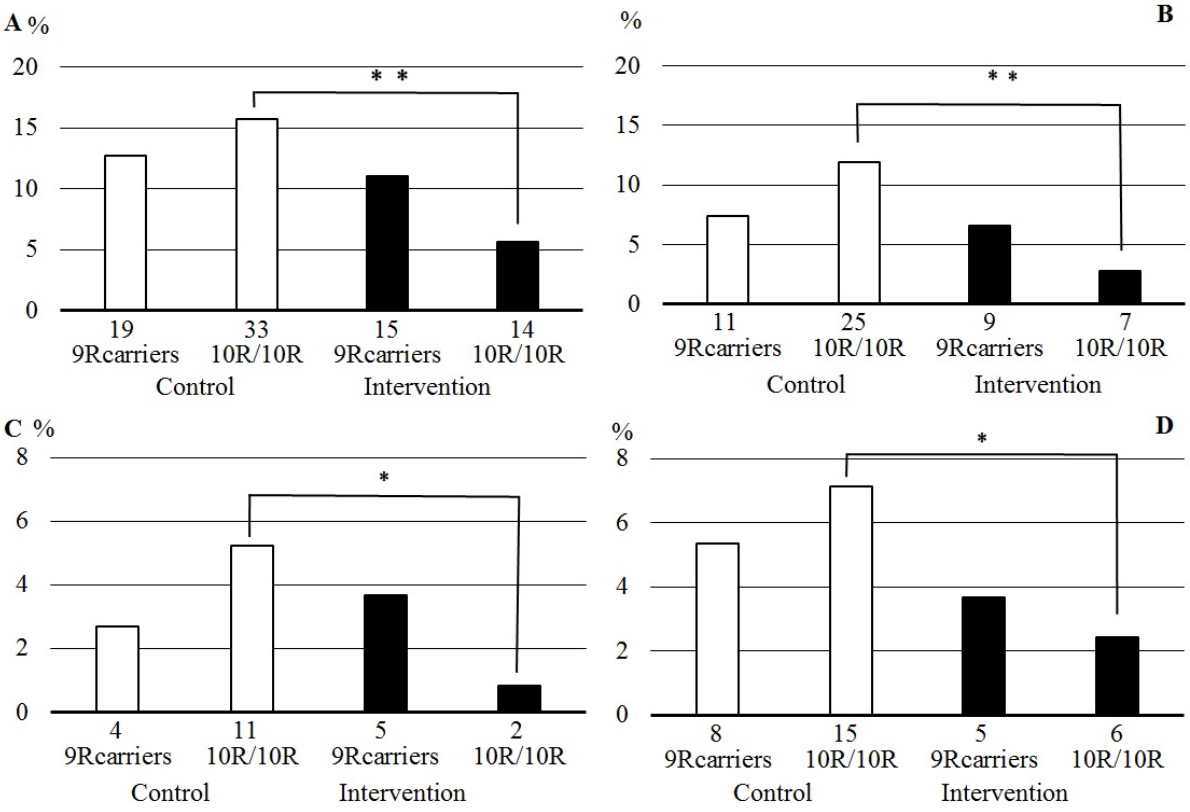


Figure 2. Proportion of females with high general traffic risk (A, occurrence of either recorded traffic offence or a collision), traffic accidents (B), passive (C), and active traffic accidents (D) by participation in intervention and DAT1 VNTR. Number of the cases presented below each column. Total number of female 9R carriers in the control group 149 and in the intervention group 136; total number of female 10R homozygotes in control group 210 and in the intervention group 247. *p<0.05, **p<0.01, significant difference.

