Chapter

# Biological Determinants of Sleep Disorders

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## Abstract

The purpose of the study is to research the effect of polymorphism of genes such as *CLOCK, ARNTL*, *PER2, NPAS2*, *DRD4, DAT*, *TNF-α*, and *NPSR1* on sleep disorders in an open population of 25–64-year-old men. We conducted screening studies of representative samples of men aged 25–64 years. The general examination was carried out according to the standard methods included in the WHO MONICA-Psychosocial Program (MOPSY). Carriers of the C/T genotype of the *CLOCK* gene more often than others reported having "satisfactory" or "poor" sleep. Carriers of the C/T genotype of the *ARNTL* gene were more likely to experience anxiety dreams, and they woke up exhausted. Carriers of the A/A genotype of the *PER2* gene were more likely to wake up two or more times per night, a total of four to seven times per week. In the population, C/T and T/T genotypes of the *NPAS2* gene were significantly more common in individuals with 7-hour sleep. Genotype 4/6 of the *DRD4* gene and genotype 9/9 of the *DAT* gene were significantly associated with sleep disturbances. Carriers of the heterozygous A/G genotype of the *TNF-α-308* gene, compared with carriers of all other genotypes, more often rated sleep as "satisfactory" (30%) than "good."

**Keywords:** population, men, sleep disorders, *CLOCK* gene, *ARNTL* gene, *PER2* gene, *NPAS2* gene, *DRD4* gene, *DAT* gene, *TNF-α* gene, *NPSR1* gene

## 1. Introduction

Sleep is a complex set of brain processes that support human physiological needs [1]. Sleep is part of the sleep-wake cycle. This cycle, consisting of approximately 8 hours of sleep at night and 16 hours of daytime wakefulness in humans, is controlled by a combination of two internal factors, that is, sleep homeostasis and circadian rhythms [2]. Unlike wakefulness, sleep is a period of inactivity and restoration of mental and physical functions. Sleep is thought to provide time for inputting information gained during waking into memory and for reestablishing communication between different parts of the brain. Sleep is also the time when other body systems replenish their energy and repair their tissues [3], and it is the key to wellness and optimal health [4–6]. People who get enough quality sleep have more energy, better cognitive function, memory, alertness, attention, and performance during the day, as

well as a healthier immune system [7]. Quality healthy sleep is one of the basic needs of people and is important for their health [8].

Circadian rhythms are a system that synchronizes all processes in living organisms that provide temporary adaptation, including sleep and wakefulness. The study of circadian rhythms and biological clocks progressed slowly until the methods that anticipated the beginning of the genomic millennium came to the aid of scientists. At the end of the last century, scientists found out that there is a biological "clockwork mechanism" in the mammalian brain that coordinates the work of the entire organism. To be more precise, these clocks are located in the suprachiasmatic nucleus (SCN) of the hypothalamus. Today we know that each SCN neuron is a miniature clock counting the circadian rhythm, and all these thousands of clocks work in unison, forcing the rest of the body's systems to obey. The SCN receives information about the illumination from special receptors located on the retina of the eye and sends corresponding signals to other organs using hormones and nerve impulses. Some SCN cells, as well as cells of many other organs, have individual molecular clocks. The "gears" in these clocks are transcription factors, the activity of which changes over the day. The synthesis of several different proteins depends on the activity of these key transcription factors, which gives rise to the circadian rhythms of the vital activity of individual cells and entire organs. A bright light turned on early at night can shift the circadian rhythm, activating *PER* gene transcription, which usually occurs in the morning. However, it should be understood that a nerve impulse represents the final crescendo of the long processes unfolding in a neuron. To understand the nature of these processes, one has to descend from the cellular to the gene level [9, 10].

In the 1960s and early 1970s, Seymour Benzer at the California Institute of Technology [11] and Ronald Konopka, one of his students, studied the genetics of Drosophila behavior and the latter discovered the first circadian rhythm gene localized in the X chromosome [12]. The gene was named *period*, or *per* (the protein encoded by this gene was respectively named PER). Scientists have found three mutant alleles for *per*, in addition to the normal "wild type." With one of them, the daily cycle of the fly was shortened to 19 hours, with the other, it was lengthened to 29 hours, while the carriers of the third "did not observe hours" at all, that is, their periods of rest and activity were of random duration [12]. The 2017 Nobel Prize in physiology or medicine was awarded to Jeffrey C. Hall, Michael Rosbash, and Michael W. Young for cloning and sequencing the *period* gene in 1984 [13, 14]. Michael Rosbash and his colleagues also noticed that the concentration of messenger RNA (mRNA) of the *per* gene increases and decreases within 24 hours. In mutants, these fluctuations accelerated or slowed down [13]. In the 90s*, new details of the mechanism were discovered—the timeless* genes, or *tim* genes, *doubletime* (Michael W. Young) [15], as well as *Clock*, *cycle,* and *Cryptochrome* genes (Michael Rosbash group) [16].

The relationship between these genes and their products is seen in **Figure 1** [17].

The clock mechanism is based on two proteins—CLOCK (CLK) and BMAL1 (-ARNT-like protein 1 in the brain and muscle; also known as ARNTL or MOP3). By dimerizing, CLOCK and BMAL1 activate the transcription of the Period *(PER*) and Cryptochrome *(CRY*) genes. In nocturnal rodents, as well as in some diurnal animals, the transcription of the *PER1* and *PER2* genes in the SCN peaks in the morning or afternoon, while for *CRY1* and *CRY2* genes, the peak is observed closer to the evening. An increase in the concentration of PER and CRY proteins in the cell triggers a feedback mechanism that blocks further synthesis of these proteins. According to recent studies, the main inhibitor of the CLOCK-BMAL1 complex is CRY, but it works only when combined with PER. During the night, cellular enzymes gradually degrade

