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Gene Polymorphisms Associated with Sleep and Cognitive Functions and Their Associations with Accident Proneness in Shift-Working Bus Drivers

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The aim of the present work was to investigate a series of single-nucleotide gene polymorphisms and their associations with accident proneness in bus drivers. The study involved 299 shift-working professional drivers for whom accident statistics were available. Polymorphisms in genes associated with the sleep-waking rhythm and cognitive and emotional functions were investigated, i.e., *CLOCK* (rs12649507), *RORA* (rs1159814), *NPAS2* (rs4851377), *NPSR1* (rs324981), *PER3* (rs2640909), *DRD3* (rs6280), *SLC6A3* (rs6347), and *DBH* (rs1611125). The study identified significant associations between accident parameters and polymorphisms in the *CLOCK*, *NPSR1*, and *SLC6A3* genes. We suggest that these are due to differences in chronotype and resistance to impairments to the sleep regime for the *CLOCK* gene and in cognitive and emotional control for the *NPSR1* and *SLC6A3* genes.

Keywords: genetic polymorphisms, single-nucleotide polymorphisms, accident proneness, driving, shift work.

Drivers of public transport – a responsible profession associated with considerable tension – require constant monitoring of traffic situations and high levels of vehicle control skills. Safe driving requires constant attention, involvement of memory, readiness to mount rapid reactions, and effective assessments of potential danger. Public transport operates from early in the morning to late at night, which means that professional drivers are required to undertake shift work. They are unable to avoid working at suboptimal times of day. Slowing of reaction times, cognitive difficulty, decreases in the level of arousal, and drowsiness can influence the quality of driving and contribute to increased risk of road traffic accidents (RTA) [Wagstaff and Sigstad Lie, 2011].

The quality of driving and the risk of being involved in an RTA depend on multiple internal and external factors. These include stable psychophysiological characteristics which are important for any professional activity: the chronotype (the preferred rhythm of sleep and waking), control of attention, and the sensory and emotional domains [Barrash et al., 2010; Johnson et al., 2014]. The risk of monotony arises in normal traffic situations, whereby the work done becomes unstable [Kiroi and Aslanyan, 2005].

Work after a night shift is a risk factor for situations involving the risk of accident [Lee et al., 2016]. Because of individual differences in the functioning of internal biological clocks, a person may be worse or better adapted to nonstandard working schedules and lack of sleep may be more or less severe [Goel et al., 2013]. The severity of the reac-

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TABLE 1. Statistics of Involvement in RTA in the Study Cohort of Drivers (number of drivers $N = 299$)

Numbers and proportions of drivers	Number of RTA			
	0	1	2	3
Involved in RTA	193	68	30	8
Proportion (%)	64.6	22.7	10	2.7
Fault RTA	248	35	14	2
Proportion (%)	82.9	11.7	4.7	0.7
No-fault RTA	229	58	11	1
Proportion (%)	76.6	19.4	3.7	0.3

tion to sleep deprivation is individual and is quite stable for any particular person [Chua et al., 2014; Spaeth et al., 2012].

Inherited contributions have now been demonstrated for many psychophysiological and neurobehavioral parameters of importance for professional activity [Puchkova and Dorokhov, 2015]. The context of these studies raises the question: can the effects of genetic differences at higher behavioral levels be identified, for example, in driving? Thus, our work sought associations between candidate genetic polymorphisms and measures of driving safety as RTA statistics. We also analyzed correlations between genetic variability at selected single-nucleotide gene polymorphisms (SNP) and chronotype parameters in drivers. The preliminary results have been published [Dorokhov et al., 2015].

The present study addressed SNP associated with the operation of biological clocks and the sleep-waking regime: *CLOCK* [Allebrandt et al., 2010] and *NPSR1* [Gafarov et al., 2015]; cognitive and affective control, impulsivity and attention: *NPSR1* [Laas et al., 2015; Neufang et al., 2015; Ruland et al., 2015]; control of the operation of the dopamine system: *SLC6A3* [Tiwari et al., 2013; Sullivan et al., 2013; Pinsonneault et al., 2011], *DRD3* [Nemoda et al., 2011], and *DBH* [Ziermans et al., 2012]. SNP in genes associated with the operation of biological clocks for which no direct association with sleep has yet been demonstrated were also selected: *RORA* (rs1159814), *NPAS2* (rs4851377), and *PER3* (rs2640909) [Hu et al., 2016]. Associations between these SNP with accident proneness were analyzed in shift-working bus drivers.

Methods. Shift-working professional drivers of shuttle buses operating in Moscow suburban depots for whom accident statistics throughout their period of work at their depots were available took part in the study. They worked six shifts each of duration 8–10 h starting at 03:30, 06:30, 09:30, 12:30, 15:30, and 17:30.