Discussion

We have previously reported that a brief intervention with focus on personal psychological risk factors, included in the driving education program but conducted by psychologists, had a significant impact on traffic safety one year after the intervention (16), and that this impact persisted throughout the following four years (17). The affective neuroscience concept (15) leads to the conclusion that much of everyday behaviour, in particular in situations with high cognitive demand, is guided by activity in evolutionally old emotive circuits that cannot be controlled in real time; nevertheless, cognitive/behavioural strategies can be constructed to mitigate their adverse outcomes. The brief intervention in driving schools aimed at enhancing awareness of impulsivity and the health risks that impulsive action can bring about, to help students to spot and acknowledge impulsive tendencies both in themselves and in others, and to guide students to monitor themselves and to encourage them to develop personal strategies for mitigation of risks borne by impulsivity in traffic (16). The study reported herein was meant to attempt independent replication in a new sample, but with one important difference from the previous study: According to the training the trainers dissemination approach, we trained the traffic school teachers to deliver the intervention session, because in everyday practice this should be much more convenient and less demanding of resources as compared to the arrangement of psychologists visiting the traffic schools. During the three-year study period a significant impact of intervention on traffic safety was indeed present. Similarly to the previous investigation, the intervention had a positive effect on involvement in traffic offenses and accidents. At variance from the previous study the effect on traffic offences as analysed separately was not statistically significant. It should however be noted that this investigation was also conducted in a different overall traffic culture as it had significantly improved. This is well illustrated by changes in the most valid and robust measure, annual mortality by traffic injuries per one million inhabitants, which in 2007 (while the first intervention study started) was 146 but by the 2016 (while the monitoring period of the

current intervention study ended) had decreased to 36. Increasing the likelihood of a floor effect the improved traffic climate may have increased the demand on statistical power. Nevertheless, the aggregate measure of traffic safety was significantly lower in the intervention group, suggesting that the training the trainers approach is feasible for the delivery of the impulsivity-focused instrument. Further studies should address the feasibility of internet-based training instruments as drivers' education varies in different settings.

The effect of intervention was not affected by consideration of a number of factors known to be associated with risk-taking in traffic. It is known that males take more risks in traffic (56), lower education level is associated with higher risk (57) and several studies, including our own, have reported higher general risks in association with alcohol-related problems (24,58,59). The findings in the present study are thus consistent with earlier research. Facets of impulsivity increase the risk in traffic (12,13,25), and the results of the present study highlight excitement seeking and fast decision-making. These two impulsivity facets were, amongst the studied impulsivity measures, also the most significant predictors of general traffic risk in our previous study on a different traffic school sample (16). Nevertheless, the association of intervention with reduction of traffic accidents and risk-taking was largely similar in multivariate analyses and apparently the acknowledgment of impulsivity in traffic can be broadly usable strategy.

It is however plausible that some subjects may be more and some less malleable to the intervention, and because our research strategy involves the aspect of precision medicine by consideration of common functional gene variants, we included two candidate genes into the study. We had previously studied the association of the 5-HTTLPR genotype with traffic behaviour and the effect of intervention (17): Previously it had been reported that the 5-HTTLPR s' allele carriers consumed more drinks at bars and the s'/s' homozygotes expressed

higher intention to drive a motor vehicle after drinking (35). Longitudinal population-representative studies have indeed described earlier and higher alcohol use in 5-HTTLPR s'/s' homozygotes (31,32). In general, lower prevalence of traffic incidents was found in the 5-HTTLPR s' allele carriers in our previous study, and the effect of intervention was largely observed in l'/l' homozygotes probably owing to the floor effect in the s'-allele carriers (17). In the present study the overall association of 5-HTTLPR with behaviour in traffic was not statistically significant. A likely explanation to this discrepancy could again be found in the overall improved traffic culture: For example, in the previous study the prevalence of speeding offences among females of the control group was 18% in the 5-HTTLPR l'/l' homozygotes vs. 4% in s'-allele carriers, but in the present study, several fold less (Table 4). However, consistently with the previous study, the female s'-allele carriers of the intervention group were the safest drivers in traffic. This also fits nicely with the notion that the 5-HTTLPR s'-allele is associated with higher social cohesion, and particularly so in females.

Among males the proportion of *DAT1* VNTR 9R carriers was higher among drunk drivers and subjects with high general traffic risk. These results support the significant role of dopaminergic system in impulse control and risk-taking behaviour (37-41) and the potentially higher risk in *DAT1* VNTR 9R allele carriers (45,46,60), including in traffic (61). In females, carrier status of the *DAT1* VNTR 9R allele had no significant association with traffic behaviour at baseline, but prevented the intervention effect to occur. This suggests that under different environmental conditions the 9R allele might appear as a risk allele even in females, and that other type of interventions may be more adequate for this group.

Limitation of the study to be borne in mind is the sample size dictated by the feasibility for an intervention study. Especially the genetic analyses should be regarded as exploratory for this reason. Also, we used several self-administered questionnaires and the

more uncomfortable questions (e.g., alcohol usage) were missed by a number of participants. Larger studies should be conducted to allow for more reliable stratified analyses.