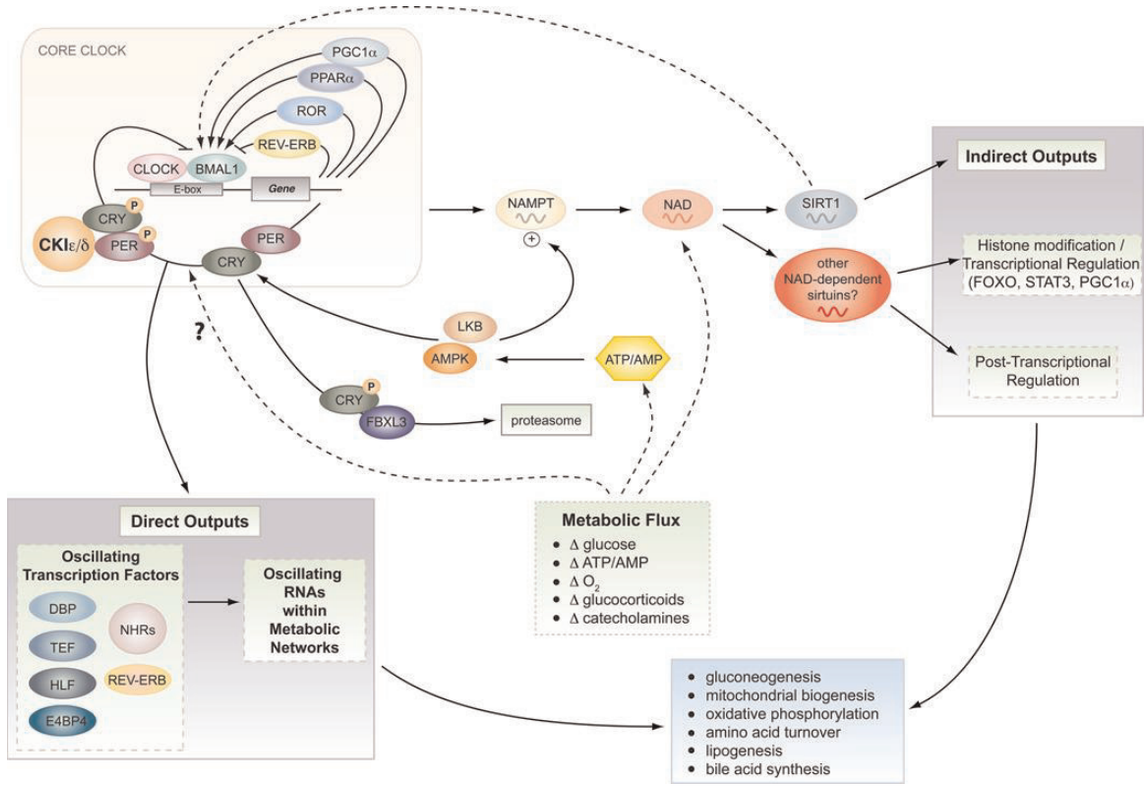


**Figure 1.**
*Direct and indirect outputs of the core clock mechanism [17].*

PER and CRY, and when their concentration reaches a critically low point, transcription is reactivated. The duration of the cycle depends on the degradation rate of PER and CRY [18, 19].

An important question is what molecular mechanisms provide a link between the light signal and the genes of the biological clock? Until recently, it was believed that phototransduction—the conversion of a light signal into an electrical signal transmitted through neurons—can only take place in the retina of the eye and only through the retinal, an active component of rhodopsin. In 2011, Fogle K.J., Parson K., et al. found that the CRY protein has the same ability, and it uses a mechanism independent of TIM and PER [20]. If the neuron in which CRY is expressed is illuminated by blue light, a complex cascade of reactions involving potassium membrane channels is triggered and an action potential is generated, that is, the neuron produces an electric signal under the direct influence of light. Control experiments have shown that this reaction has nothing to do with opsin, the visual pigment in Drosophila. However, if in the course of an experiment the other neurons not previously possessing photosensitivity are forced to synthesize CRY, they also start generating signals in response to flashes of light [20].

Mention should also be made of the *NPAS2* gene, which is located on chromosome 2 in 2q11.2, one of the most important circadian genes. NPAS2 forms heterodimers with BMAL1 and then activates the circadian genes *PER* and *CRY*, which are essential for maintaining biological rhythms in many organisms. Animal studies have shown that loss of normal function of the *NPAS2* gene can cause defects in several aspects of the circadian system, such as sleep patterns and behavior [21].

According to the current understanding of the neurophysiology of sleep, monoamines, one of which is dopamine, also play an important role [22]. Damage to central

dopaminergic synaptic transmission plays an important role in the onset of severe neuropsychiatric disorders [23]. People with these conditions have serious sleep disturbances such as excessive daytime sleepiness [24], rapid eye movements disrupting sleep behavior [25], a decrease in the period of paradoxical sleep, and disrupted sleep architecture [26]. In general, all these observations suggest that dopamine plays an important role in the regulation of the sleep-wake cycle [27]. The evidence that increased extracellular dopamine is one of the key mechanisms of wakefulness activation also associates reduced dopamine metabolism with sleep disturbances [28].

TNF-α (tumor necrosis factor-) is a pro-inflammatory cytokine that contributes to the formation of atherosclerotic plaque. Although early reviews showed a contradictory association with CHD (coronary heart disease) [29], the replacement of G (guanidine) by A (adenine) at position 308 in the promoter region is now associated with increased TNF-α production [30], as well as with increased inflammatory response after cardiac surgery [31], insulin resistance [32], CHD, in individuals with type II diabetes [33] and increased C-reactive protein in individuals with vital exhaustion, defined as a condition with excessive fatigue, difficulty falling asleep, general malaise, apathy, irritability, and lack of energy [34]. In addition, it was suggested that variability in location 308 may affect the development of depressive symptoms, for example, allele A is more common in depressed patients than in controls [35]. There is an association between obstructive sleep apnea and the presence of the A allele [36].

The neuropeptide S receptor (NPSR1) is a metabotropic G protein receptor with seven transmembrane helices [37]. The receptor was first described in 2002, and in 2004 neuropeptide S (NPS) was identified as a ligand [38, 39]. NPS refers to neuropeptides, a diverse group of neurons expressing signaling molecules involved in various brain functions. Studies in rats have shown that administration of NPS greatly induces wakefulness and reduces the occurrence of all stages of sleep [38, 40]. NPS appears to be expressed only in certain brain regions [41–46]. The highest concentration of NPS precursor mRNA was found in brainstem neurons adjacent to the locus coeruleus, in the parabrachial nuclei of the variolar bridge, and the sensory nucleus of the trigeminal nerve [39, 40]. The locus coeruleus and parabrachial nuclei belonging to the ascending activating reticular system, as well as the sensory nucleus of the trigeminal nerve, are known for their contribution to modulating the sleep/wake cycle [42, 43]. Compared to NPS, the expression pattern of NPSR1, a precursor of mRNA, is more widely distributed in the brain. It covers important centers of the sleep/wake system in the hypothalamus and thalamus and is also present in the cortex and amygdala. In particular, it can be found in hypothalamic regions such as the peripheral region (red band) and the tuberomammillary nucleus, which are known for their expression of orexin and histamine, respectively [44–46]. In addition, NPSR1 mRNA was found in key regions responsible for sleep induction. At the molecular level, the receptor activates protein kinases and increases intracellular cAMP and $Ca^{2+}$ levels [38]. Thus, NPS is believed to modulate the neurotransmission of the expressing neurons NPSR1. Although the NPS system appears to play a critical role in modulating sleep, most of the conclusions have been drawn from rodent studies, and data on its effect on sleep in humans are limited. A single rs324981 nucleotide polymorphism (lying at triplet position 107 of the *NPSR1* gene on chromosome 7p14.3) provides an opportunity for a noninvasive study of the effect of NPS/NPSR1 in the human body. The SNP T allele leads to the exchange of amino acids in the active center of the receptor binding site (Asn ≥ Ile) [47]. This, in turn, leads to an approximately tenfold increase in the sensitivity of neuropeptide S [47]. The T allele has already been identified as a risk factor for the development of asthma and panic disorder [48–50]. Gottlieb et al. [51] conducted a whole-genome

sequencing that examined sleep parameters—falling asleep time and sleep duration. They found a connection between rs324981 and the time a person goes to bed. This study showed a delay in the moment of falling asleep among T-allele carriers.

Thus, our study aimed to investigate the effect of polymorphism of circadian rhythm genes (i.e., *CLOCK*, *ARNTL*, *PER2,* and *NPAS2*), dopamine receptor genes (i.e., *DRD4, DAT*), pro-inflammatory cytokines (*TNF-α* gene), and the *NPSR1* gene on sleep disorders in an open population of 25–64-year-old men.

## 2. Materials and methods

We conducted screening studies of representative samples of the population aged 25–64 years in one of the districts of Novosibirsk city (the budget theme No. AAAA17–117112850280-2):

at screening II in 1988–1989, 725 men were examined, average age—43.4 ± 0.4 years, the response rate was 71.3%;

at screening III in 1994–1995, 647 men were examined, average age—44.3 ± 0.4 years, the response rate was 82.1%;

at screening IV in 2003–2005, 576 men were examined, average age—54.23 ± 0.2 years, the response rate was 61%;

at screening V in 2013–2016, 427 men were examined, the average age—34 ± 0.4 years, the response rate was 71%;

at screening VI in 2016–2018, 275 men were examined, average age—49 ± 0.4 years, the response rate was 72%.