Accident statistics included the number of RTA while working at their depots and whether incidents were fault or no-fault according to RTA protocols. The group included all workers with the largest numbers of RTA and some with the best RTA statistics. For some participants, the amount of work experience at the depot was known, and the ratio of the number of RTA to the experience of work (days) was determined. Subjects, having given consent, also provided biological specimens for genotyping. Genetic studies evaluated SNP in the following genes: *CLOCK* (rs12649507), *RORA* (rs1159814), *NPAS2* (rs4851377), *NPSR1* (rs324981), *PER3* (rs2640909), *SLC6A3* (rs6347), and *DBH* (rs1611125). Genomic DNA was extracted from cell samples from the mucous membrane of the cheek.

Genotyping of SNP was performed using the real-time polymerase chain reaction (PCR) by the TaqMan method. PCR was run on a Step One Plus amplifier. The 25-μl mix for amplification contained 2.5 μl 10× PCR buffer, 2.5 μl 25 mM $MgCl_2$, 2.5 μl 2.5 mM dNTP solution, each primer at 10 pM (TaqMan primers), each probe at 4 pM, 1.25 U of thermostable DNA polymerase (Taq polymerase, Fermentas), 0.1–0.2 μg of genomic DNA, and deionized water to 25 μl. Genotyping was performed at the Laboratory for Molecular Genetics of Inherited Diseases, Institute of Molecular Genetics, Russian Academy of Sciences.

The study was performed in compliance with the standards of the Helsinki Declaration and was approved by the Ethics Committee of the Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences; all participants provided informed consent to take part.

Results were analyzed statistically in Statistica 7.0. Differences were regarded as significant at $p < 0.05$.

Results. A total of 299 male drivers aged 21–68 (mean 45.8 ± 11.8) years took part in the study. Accident statistics showed that 35.4% of drivers had been involved in at least one RTA while working; the maximum number of RTA per driver was three (eight drivers, 2.7%). A total of 152 RTA were recorded, of which 69 (45.4%) were the drivers' fault. A total of 82.9% of the drivers of this group were not responsible for any accidents (Table 1).

Accidents which were not the driver's fault could not always be regarded as analogous to fault accidents, so the analysis used both the total number of accidents and the number of accidents stratified on the basis of fault/no fault.

Work experience was known for 162 drivers at the depot at the moment of collecting accident statistics. This

TABLE 2. Frequencies of Minor Variants of the Study Polymorphisms in the Group of Drivers

SNP	Allelic variants	Minor allele	Minor homozygotes, %	Frequency of minor allele, %	Number of specimens, *N*
CLOCK (rs12649507)	A/G	A*	14.1	34.5	213
RORA (rs1159814)	C/T	C	25.9	45.0	212
NPSRI (rs324981)	A/T	A*	21.0	47.9	214
PER3 (rs2640909)	C/T	C*	3.3	22.2	212
DRD3 (rs6280)	C/T	C	10.0	26.3	150
SLC6A3 (rs6347)	A/G	G*	7.8	28.4	153
DBH (rs1611125)	C/T	C*	23.8	48.3	151
NPAS2 (rs4851377)	C/T	C*	19.2	45.7	151

*Alleles complying with Hardy–Weinberg equilibrium.

ranged from 20 to 4382 days (mean 1512 ± 1424 days, median 839 days). The accident proneness coefficient was calculated for these drivers, i.e., the ratio of the number of RTA to the amount of work experience in days. The mean values of this coefficient was 0.00098 ± 0.00248 (median 0, lower and upper quartiles 0 and 0.001). Overall, this parameter characterizes professional drivers as quite safe – most, even with long experience, had had small numbers of RTA.

Analysis of the link between the number of RTA and age showed a small but significant correlation ($r = 0.30$, $p < 0.001$). However, we suggest that this is due to the greater duration of work experience among older drivers: in the study group, only drivers more than 42 years old had had work experience of more than 2500 days, while drivers aged less than 30 years had no more than 1100 days of experience.

Overall, the group showed a smooth increase in the number of accidents with increases in work experience. Although there were drivers with little experience who had been involved in three RTA as well as four drivers with more than 3000 days of experience who had had no RTA, the general trend was for a significant increase in the number of RTA (Kruskal–Wallis test. H(3; 160) = 13.9935, $p < 0.01$), regardless of blame (Fig. 1). The uniform trend was lost when statistics were stratified in terms of blame for RTA, partly because of the small numbers of drivers with two or more RTA in each category.