In conclusion, the brief intervention conducted by driving school teachers had a significant impact on traffic safety and could be a part of curricula at driving schools. An important aspect in planning interventions is to recognize the differences, in part genetic, within the target population that suggest the necessity of combining a variety of measures.

Acknowledgements

We are grateful to the EPSTB participants, and the whole study team. This work was supported by the Estonian Research Council (3.2.1002.11-0002, TerVE VIGA and IUT20-40], the EC FP7 project Aggressotype (FP7-Health-2013-Innovation-1, 602805), the EC Horizon 2020 projects CoCA (H2020-PHC-2015-667302) and Eat2beNICE (H2020-SFS-2016-728018), and the Estonian Road Administration.

Authors' Contribution

JH and DE designed the study and coordinated data collection. MV peformed genotyping genotyping. KL, TT and DE contributed to the literature searches, and conducted statistical analysis. KL wrote the first draft of the manuscript. All authors contributed to the acquisition and analysis of the data, critically reviewed the content, edited the draft manuscript, and approved the final version for publication.

Financial Support

Financial support was received from the Estonian Research Council (3.2.1002.11-0002, TerVE VIGA and IUT20-40], the EC FP7 project Aggressotype (FP7-Health-2013-Innovation-1, 602805), the EC Horizon 2020 projects CoCA (H2020-PHC-2015-667302) and Eat2beNICE (H2020-SFS-2016-728018), and the Estonian Road Administration.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Ethical Standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

References

1. WORLD HEALTH ORGANIZATION (WHO). Global Health Estimates 2015: Deaths by Cause, Age, Sex, by Country and by Region, 2000-2015. Geneva: World Health Organization; 2016.

2. OUIMET MC, PRADHAN AK, BROOKS-RUSSELL A, EHSANI JP, BERBICHE D, SIMONS- MORTON BG. Young drivers and their passengers: A systematic review of epidemiological studies on crash risk. Journal of Adolescent Health 2015;**57(S1)**:S24–35.

3. PANAYIOTOU G. The bold and the fearless among us: elevated psychopathic traits and levels of anxiety and fear are associated with specific aberrant driving behaviors. Accident Analysis & Prevention 2015;**79**:117–125.

4. SAGBERG F, SELPI S, PICCININI GF, ENGSTRÖM J. A review of research on driving styles and road safety. Human Factors 2015;**57**:1248–1275.

5. SMORTI M, GUARNIERI S. Do aggressive driving and negative emotional driving mediate the link between impulsiveness and risky driving among young Italian drivers? The Journal of Social Psychology 2016;**156**:669–673.

6. FUERMAIER AB, TUCHA L, EVANS BL, KOERTS J, DE WAARD D, BROOKHUIS K, ASCHENBRENNER S, THOME J, LANGE KW, TUCHA O. Driving and attention deficit hyperactivity disorder. Journal of Neural Transmission 2017;**124(S1)**:55–67.

7. MAES S, BOERSMA SN. Applications in health psychology: How effective are interventions? In: Sutton S, Baum A, Johnston M, editors. The Sage Handbook of Health Psychology, London: Sage; 2004, p. 299−325.

8. FRANK RG, LEE AM. Accidents and unintentional injuries. In: Ayers S, Baum A, McManus C, Newman S, Wallston K, Weinman J, West R, editors. Cambridge

Handbook of Psychology, Health and Medicine, New York: Cambridge University Press; 2007, p. 527−529.

9. MAKEHAM P. Traffic education strategy. In: von Holst H, Nygren Å, Andersson ÅE, editors. Transportation. Traffic Safety and Health: Human Behaviour, Berlin Heidelberg: Springer- Verlag; 2000, p. 133−162.

10. CARNEY C, MCGEHEE D, LEE J, REYES M, RABY M. Using an event-triggered video intervention system to expand the supervised learning of newly licensed adolescent drivers. American Journal of Public Health 2010;**100(6):**1101–1106.

11. JONAH BA. Sensation seeking and risky driving: a review and synthesis of the literature. Accident Analysis & Prevention 1997; **29(5)**:651–665.

12. BARKLEY RA, COX DA. Review of driving risks and impairments associated with attention-deficit/hyperactivity disorder and the effects of stimulant medication on driving performance. Journal of Safety Research 2007;**38(1)**:113–128.