The general examination in 1988–1989, 1994–1995, 2003–2005, 2013–2016, and 2016–2018 was conducted according to standard methods included in the WHO MONICA-Psychosocial Program (MOPSY) [52].

A standard Jenkins questionnaire was used to study sleep disorders. Statistical analysis was performed using the SPSS software package version 20 [53].

Genotyping of the studied polymorphisms of circadian rhythm genes (*CLOCK, ARNTL*, *PER2, NPAS2* genes) (screening V), as well as those related to the dopaminergic system (*DRD4, DAT* genes) (screening III) and inflammatory pro-cytokines (*TNF-α*) (screening III) and *NPSR1* gene (screening IV), was performed in the Laboratory of Molecular Genetic Studies of the Research Institute of Therapy and Preventive Medicine—Branch of ICG SB RAS, Novosibirsk [Vladimir N. Maksimov, Doctor of Sciences (Medicine), is the head of the laboratory].

The distribution of traits and their quantitative characteristics was analyzed. Simple relations between variables were analyzed (contingency tables). The hypothesis of independence of factors A and B or homogeneity of factor B in relation to the levels of factor A was tested using the method of contingency table construction. The reliability of the independence of the factors was assessed by the criterion $\chi^2$ [54]. Reliability was accepted at a significance level of $p \leq 0.05$.

## 3. Results

In the male population under study, the rate of sleep disturbances ("poor" and "very poor" sleep) in the group aged 25–34 years in 1988–1989 was 5.4%; in 1994–1995, it went down to 3.6%; and in 2013–2016, it reached 4.3%. In the group

aged 35–44 years, the rate of sleep disorders in 1988–1989 was 9.5%; in 1994–1995, it was 9.3%; in 2013–2016, it decreased to 4.2%; and later in 2016–2018, it grew dramatically to 11%. In the group aged 45–54 years, the rate of sleep disorders was 11% in 1988–1989, 9.8% in 1994–1995, in 2003–2005, it rose to 12.5%, and fall to 4.9% in 2016–2018. Among men in the older age group of 55–64 years, the rate of sleep disturbance was the highest in 1988–1989—2020.8%; in 1994–1995, it plummeted to 12.1%; in 2003–2005, it showed a slight decrease to 11.8%; and finally, in 2015–2018, it rose to 19.7% (**Table 1**).

### 3.1 Association of polymorphism of the rs2412646 CLOCK gene with sleep disorders

**Table 2** shows the frequency distribution of rs2412646 genotypes of the *CLOCK* gene among men aged 25–44 years in Novosibirsk. In the studied population of 25–44-year-old men, the most common was the homozygous C/C genotype of the *CLOCK* gene—50.3%, the heterozygous C/T genotype was found in 42.5% and the T/T genotype in only 7.2%.

The respondents were asked how well they sleep. Among carriers of the C/T genotype, the response "satisfactory" (36.8%) and "poorly" (5.3%) sounded more often than among carriers of all other genotypes ($\chi^2 = 9.44$ df = 4 $p < 0.05$). Carriage of the T/T and C/T genotype of rs2412646 *CLOCK* gene was most frequently combined with carriage of the A/A genotype of rs934945 *PER2* gene among men aged 25–44 years in Novosibirsk (30.8% and 46.2%, respectively). The carriers of the C/C genotype of rs2412646 *CLOCK* gene most often had A/G and G/G genotypes of rs934945 *PER2* gene (68.4% and 68.9%, respectively) ($\chi^2 = 27.18$ df = 4 $p = 0.001$).

### 3.2 Association of the polymorphism of the rs2278749 ARNTL gene with sleep disorders

**Table 3** shows the frequency distribution of rs2278749 genotypes of the *ARNTL* gene among 25–44-year-old men in Novosibirsk.

The most common rs2278749 genotype of the *ARNTL* gene was the homozygous C/C genotype—74.9%, the second most common was the heterozygous C/T genotype—22.3%, while only 2.8% of individuals in the population had the homozygous T/T genotype.

The question was asked: "How often in the last month have you had disturbing dreams while sleeping?" Only C/T genotype carriers experienced such disturbances for 22 days or more, that is, 7.5%; 27.5% of C/T genotype and 20% of T/T genotype carriers reported having disturbing dreams 4–7 days per month, while C/C genotype carriers more often than others responded that they had no disturbing dreams at all, that is, 42.5% ($\chi^2 = 16.35$ df = 8 $p < 0.05$).

The question, "During the past month, how often did you wake up after a normal sleep, feeling tired or exhausted?", showed that 40% of T/T genotype carriers experienced such problems one to three times a month, while C/T genotype carriers (7.5%) experienced this problem more often than others, from 15 days to 3 weeks ($\chi^2 = 18.71$ df = 8 $p < 0.01$). Men carrying the heterozygous C/T genotype (57.3%) were more likely than others to wake up feeling exhausted and tired ($\chi^2 = 19.52$ df = 4 $p = 0.001$).

| **Question/Attitude: Do you sleep well? Rate the quality of your sleep.** | | **25–34** | | **35–44** | | **45–54** | | **55–64** | | **25–64*** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Screening | | N | % | N | % | N | % | N | % | N | % |
| Screening II 1988–1989 | 1. Very good | 35 | 17.2 | 16 | 8 | 10 | 5.8 | 8 | 5.4 | 69 | 9.5 |
| | 2. Good | 101 | 49.5 | 91 | 45.7 | 59 | 34.1 | 42 | 28.2 | 293 | 40.5 |
| | 3. Satisfactory | 57 | 27.9 | 73 | 36.7 | 85 | 49.1 | 68 | 45.6 | 283 | 39 |
| | 4. Poor | 10 | 4.9 | 17 | 8.5 | 15 | 8.7 | 30 | 20.1 | 72 | 9.9 |
| | 5. Very poor | 1 | 0.5 | 2 | 1 | 4 | 2.3 | 1 | 0.7 | 8 | 1.1 |
| | Total | 204 | 100 | 199 | 100 | 173 | 100 | 149 | 100 | 725 | 100 |
| | $\chi^2$ = 68,611 df = 12 p < 0.001 | | | | | | | | | | |
| Screening III 1994–1995 | 1. Very good | 9 | 5.4 | 11 | 6.4 | 8 | 5.6 | 8 | 4.8 | 36 | 5.6 |
| | 2. Good | 97 | 58.1 | 72 | 41.9 | 55 | 38.5 | 75 | 45.5 | 299 | 46.2 |
| | 3. Satisfactory | 55 | 32.9 | 73 | 42.4 | 66 | 46.2 | 62 | 37.6 | 256 | 39.6 |
| | 4. Poor | 5 | 3 | 14 | 8.1 | 13 | 9.1 | 17 | 10.3 | 49 | 7.6 |
| | 5. Very poor | 1 | 0.6 | 2 | 1.2 | 1 | 0.7 | 3 | 1.8 | 7 | 1 |
| | Total | 167 | 100 | 172 | 100 | 143 | 100 | 165 | 100 | 647 | 100 |
| | $\chi^2$ = 20,148 df = 12 p < 0.001 | | | | | | | | | | |
| Screening IV 2003–2005 | 1. Very good | | | | | 10 | 3.3 | 10 | 3.7 | 20 | 3.5 |
| | 2. Good | | | | | 104 | 34.2 | 73 | 26.8 | 177 | 30.7 |
| | 3. Satisfactory | | | | | 152 | 50 | 157 | 57.7 | 309 | 53.6 |
| | 4. Poor | | | | | 37 | 12.2 | 29 | 10.7 | 66 | 11.5 |
| | 5. Very poor | | | | | 1 | 0.3 | 3 | 1.1 | 4 | 0.7 |
| | Total | | | | | 304 | 100 | 272 | 100 | 576 | 100 |
| | $\chi^2$ = 5720 df = 4 p > 0.05 | | | | | | | | | | |
| Screening V 2013–2016 | 1. Very good | 25 | 15.2 | 28 | 10.7 | | | | | 53 | 12.4 |
| | 2. Good | 78 | 47.6 | 126 | 48.3 | | | | | 205 | 48 |
| | 3. Satisfactory | 54 | 32.9 | 96 | 36.8 | | | | | 151 | 35.4 |
| | 4. Poor | 6 | 3.7 | 10 | 3.8 | | | | | 16 | 3.7 |
| | 5. Very poor | 1 | 0.6 | 1 | 0.4 | | | | | 2 | 0.5 |
| | Total | 164 | 100 | 261 | 100 | | | | | 427 | 100 |
| | $\chi^2$ = 2200 df = 4 p > 0.05 | | | | | | | | | | |
| Screening VI 2016–2018 | 1. Very good | | | 6 | 8.3 | 3 | 3.7 | 0 | 0 | 9 | 3.3 |
| | 2. Good | | | 32 | 44.4 | 35 | 43.2 | 35 | 28.7 | 102 | 37.1 |
| | 3. Satisfactory | | | 26 | 36.1 | 39 | 48.1 | 63 | 51.6 | 128 | 46.5 |
| | 4. Poor | | | 8 | 11.1 | 4 | 4.9 | 24 | 19.7 | 36 | 13.1 |
| | 5. Very poor | | | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Total | | | 72 | 100 | 81 | 100 | 122 | 100 | 275 | 100 |
| | $\chi^2$ = 24.636 df = 6 p < 0.001 | | | | | | | | | | |