Genotyping results were in accord with Hardy–Weinberg equilibrium for the polymorphisms of all genes apart from *RORA* and *DRD3*, which can be linked with the small cohort size or specific work-related features (Table 2). In seeking associations between genotype and measures of accident proneness we conduced correlation analysis (gamma correlation) between the numbers of the minor alleles (0, 1, or 2) and the number of accidents and the accident proneness coefficient. The distributions of genotypes in

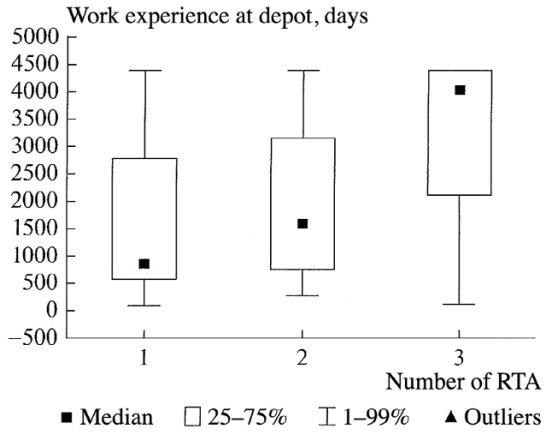

Fig. 1. Interaction between statistics for RTA in which drivers were involved and work experience at depots (number of drivers $N = 162$, total number of RTA = 152).

groups involved and not involved in accidents and being to blame and not to blame for accidents (2×2 and 2×3 χ^2 test and the Fisher–Friedman–Holton exact test) were also assessed.

Significant correlations ($p < 0.05$) were found for polymorphisms of the *CLOCK*, *RORA*, *NPSR1*, *SLC6A3*, and *DRD3* genes. After application of the Bonferroni correction for multiple comparisons, the following associations were significant: for the number of RTA in which the driver was at fault and the minor allele of the SNP of the *CLOCK* gene, $r = –0.32$; for the number of RTA in which the driver was not at fault and the minor allele of the SNP of the *NPSR1* gene, $r = 0.30$, and the SNP of the *SLC6A3* gene, $r = 0.45$. Similar correlation coefficients were also obtained for the accident proneness coefficient for all SNP: –0.37 for the *CLOCK* gene, 0.31 for the *NPSR1* gene, and 0.52 for the *SLC6A3* gene.

TABLE 3. Frequencies of Allelic Variants of SNP of the *NPSR1* Gene among Drivers Involved and Not Involved in RTA

	AA genotype	AT genotype	TT genotype
Involved in RTA	23	44	20
Not involved in RTA	22	71	34

TABLE 4. Frequencies of Allelic Variants of the *CLOCK* Gene among Drivers Involved in Fault and No-Fault RTA

	AA genotype	AG genotype	GG genotype
Fault RTA	4	14	27
no-fault RTA	26	73	69

Table analysis (Tables 3 and 4) showed that the linkage of the risk of being involved in an RTA was significantly greater for carriers of the minor allele of the *NPSR1* gene – the odds ratio (OR) was 2.42 ($df = 1, \chi^2 = 7.65, p < 0.01$). In the case of the polymorphism of the *CLOCK* gene, there was also a significant decrease in the risk of being at fault in an RTA in carriers of the minor allele, with OR = 0.46 ($df = 1$, $\chi^2 = 5.14, p < 0.05$).

Discussion. Carriers of the minor allele of the *CLOCK* gene were less often responsible for RTA, while carriers of the minor alleles of the *NPSR1* and *SLC6A3* genes were more often involved in no-fault RTA.

This study provided the first evidence of an association between measures of driving safety – accident proneness – and various single-nucleotide polymorphisms. Associations with behavioral and cognitive parameters have already been demonstrated for these polymorphisms. In the case of the A allele of the SNP of the *CLOCK* gene this was with parameters of the sleep-waking rhythm, with a shorter mean duration of sleep [Allebrandt et al., 2010; Dorokhov et al., 2015]; the minor allele of the SNP of *NPSR1* was associated with lower anxiety and impulsivity, as well as control of emotions and attention, and sleep disturbance [Gafarov et al., 2015; Neufang et al., 2015; Laas et al., 2015; Ruland et al., 2015]. The *SLC6A3* polymorphism studied here has been shown to have a functional influence on the operation of the dopaminergic system [Tiwari et al., 2013; Sullivan et al., 2013; Pinsonneault et al., 2011].

Conclusions. Carriers of the minor variant of the polymorphism in the *CLOCK* gene were more rarely at fault in RTA, while carriers of the minor alleles of the *NPSR1* and *SLC6A3* genes were more often involved in no-fault RTA, evidently due to lesser ability to perceive and avoid dangerous situations developing in traffic.

As all the drivers in the group studied here worked a shift pattern, i.e., they did not have a regular sleep and waking regime, it is logical that the link seen here between accident proneness and the *CLOCK* gene polymorphism is due to different preferences in this regime and resistance to its derangement. In the cases of the *SLC6A3* and *NPSR1* SNP, this associated is probably due to differences in the effectiveness of the emotional and cognitive control systems, which is important for adaptation to traffic situations.

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