13. BICAKSIZ P, ÖZKAN T. Impulsivity and driver behaviors, offences and accident involvement: A systematic review. Transportation Research Part F 2016;**38**:194–223.

14. ALAVI SS, MOHAMMADI MR, SOURI H, KALHORI SM, JANNATIFARD F, SEPAHBODI G. Personality, driving behavior and mental disorders factors as predictors of road traffic accidents based on logistic regression. Iranian Journal of Medical Sciences 2017;**42(1)**:24–31.

15. PANKSEPP J. Affective Neuroscience: The Foundations of Human and Animal Emotions. New York, NY, US: Oxford University Press; 1998.

16. PAAVER M, EENSOO D, KAASIK K, VAHT M, MÄESTU J, HARRO J. Predicting risky driving: A novel and efficient brief intervention focusing on acknowledgement of personal risk factors. Accident Analysis & Prevention 2013;**50**:430–437.

17. EENSOO D, PAAVER M, VAHT M, LOIT HM, HARRO J. Risky driving and the persistent effect of a randomized intervention focusing on impulsivity: The role of the serotonin transporter promoter polymorphism. Accident Analysis & Prevention 2018;**113**:19–24.

18. EVENDEN JL. Varieties of impulsivity. Psychopharmacology. 1999;146:348–361.

19. TAKAHASHI H, TAKANO H, CAMERER CF, IDENO T, OKUBO S, MATSUI H, TAMARI Y, TAKEMURA K, ARAKAWA R, KODAKA F, YAMADA M, EGUCHI Y, MURAI T, OKUBO Y, KATO M, ITO H AND SUHARA T. Honesty mediates the relationship between serotonin and reaction to unfairness. Proceedings of the National Academy of Sciences of the United States of America 2012;**109**:4281–4284.

20. CUNNINGHAM KA, ANASTASIO NC. Serotonin at the nexus of impulsivity and cue reactivity in cocaine addiction. Neuropharmacology 2014;**76(B)**:460–478.

21. TOMSON K, VAHT M, LAAS K, VEIDEBAUM T, HARRO J. Effect of a human serotonin 5-HT_{2A} receptor gene polymorphism on impulsivity: Dependence on cholesterol levels. Journal of Affective Disorders 2016;**206**:23–30.

22. LAAS K, KIIVE E, MÄESTU J, VAHT M, VEIDEBAUM T, HARRO J. Nice guys: Homozygocity for the TPH2 -703G/T (rs4570625) minor allele promotes low aggressiveness and low anxiety. Journal of Affective Disorders 2017;**215**:230–236.

23. EENSOO D, PAAVER M, PULVER A, HARRO M, HARRO J. Low platelet MAO activity associated with high dysfunctional impulsivity and antisocial behavior: Evidence from drunk drivers. Psychopharmacology 2004; **172(3):**356–358.

24. PAAVER M, EENSOO D, PULVER A. HARRO J. Adaptive and maladaptive impulsivity, platelet monoamine oxidase (MAO) activity and risk-admitting in different types of risky drivers. Psychopharmacology 2006;**186(1)**:32−40.

25. LUHT K, EENSOO D, TOODING LM, HARRO J. The association of measures of the serotonin system, personality, alcohol use, and smoking with risk-taking traffic behavior in adolescents in a longitudinal study. Nordic Journal of Psychiatry 2018;**72(1)**:9–16.

26. LESCH K-P, BENGEL D, HEILS A, SABOL SZ, GREENBERG BD, PETRI S, BENJAMIN J, MÜLLER CR, HAMER DH and MURPHY DL. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science 1996;**274**:1527–1531.

27. CANLI T, LESCH K. Long story short: The serotonin transporter in emotion regulation and social cognition. Nature Neuroscience 2007;**10**:1103–1109.

28. BRODY G, BEACH S, PHILIBERT R, CHEN Y, MURRY V. Prevention effects moderate the association of 5-HTTLPR and youth risk behavior initiation: gene × environment hypotheses tested via a randomized prevention design. Child Development 2009:**80(3);**645–661.

29. STEIGER H, JOOBER R, ISRAËL M, YOUNG SN, NG YING KIN NM, GAUVIN L, BRUCE KR, JONCAS J and TORKAMAN-ZEHI A. The 5HTTLPR polymorphism, psychopathologic symptoms, and platelet [^{3}H]paroxetine binding in bulimic syndromes. International Journal of Eating Disorders 2005;**37(1):**57–60.