*Note *—for screening IV, 45–64 years old, for screening V, 25–44 years old, for screening VI, 35–64 years old.*

**Table 1.**
*Prevalence of sleep disorders in an open population of males 25–64 years of age for the period 1988–2018.*

| **rs2412646 genotypes of the *CLOCK* gene** | **N** | **%** |
|---|---|---|
| T / T | 13 | 7.2 |
| C/T | 76 | 42.5 |
| C/C | 90 | 50.3 |
| Total | 179 | 100 |
| The rs2412646 alleles of the *CLOCK* gene | N | % |
| T | 102 | 28.5 |
| C | 256 | 71.5 |
| Total | 358 | 100 |
| Hardy-Weinberg equilibrium | $\chi^2$ = 0.0409, p = 0.7344 q = 0.2656 | |

**Table 2.**
*rs2412646 polymorphism of the* CLOCK *gene in a population of men aged 25–44 years (screening V).*

| **Genotypes rs2278749 of the *ARNTL* gene** | **N** | **%** |
|---|---|---|
| T / T | 5 | 2.8 |
| C/T | 40 | 22.3 |
| C/C | 134 | 74.9 |
| Total | 179 | 100 |
| Allele rs2278749 of the *ARNTL* gene | N | % |
| T | 50 | 14 |
| C | 308 | 86 |
| Total | 358 | 100 |
| Hardy-Weinberg equilibrium | $\chi^2$ = 10.134 p = 0.8641 q = 0.1359 | |

**Table 3.**
*Polymorphism rs2278749 of the* ARNTL *gene in an open population of men aged 25–44 years (screening V).*

## 3.3 Association of the polymorphism of the rs934945 PER2 gene with sleep disorders

The prevalence of polymorphic variants of candidate gene of rs934945 *PER2* gene was as follows—homozygous genotype A/A—4.47%, heterozygous genotype A/G—30.17%, and homozygous genotype G/G—65.36%. **Table 4** shows the frequency distribution of the rs934945 *PER2* gene among 25–44-year-old men in Novosibirsk.

Among individuals with the homozygous A/A genotype of the *PER2* gene, there was a growing tendency for anxiety dreams during sleep a total of 4–7 days per month (12.5%), compared with A/G genotype carriers (7.4%) and G/G genotype carriers (12%) ($\chi^2$ = 13.83 df = 8 p = 0.08).

The carriers of the G/G genotype of the *PER2* gene were significantly less likely to wake up at night (51.9%) compared to men carrying the A/G (38.5%) and A/A (37.5%) genotypes. In contrast, men carrying the AA genotype were more likely (25%) to wake up (two or more times per night), a total of four to seven times per week, compared to the carriers of the A/G genotype (20.4%) and the G/G genotype (13.2%) ($\chi^2$ = 25.76 df = 8 p = 0.004).

| **Genotypes of rs934945 *PER2* gene** | **N** | **%** |
|---|---|---|
| A/A | 8 | 4.47 |
| A/G | 54 | 30.17 |
| G/G | 117 | 65.36 |
| Total | 179 | 100 |
| Alleles rs934945 of the *PER2* gene | N | % |
| A | 62 | 17.3 |
| G | 296 | 82.7 |
| Total | 358 | 100 |
| Hardy-Weinberg equilibrium | $\chi^2$ = 0.4852 p = 0.8128 q = 0.1872 | |

**Table 4.**
*Polymorphism of rs934945* PER2 *gene in an open population of men aged 25–44 years (screening V).*

In the population of men aged 25–44 years, individuals carrying the A/A genotype of the *PER2* gene tended to have a shorter sleep duration of 5 hours or less (62.5%) compared to carriers genotypes A/G (57.4%) and G/G (41.9%) ($\chi^2$ = 9.21 df = 10 p = 0.51).

### 3.4 Association of the polymorphism of the rs934945 PER2 gene with sleep disorders

The prevalence of polymorphic variants of the candidate gene NPAS2 rs4851377 in the population was as follows—homozygous C/C genotype in 13.4%, heterozygous C/T genotype in 53.6%, and homozygous genotype T/T in 32.9%. The C allele of the NPAS2 candidate gene was found in 40.3% of men, and the T allele was found in 59.7% of men (**Table 5**).

In the population, the C/C genotype of the NPAS2 rs4851377 gene was significantly more common in those who slept at least 8 hours per night (33.3%) and 9 hours per night (33.3%), and the C/T and T/T genotypes were in those with 7 hours of sleep (50% and 53.3%, respectively) ($\chi^2$ = 18.425 df = 10 $p < 0.05$).

It was found that 6-hour sleep 4.5 times (95% CI 0.735–27.536) was significantly more often observed among the carriers of the T allele than the C allele who had 9-hour sleep ($\chi^2$ = 6.142 df = 1 $p < 0.05$); also 7-hour sleep versus 9-hour sleep was four times more often (95% CI 0.66–24.245) in carriers of the T allele than in carriers of the C allele ($\chi^2$ = 5.488 df = 1 $p < 0.05$).