30. PAAVER M, KURRIKOFF T, NORDQUIST N, ORELAND L, HARRO J. The effect of 5-HTT gene promoter polymorphism on impulsivity depends on family relations in girls. Progress in Neuro-Psychopharmacology & Biological Psychiatry 2008;**32(5):**1263–8.

31. MERENÄKK L, MÄESTU J, NORDQUIST N, PARIK J, ORELAND L, LOIT H-M and HARRO J. Effects of the serotonin transporter (5-HTTLPR) and α_{2A}-adrenoceptor (C-1291G) genotypes on substance use in children and adolescents: a longitudinal study. Psychopharmacology 2011**;215**:13–22.

32. VAHT M, MERENÄKK L, MÄESTU J, VEIDEBAUM T, HARRO J. Serotonin transporter gene promoter polymorphism (5-HTTLPR) and alcohol use in general population: interaction effect with birth cohort. Psychopharmacology 2014;**231**:2587–2594.

33. DE OLIVEIRA CE, ODA JM, ARIZA CB, GUEMBAROVSKI RL, HIRATA BK, DE ALMEIDA FC, ANDRÉ ND, FUNGARO MH and WATANABE MA. Genetic polymorphism in the promoter region of serotonin transporter: implications for ethanol abuse in children and adolescents. Journal of the Canadian Academy of Child and Adolescent Psychiatry 2016;**25(1)**:43–9.

34. COPE LM, MUNIER EC, TRUCCO EM, HARDEE JE, BURMEISTER M, ZUCKER RA and HEITZEG MM. Effects of the serotonin transporter gene, sensitivity of response to alcohol, and parental monitoring on risk for problem alcohol use. Alcohol 2017;**59**:7–16.

35. THOMBS DL, O'MARA RJ, HOU W, WAGENAAR AC, DONG HJ, MERVES ML, GOLDBERGER BA, WEILER RM, DODD VJ and CLAPP JD. 5-HTTLPR genotype and associations with intoxication and intention to drive: results from a field study of bar patrons. Addiction Biology 2011;**16**:133–141.

36. HOMBERG JR, LESCH K-P. Looking on the bright side of serotonin transporter gene variation. Biological Psychiatry 2011;**69**:513–9.

37. DE WIT H, ENGGASSER J, RICHARDS J. Acute administration of d-amphetamine decreases impulsivity in healthy volunteers. Neuropsychopharmacology 2002;**27(5)**:813–825.

38. FRIEDEL R. Dopamine dysfunction in borderline personality disorder: A hypothesis. Neuropsychopharmacology 2004;29(6):1029–1039.

39. THAPAR A, O'DONOVAN M, OWEN MJ. The genetics of attention deficit hyperactivity disorder. Human Molecular Genetics 2005;**14(S2):**R275–R282.

40. FRIED R, PETTY CR, SURMAN CB, REIMER B, ALEARDI M, MARTIN JM, COUGHLIN JF and BIEDERMAN J. Characterizing impaired driving in adults with attention-deficit/hyperactivity disorder: A controlled study. Journal of Clinical Psychiatry 2006;**67(4):**567–574.

41. CONGDON E, LESCH KP, CANLI T. Analysis of DRD4 and DAT polymorphisms and behavioral inhibition in healthy adults: Implications for impulsivity. American Journal of Medical Genetics 2008(B);**147**:27–32.

42. CHEN N, REITH ME. Structure and function of the dopamine transporter. European Journal of Pharmacology 2000;**405(1):**329–339.

43. FUKE S, SUO S, TAKAHASHI N, KOIKE H, SASAGAWA N, ISHIURA S. The VNTR polymorphism of the human dopamine transporter (DAT1) gene affects gene expression. The Pharmacogenomics Journal 2001;**1**:152–156.

44. MILL J, ASHERSON P, BROWES B, D'SOUZA U, CRAIG I. Expression of the dopamine transporter gene is regulated by the 39 UTR VNTR: Evidence from brain and

lymphocytes using quantitative RT-PCR. American Journal of Medical Genetics 2002;**114**:975–979.

45. VANNESS SH, OWENS MJ, KILTS CD. The variable number of tandem repeats element in DAT1 regulates in vitro dopamine transporter density. BMC Genet 2005;**6**:55.

46. VAN DE GIESSEN EM, DE WIN ML, TANCK MT, VAN DEN BRINK W, BAAS F, BOOIJ J. Striatal dopamine transporter availability associated with polymorphisms in the dopamine transporter gene SLC6A3. Journal of Nuclear Medicine 2009;**50(1)**:45–52.

47. FARAONE SV, SPENCER TJ, MADRAS BK, ZHANG-JAMES Y, BIEDERMAN J. Functional effects of dopamine transporter gene genotypes on in vivo dopamine transporter functioning: a meta-analysis. Molecular Psychiatry 2014;**19(8)**:880–889.

48. HEITLAND I, OOSTING RS, BAAS JM, MASSAR SA, KENEMANS JL, BÖCKER KB. Genetic polymorphisms of the dopamine and serotonin systems modulate the neurophysiological response to feedback and risk taking in healthy humans. Cognitive, Affective, & Behavioral Neuroscience 2012;**12(4)**:678–691.

49. FORBES E, BROWN S, KIMAK M, FERRELL R, MANUCK S, HARIRI A. Genetic variation in components of dopamine neurotransmission impacts ventral striatal reactivity associated with impulsivity. Molecular Psychiatry 2009;**14(1)**:60–70.

50. DICKMAN SJ. Functional and dysfunctional impulsivity: personality and cognitive correlates. Journal of Personality and Social Psychology 1990;**58**:95–102.

51. LAAS K, REIF A, HERTERICH S, EENSOO D, LESCH KP, HARRO J. The effect of a functional NOS1 promoter polymorphism on impulsivity is moderated by platelet MAO activity. Psychopharmacology 2010;**209(3)**:255–261.

52. EENSOO D, PAAVER M, HARRO M, HARRO J. Predicting drunk driving: Contribution of alcohol use and related problems, traffic behaviour, personality and platelet monoamine oxidase (MAO) activity. Alcohol and Alcoholism 2005;**40(2)**:140–146.

53. ANCHORDOQUY HC, MCGEARY C, LIU L, KRAUTER KS, SMOLEN A. Genotyping of three candidate genes after whole-genome preamplification of DNA collected from buccal cells. Behavior Genetics 2003;**33(1)**:73–78.

54. TOMSON K, MERENÄKK L, LOIT H-M, MÄESTU J, HARRO J. The relationship between serotonin transporter gene promoter polymorphism and serum lipid levels at young age in a longitudinal population-representative study. Progress in Neuro-Psychopharmacology & Biological Psychiatry 2011;**35**:1857–1862.

55. MAKSIMOV M, VAHT M, MURD C, HARRO J, BACHMANN T. Brain dopaminergic system related genetic variability interacts with target/mask timing in metacontrast masking. Neuropsychologia 2015;**71**:112–118.

56. TURNER C, MCCLURE R. Age and gender differences in risk-taking behaviour as an explanation for high incidence of motor vehicle crashes as a driver in young males. International Journal of Injury Control and Safety Promotion 2003;**10(3)**:123–130.

57. HARPER S, CHARTERS T, STRUMPF E. Trends in socioeconomic inequalities in motor vehicle accident deaths in the United States, 1995–2010. American Journal of Epidemiology 2015;**182(7)**:606–614.

58. EENSOO D, HARRO M, PULLMANN H, ALLIK J, HARRO J. Association of traffic behavior with personality and platelet monoamine oxidase activity among schoolchildren. Journal of Adolescent Health 2007;**40(4)**:311–317.

59. VAN DYKE N, FILLMORE MT. Alcohol effects on simulated driving performance and self-perceptions of impairment in DUI offenders. Experimental and Clinical Psychopharmacology 2014;**22**:484–493.

60. GUO G, CAI T, GUO R, WANG H, HARRIS KM. The dopamine transporter gene, a spectrum of most common risky behaviors, and the legal status of the behaviors. PLoS One 2010;**5(2)**:e9352.

61. TOKKO T, EENSOO D, VAHT M, LESCH KP, REIF A, HARRO J. Relapse of drunk driving and association with traffic accidents, alcohol-related problems, and biomarkers of impulsivity. Acta Neuropsychiatrica 2019;**31**:84–92. doi: 10.1017/neu.2018.30