### 3.5 Association of VNTR polymorphisms of the DRD4 gene and VNTR polymorphism of the DAT gene with sleep disorders (screening III)

In an open population of men aged 25–64 years, the frequency of homozygous genotype 4/4 of the dopamine receptor subtype 4 (*DRD4*) gene was 57.9%; genotype 2/2 was less frequent—6.1%, genotype 2/4—12.5%, and genotype 3/4—5.6%; even less frequent were—genotype 4/6—4.2%, genotype 2/6, genotypes 4/7 and 6/6 were present in equal proportions—2.1%. The frequency distribution of alleles showed that allele 4 prevails—found in 70.7%, allele 2 was found in 14%, allele 6—in 6%. The remaining alleles account for 0.8%–5.4% (**Table 6**).

| **Genotypes of rs4851377 *NPAS2* gene** | **N** | **%** |
|---|---|---|
| C/C | 24 | 13.4 |
| C/T | 96 | 53.6 |
| T/T | 59 | 32.9 |
| Total | 179 | 100 |
| Alleles of rs4851377 *NPAS2* gene | N | % |
| C | 144 | 40.3 |
| T | 214 | 59.7 |
| Total | 358 | 100 |
| Hardy-Weinberg equilibrium | $\chi^2$ = 0.2221 p = 0.6453 q = 0.3547 | |

**Table 5.**
*Polymorphism of rs4851377* NPAS2 *gene in an open population of men aged 25–44 years (screening V).*

The sleep self-assessment distribution of VNTR carriers of *DRD4* gene polymorphism is presented in **Table 6**. Carriers of genotype 2/4 of the *DRD4* gene were more likely to respond that their sleep was "good" (16.2%) than "satisfactory" (7.9%), both compared with carriers of all other genotypes ($\chi^2$ = 5.626 υ = 1 p < 0.01) and compared with carriers of genotype 4/4 ($\chi^2$ = 4.507 υ = 1 p < 0.05). Self-assessment of sleep as "poor/good" revealed a significant difference between carriers of genotypes 2/4 and 4/6; in the former, self-assessment of sleep as "good" predominated (16.2%), and the latter more often assessed their sleep as "poor" (28.6%) ($\chi^2$ = 5.714 df = 1, p < 0.05). For the "good/very good" sleep self-assessment, the "very good" (15.4%) rather than "good" (4.3%) responses were more common among genotype 3/4 carriers ($\chi^2$ = 5.199 υ = 1 p < 0.05). Among carriers of genotype 4/4, sleep is more often assessed as "satisfactory" (61.8%) than "poor" (38.1%), as in comparison with carriers of all other genotypes ($\chi^2$ = 7.687 υ = 1, p < 0.01), and in comparison with carriers of genotype 4/6 OR = 12.7 (95% CI 4.1–38.7); ($\chi^2$ = 26.941 υ = 1 p < 0.0001). A reliable result was obtained when comparing carriers of genotype 4/4, in which the score "good sleep" prevails (60%), with carriers of genotype 2/6, in which the score "poor sleep" prevails (7.1%), OR = 10, 4 (95% CI 1.6–67.1); ($\chi^2$ = 8.772 df = 1 p < 0.01). Self-assessment of sleep as "good" (60%) in carriers of genotype 4/4 is more common than assessment as "very good" (40%) among carriers of all other genotypes ($\chi^2$ = 6.664 υ = 1 p < 0.01) and in comparison with carriers of genotype 2/4 (15.4%) ($\chi^2$ = 5.223 υ = 1 p < 0.05). Among carriers of genotype 4/6, there is an increase in responses of "poor sleep" (28.6%) rather than "satisfactory" (3.6%) in comparison with carriers of all other genotypes OR = 10.6 (95% CI 3.6–30.4); ($\chi^2$ = 26.217 υ = 1 p < 0.0001); with carriers of genotype 2/4 OR = 5.2 (95% CI 1.2–21.5); ($\chi^2$ = 5.461 υ = 1 p < 0.05).

Carriers of allele 2 of the *DRD4* gene more often assessed their sleep as "good" (15.4%) than "poor" (9.5%), in comparison with carriers of all other alleles ($\chi^2$ = 5.739 υ = 1 p < 0, 01) as well as with carriers of allele 3 ($\chi^2$ = 4.790 υ = 1 p < 0.05). Carriers of allele 4 were more "satisfied" (71.5%) with their sleep than carriers of all other alleles ($\chi^2$ = 4.101 υ = 1 p < 0.05). Carriers of allele 5 rated their sleep as "very poor" (7.1%) much more often than "satisfactory" (0.9%), in comparison with carriers of all other alleles OR = 8.3 (95% CI 0.8–86.1); ($\chi^2$ = 4.541 υ = 1 p < 0.05).

| Genotype | Population | | Sleep self-assessment | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Very good | | Good | | Satisfactory | | Poor | | Very poor | |
| | n | % | n | % | n | % | n | % | n | % | n | % |
| 2/2 | 26 | 6.1 | 3 | 11.5 | 11 | 5.9 | 11 | 6.7 | 0 | 0 | 1 | 14.3 |
| 2/3 | 1 | 0.2 | 0 | 0 | 0 | 0 | 1 | 0.6 | 0 | 0 | 0 | 0 |
| 2/4 | 53 | 12.5 | 4 | 15.4 | 30 | 16.2 | 13 | 7.9 | 5 | 11.9 | 1 | 14.3 |
| 2/5 | 2 | 0.5 | 0 | 0 | 2 | 1.1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2/6 | 10 | 2.4 | 0 | 0 | 2 | 1.1 | 5 | 3 | 3 | 7.1* | 0 | 0 |
| 2/7 | 1 | 0.2 | 0 | 0 | 1 | 0.5 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3/3 | 8 | 1.9 | 0 | 0 | 4 | 2.2 | 4 | 2.4 | 0 | 0 | 0 | 0 |
| 3/4 | 24 | 5.6 | 4 | 15.4* | 8 | 4.3 | 8 | 4.8 | 4 | 9.5 | 0 | 0 |
| 3/6 | 3 | 0.7 | 0 | 0 | 1 | 0.5 | 1 | 0.6 | 1 | 2.4 | 0 | 0 |
| 3/7 | 2 | 0.5 | 0 | 0 | 0 | 0 | 2 | 1.2 | 0 | 0 | 0 | 0 |
| 4/4 | 246 | 57.9 | 13 | 40 | 111 | 60 | 102 | 61.8*** | 16 | 38.1 | 4 | 57.1 |
| 4/5 | 4 | 0.9 | 0 | 0 | 1 | 0.5 | 1 | 0.6 | 1 | 2.4 | 1 | 14.3 |
| 4/6 | 18 | 4.2 | 0 | 0 | 0 | 0 | 6 | 3.6 | 12 | 28.6** | 0 | 0 |
| 4/7 | 9 | 2.1 | 0 | 0 | 6 | 3.2 | 3 | 1.8 | 0 | 0 | 0 | 0 |
| 4/8 | 1 | 0.2 | 0 | 0 | 0 | 0 | 1 | 0.6 | 0 | 0 | 0 | 0 |
| 5/5 | 3 | 0.7 | 0 | 0 | 2 | 1.1 | 1 | 0.6 | 0 | 0 | 0 | 0 |
| 5/6 | 2 | 0.5 | 2 | 7.7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6/6 | 9 | 2.1 | 0 | 0 | 5 | 2.7 | 4 | 2.4 | 0 | 0 | 0 | 0 |
| 7/7 | 3 | 0.7 | 0 | 0 | 1 | 0.5 | 2 | 1.2 | 0 | 0 | 0 | 0 |
| $\chi^2$ = 161.067 df = 72 p = 0.0001 | | | | | | | | | | | | |
| **Allele** | **Population** | | **Very good** | | **Good** | | **Satisfactory** | | **Poor** | | **Very poor** | |
| | n | % | n | % | n | % | n | % | n | % | n | % |
| 2 | 26 | 6.1 | 10 | 19.2 | 57 | 15.4 | 41 | 12.4 | 8 | 9.5 | 3 | 2.4 |
| 3 | 9 | 2.1 | 4 | 7.7 | 17 | 4.6 | 20 | 6.1 | 5 | 6.0 | 0 | 0 |
| 4 | 323 | 76.0 | 34 | 65.4 | 267 | 72.2 | 236 | 71.5 | 54 | 64.3 | 10 | 71.4 |
| 5 | 9 | 2.1 | 2 | 3.8 | 7 | 1.9 | 3 | 0.9 | 1 | 1.2 | 1 | 7.1 |
| 6 | 42 | 9.9 | 2 | 3.8 | 13 | 3.5 | 20 | 6.1 | 16 | 19.0 | 0 | 0 |
| 7 | 15 | 3.5 | 0 | 0 | 9 | 2.4 | 9 | 2.7 | 0 | 0 | 0 | 0 |
| 8 | 1 | 0.2 | 0 | 0 | 0 | 0 | 1 | 0.3 | 0 | 0 | 0 | 0 |
| $\chi^2$ = 46,555 df = 24 p = 0.004 | | | | | | | | | | | | |

**-p < 0,05; **-p < 0,01; ***-p < 0,001*

**Table 6.**
*Frequencies of genotypes and alleles of VNTR polymorphism of the* DRD4 *gene in the population and their association with sleep disorders (screening III).*

### 3.6 Carriers of allele 6 of the *DRD4* gene more often rated their sleep as "poor" (19%)

a. rather than "satisfactory" (6.1%) in carriers of all other alleles OR = 3.6 (95% CI 1.7–7.4); ($\chi^2$ = 14.224 υ = 1 p < 0.0001) and in comparison with carriers of allele 2 ($\chi^2$ = 8.097 υ = 1 p < 0.004), allele 3 ($\chi^2$ = 3.905 υ = 1 p < 0.05), allele 4 ($\chi^2$ = 12.665 υ = 1 p < 0, 00001);

b. rather than "good" (3.5%) compared to carriers of all other alleles OR = 6.4 (95% CI 2.9–14); ($\chi^2$ = 27.626 υ = 1 p < 0.0001); carriers of allele 2 ($\chi^2$ = 19.379 υ = 1 p < 0.0001), allele 3 ($\chi^2$ = 5.437 υ = 1 p < 0.05), allele 4 ($\chi^2$ = 5.048 υ = 1 p < 0.05), and allele 5 ($\chi^2$ = 5.147 υ = 1 p = 0.05);

c. and also rather "very good" (3.8%) in comparison with carriers of all other alleles OR = 5.8 (95% CI 1.2–26.7); ($\chi^2$ = 6.463 υ = 1 p < 0.01) (**Table 6**).

In the frequency distribution of VNTR genotypes of the *DAT* gene polymorphism in a population of men aged 25–64 years with different self-assessed sleep, no significant differences were found. Positive sleep assessments were more common—41.9% of carriers of genotype 9/10 had "good" sleep, among carriers of genotype 10/10, "very good" sleep was observed in 61.5%. Among the carriers of the 9/9 genotype, 8.3% considered their sleep to be "poor" ($\chi^2$ = 25.486 υ = 32 p > 0.05). The distribution of the remaining carriers of various *DAT* genotypes and self-assessment of sleep did not exceed 3.8% (**Table 7**).

There were no significant differences in the frequency distribution of the *DAT* gene alleles in a population of men aged 25–64 years, with different self-assessment of sleep. There was a tendency for an increase in positive responses 78.8% of carriers of allele 10 reported having "very good" sleep, while 26.4% carriers of allele 9 assessed their sleep as "poor." The distribution of the other alleles of the *DAT* gene did not exceed 3.8% ($\chi^2$ = 16.777 υ = 20 p > 0.05) (**Table 7**).

### 3.7 Association of G308A polymorphism of TNF- α gene with sleep disorders

The frequency of genotypes G308A polymorphism of the tumor necrosis factor *TNF-α* gene in the male population of Novosibirsk city is presented in **Table 8**. The G/G genotype of the *TNF-α* gene was found in 79.1% of individuals, the A/G genotype was found in 19% of cases, and finally, the A/A genotype was only found in 1.9% of men. In the population, 88.6% of men had allele G and only 11.4% had allele A (**Table 8**).

There was a tendency that the G/G genotype of the *TNF-α* gene was more common not only in individuals with very good (88.2%) and good (83.3%) sleep but also in the group with sleep disorders: poor (84.2%) and very poor (100%) sleep. The genotype A/G of the *TNF-α* gene was most often found among men who consider their sleep "satisfactory" (30%). The A/A genotype of the *TNF-α* gene did not exceed 5.3% among all categories of sleep disorders ($\chi^2$ = 12,012 df = 8 p = 0,151).

There was also a tendency toward a more frequent occurrence of the G allele of the *TNF-α* gene among all categories of men with different assessments of their sleep, and the A allele of the *TNF-α* gene in the group of men who rate their sleep as satisfactory (17.5%) ($\chi^2$ = 9.451 df = 4 p = 0.051).

Comparative analysis in groups with various self-assessment of sleep showed that among carriers of genotype G/G gene *TNF-α*, in comparison with carriers of all other genotypes, assessment of sleep as "good" (98.3%) rather than satisfactory (67.5%) $\chi^2$ = 36,943 df = 1 p = 0.0001; OR = 27,685 (95% CI 6339–120,906), and "good" (98.3%), followed by "poor" (84.2%) $\chi^2$ = 9151 df = 1 p = 0.02; OR = 10,781 (95% CI

| Genotype | Sleep self-assessment | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Population | | Very good | | Good | | Satisfactory | | Poor | | Very poor | |
| | n | % | n | % | n | % | n | % | n | % | n | % |
| 8/8 | 4 | 1 | 1 | 3.8 | 1 | 0.6 | 2 | 1.2 | 0 | 0 | 0 | 0 |
| 9/9 | 15 | 3.7 | 0 | 0 | 2 | 1.1 | 10 | 6.2 | 3 | 8.3 | 0 | 0 |
| 6/10 | 3 | 0.7 | 0 | 0 | 1 | 0.6 | 2 | 1.2 | 0 | 0 | 0 | 0 |
| 8/10 | 1 | 0.2 | 0 | 0 | 1 | 0.6 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9/10 | 149 | 36.6 | 9 | 34.6 | 75 | 41.9 | 51 | 31.7 | 13 | 36.1 | 1 | 20 |
| 10/10 | 223 | 54.8 | 16 | 61.5 | 90 | 50.3 | 94 | 58.4 | 19 | 52.8 | 4 | 80 |
| 10/11 | 4 | 1.0 | 0 | 0 | 3 | 1.7 | 1 | 0.6 | 0 | 0 | 0 | 0 |
| 10/12 | 1 | 0.2 | 0 | 0 | 1 | 0.6 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11/11 | 7 | 1.7 | 0 | 0 | 5 | 2.8 | 1 | 0.6 | 1 | 2.8 | 0 | 0 |
| $\chi^2$ = 25.486 df = 32 p = 0.786 | | | | | | | | | | | | |
| **Allele** | **Sleep self-assessment** | | | | | | | | | | | |
| | **Population** | | **Very good** | | **Good** | | **Satisfactory** | | **Poor** | | **Very poor** | |
| | **n** | **%** | **n** | **%** | **n** | **%** | **n** | **%** | **n** | **%** | **n** | **%** |
| 6 | 3 | 0.4 | 0 | 0 | 1 | 0.3 | 2 | 0.6 | 0 | 0 | 0 | 0 |
| 8 | 9 | 1.1 | 2 | 3.8 | 3 | 0.8 | 4 | 1.2 | 0 | 0 | 0 | 0 |
| 9 | 179 | 22 | 9 | 17.3 | 79 | 22.1 | 71 | 22 | 19 | 26.4 | 1 | 10 |
| 10 | 604 | 74.2 | 41 | 78.8 | 261 | 72.9 | 242 | 75.2 | 51 | 70.8 | 9 | 90 |
| 11 | 18 | 2.2 | 0 | 0 | 13 | 3.6 | 3 | 0.9 | 2 | 2.8 | 0 | 0 |
| 12 | 1 | 0.1 | 0 | 0 | 1 | 0.3 | 0 | 0 | 0 | 0 | 0 | 0 |
| $\chi^2$ = 16.777 df = 20 p = 0.667 | | | | | | | | | | | | |

**Table 7.**
*Frequencies of VNTR genotypes and alleles of the* DAT *gene polymorphism in the population and their association with sleep disorders (screening III).*

1672–69,537) is much more common. On the contrary, among carriers of the heterozygous A/G genotype of the *TNF-α* gene, in comparison with carriers of all other genotypes, sleep was more often satisfactory (30%) than good (15.2%) $\chi^2$ = 6756 df = 1 p = 0.009. A comparative analysis between the carriers of different genotypes of *TNF-α* helped to reveal that the carriers of the genotype G/G reported good sleep (84.6%) rather than satisfactory (69.2%), more often, whereas in carriers of genotype A/G, on the contrary, satisfactory sleep (30.8%), rather than good sleep (15.4%) $\chi^2$ = 7013 df = 1 p = 0,008; OR = 2434 (95%CI 1247–4751) prevails. Comparative analysis of carriers of the G and A alleles of the *TNF-α* gene among men with different self-assessment of sleep did not show significant differences.

### 3.8 Association of polymorphism of the rs324981 NPSR1 gene with sleep disorders (screening IV)

In an open population of men aged 45–64 years, the frequency of homozygous C/C genotype of the *neuropeptide S* gene (*NPSR1* rs324981 gene) was 19.4%, with a lower

| ***TNF-α-308G/A* genotype** | **n** | **%** |
|---|---|---|
| G/G | 204 | 79.1 |
| A/G | 49 | 19 |
| A/A | 5 | 1.9 |
| Total | 258 | 100 |
| Allele of *TNF-α-308G/A* gene | n | % |
| G | 457 | 88.6 |
| A | 59 | 11.4 |
| Total | 516 | 100 |
| Hardy-Weinberg equilibrium | $\chi^2$ = 1.0964, p = 0.8878 q = 0.1122 | |

**Table 8.**
Tumor necrosis factor TNF-gene α *G308A polymorphism (screening III).*

| ***NPSR1* rs324981 genotype** | **n** | **%** |
|---|---|---|
| C/C | 7 | 19.4 |
| C/T | 19 | 52.8 |
| T/T | 10 | 27.8 |
| Total | 36 | 100 |
| The allele of the *NPSR1* gene rs324981 | n | % |
| C | 33 | 45.8 |
| T | 39 | 54.2 |
| Total | 72 | 100 |
| Hardy-Weinberg equilibrium | $\chi^2$ = 0.1859, p = 0.6163 q = 0.3832 | |

**Table 9.**
NPSR1 *gene rs324981 polymorphism in 45–64-year-old male population (screening IV).*

frequency of T/T genotype—27.8%, the most common in men was the heterozygous C/T genotype—52.8%. The frequency distribution of alleles showed that among men the T-allele prevails—54.2% and C-allele was found in 45.8% of individuals (**Table 9**).

An associative analysis of the allele frequencies of the neuropeptide S polymorphism of the *NPSR1* rs324981 gene in men with different self-assessment of sleep revealed that T-allele carriers more frequently rated their sleep as "satisfactory"—in 69% of cases, while C-allele carriers did it only in 57.1% of cases. In addition, carriers of the C-allele are more often satisfied with their sleep, rating it as "good"—28.6% and "very good" than carriers of the T-allele who have "good" sleep only in 20.7% of cases ($\chi^2$ = 15,713 df = 8, $p < 0.05$).

## 4. Discussion

Sleep plays an important role in promoting health. Studies conducted over the past decade have confirmed that sleep disturbance has a powerful effect on the risk of infectious diseases, the occurrence and progression of some major diseases, including

cardiovascular diseases and cancer, as well as the incidence of depression [55, 56]. In Russia, about 45% of adults are dissatisfied with their sleep, and almost 20% need serious treatment for sleep disorders [57]. In our population, the level of sleep disorders turned out to be high and had the following trend—it demonstrated a decrease from 1988 to 1989 to 1994–1995 and an increase in 2003–2018. The increase in sleep disturbance in 2003–2018 was observed mainly in the older age groups (45–64 years old). Problems with sleep in the population can only get worse every year. The rapid emergence of "24/7" communities, working 24 hours a day, 7 days a week, participation in round-the-clock events, increased nighttime use of television, the internet, and mobile phones, means that an adequate uninterrupted nighttime sleep is becoming rare. The proportion of workers with circadian rhythm disturbances who are needed to service "24/7" communities is likely to increase [58], which determined the feasibility of studying biological determinants of sleep disorders.

The *CLOCK* gene encoding the positive transcription factor CLOCK is among the major circadian rhythm genes. CLOCK protein with binding partner BMAL1, *BMAL1* gene product, forms a transactivation dimer influencing the promoter of controlled genes [59]. In our study, we examined the associative relationship between sleep disorders and various polymorphic variants of the rs2412646 genotype of the *CLOCK* gene among men aged 25–44 years. The response of having "satisfactory" sleep (36.8%) and "poor" sleep (5.3%) was found to be more frequent among C/T genotype carriers than among carriers of all other genotypes ($\chi^2 = 9.44$ df = 4 $p < 0.05$). The frequency distribution of rs2412646 genotypes of the *CLOCK* gene depending on the rs934945 genotype of the *PER2* gene is also of interest. We found that carriers of the rs2412646 genotype of the *CLOCK* C/T gene were most often carrying the A/A rs934945 genotype of the *PER2* gene in the male population of 25–44 years old. Our results are supported by the findings obtained by other researchers regarding the association of the *CLOCK* gene with insomnia [60, 61] and preference for a particular sleep-wake cycle [62–64].

The *ARNTL* gene is a key element of the positive feedback loop of the molecular circadian oscillator [65]. According to the activity of some genes, Jun Z. Li, et al. 2013 [66] were able to determine that in individuals suffering from affective disorders circadian rhythms are interrupted, and "night" genes were expressed during the day. It has been suggested that desynchronization may occur due to a disruption in the connection between individual circadian genes. Considering the association of genotypes of the rs2278749 *ARNTL* gene with affective disorders, we have identified several components that have shown the strongest association with polymorphic variants of the gene under study. It turned out that carriers of the genotype C/T had problems with sleep, especially since they had much more anxiety dreams during the month. Men, carriers of the T allele, both homozygotes (T/T genotype) and heterozygotes (C/T genotype) were more likely to wake up tired or exhausted. Thus, our results confirm the data of other researchers concerning the identification of individual polymorphic variants of the *ARNTL* gene, leading to possible desynchronization and disruption of the circadian rhythm and, accordingly, leading to affective disorders [66].

Examining the association of *PER2* gene genotypes with sleep characteristics, we found that among carriers of the A/A genotype there is a tendency to have more anxiety dreams in comparison with carriers of other genotypes. Moreover, A/A genotype carriers were more likely to wake up during the night, and the tightest sleep was observed in men who were A/G and G/G genotype carriers. Sleep deprivation (5 hours or less) also occurred more frequently in individuals whose genotype contained the

homozygous A allele. Our results overlap in part with those obtained by Ojeda D.A., et al. [67], who studied the association of the *PER2* gene (rs934945) with circadian rhythms in healthy individuals, students at Columbia University. The *PER2* gene (rs934945) showed a statistically significant association with two subscales of the morning sleepiness scale, that is, "activity planning" and "morning alertness." The association of rs934945 with "morning restlessness" was first shown.

The most common in the population was the heterozygous genotype of the candidate gene *NPAS2 C/T*—53.3%, followed by the homozygous genotype T/T, with both variants of the candidate gene more common among men who had enough sleep for only 7 hours a day. The C/C genotype of the *NPAS2* candidate gene was significantly more common in those who slept for at least 8 hours (33.3%) or 9 hours (33.3%) per day. The major T allele of the candidate gene *NPAS2* was 4.5 times more common in men who sleep 6 hours a day and four times more common in men with 7 hours of sleep. Thus, the obtained data indicate that rs4851377 of the candidate NPAS2 gene determines whether men are night owls or early birds [21].

According to the literature devoted to genetic research, it has been established that some mental and emotional characteristics of a person are associated with polymorphism of the 3rd exon of the gene of the neurotransmitter system of the dopamine receptor, subtype 4 (*DRD4*) [68] and the dopamine transporter gene (*DAT*) [69].

When considering the occurrence of identified polymorphic variants of *DRD4* candidate genes in people with sleep disorders, it was found that carriers of the genotype 4/4 were more likely to believe that they had either good or satisfactory sleep. Carriers of genotypes 2/4 and 3/4 were more likely to rate their sleep positively, while men with genotypes 2/6 and 4/6, on the contrary, were dissatisfied with their sleep and rated it as "poor" more often.

When comparing the "short" and "long" alleles of the *DRD4* gene, we observed approximately the same pattern: carriers of the "long" allele 6 more often evaluated their sleep as "poor," carriers of allele 2 believed that their sleep was "very good," and carriers of the most common in the population allele 4 mostly reported having "good" sleep.

According to the current understanding of the biosynthesis of dopamine, it is known that just one sleepless night is enough for its level in the brain to increase [70]. The findings of the study conducted by Nora Volkow et al. suggest that dopamine in the human brain is involved in the so-called adaptation process, which leads to sleep disturbance. The researchers also found that the amount of dopamine in the brain is associated with feelings of fatigue and physical ability to perform cognitive tasks. However, the study also found that increased levels of dopamine in the brain cannot compensate for cognitive disorders caused by lack of sleep. On the other hand, according to the literature, people with the "long" form of the *DRD4* gene (six or more repeat units) have a lowered affinity of dopamine receptors and a reduced number of receptors. These individuals are less sensitive to dopamine. This means that they need more stimulation to get the same reaction than people with a "short" gene [71]. It can be assumed that "stimulating wakefulness," for example, a sleepless night, is one of the "ways" to naturally raise the level of dopamine, to receive a "reward" by the brain, for which we later have to pay with insomnia.

When analyzing the frequency distribution according to the conjugation tables of genotypes and alleles of the VNTP polymorphism of the *DAT* gene in a population of 25–64-year-old men, with different sleep self-assessment, no significant differences were found. There was only a tendency toward an increased number in the positive assessments of sleep-in carriers of genotype 9/10 and genotype 10/10 of the *DAT*

gene. Negative sleep assessments were slightly more frequent in carriers of genotype 9/9 of the *DAT* gene. There was a tendency for an increase in the number of those who reported their sleep to be "very good" among the carriers of allele 10 and "poor" among the carriers of allele 9. Dopamine uptake is carried out through active transmembrane transfer using the dopamine protein transporter, it has been experimentally established that disabling the *DAT* gene in mice leads to a reduction in the paradoxical sleep phase (REM sleep) and promotes early awakening [28]. In individuals containing a short variant of the *DAT* gene in the genome, the reuptake of dopamine is altered [72], and there is reason to believe that an increase in free dopamine contributes to an increase in the period of wakefulness, but, as mentioned above, does not contribute to either physical or mental rest [70]. Probably, for this reason, men carrying the genotype 9/9 of the *DAT* gene are *more* likely to evaluate their sleep negatively.

In the Novosibirsk population, the most frequent genotype-G308A of the tumor necrosis factor *TNF-α* gene polymorphism was the G/G genotype—it was observed in almost 80% of men. Moreover, the same genotype was predominant in all groups differing in sleep quality. Comparative analysis showed that individuals with homozygous genotype G /G are much more likely to give positive assessments of their sleep, unlike carriers of all other genotypes combined, who evaluate sleep as satisfactory or even poor. In addition, heterozygous carriers with the A/G genotype are less likely to positively assess sleep than carriers of the homozygous G/G genotype. Although there are no direct analogous studies in the world literature, nevertheless, studies devoted to the study of obstructive sleep apnea can provide indirect evidence. According to various authors, insomnia occurs in 42%–54.9% of patients with sleep breathing disorders [73]. A meta-analysis published in 2012 showed an association between 308G/A and obstructive sleep apnea—the presence of the 308 A allele increases the risk of developing obstructive sleep apnea by 65%, compared with individuals with a homozygous G/G genotype (OR = 1.65, 95% CI = 1.02–2.68, p = 0.04) [36].

In the study population, we found an association between some polymorphic variants of the *NPSR1* gene and sleep self-assessment among men. The presence of the T allele in the genotype was found to contribute to poorer sleep quality among men. Our results are confirmed by the results obtained by Spada J et al. [74], who proved that the study participants with the homozygous T/T genotype had significantly shorter sleep/rest times than individuals carrying the C allele in the genotype. These findings are confirmed by the studies by G.W.A. Gottlieb et al. [51], who found associations between rs324981 and sleep. Our conclusion about the relationship between sleep quality and rs324981 is also consistent with studies on rats since the direct introduction of NPS into the rat brain strongly affects sleep architectonics. Already within the first hour after injecting NPS into the brain, wakefulness time was lengthened, while the rapid eye movement phase, as well as the slow sleep phase, were shortened [39]. Similar results were obtained by Zhao et al. [40], who reported a decrease in sleep phases. These studies indicate that NPS can inhibit different sleep phases that were previously thought to be independently regulated [75]. However, the causal relationship can be much more complicated, as it is still unknown how rs324981 polymorphism acts in ontogeny. There is a hypothesis that various compensatory mechanisms may be induced simultaneously with the loss of allele function, or there may be interaction with other unknown genetic or environmental factors, which may explain the lack of association with sleep disturbances in heterozygotes [76, 77].

## 5. Conclusions

1. It was found that 25–64-year-old men scored high in sleep disturbance. We observed the following trend—firstly, a decline from 1988 to 1989 to 1994–1995 (11% and 8.6%, respectively) and later growth in 2003–2018 (13.1%). The increase in sleep disturbance in 2003–2018 was observed mainly in the older age groups (45–64 years old).

2. The most common genotype in the population was C/C rs2412646 of the *CLOCK* gene (50.3%), C/T was found in 42.5%, and the genotype T/T in 7.2%. Carriers of the C/T genotype of the *CLOCK* gene more often than others reported having "satisfactory" or "poor" sleep. Carriage of the rs2412646 genotype of the *CLOCK* T/T and C/T gene was most often combined with carriage of the A/A rs934945 genotype of the *PER2* gene. Carriers of the C/C rs2412646 genotype of the *CLOCK* genotype most often had the A/G and G/G rs934945 genotypes of the *PER2 gene.*

3. The most common genotype rs2278749 of the *ARNTL* gene was the homozygous C/C genotype (74.9%), the C/T genotype was found in 22.3% of individuals, and 2.8% were the carriers of the T/T genotype. Carriers of the C/T genotype were more likely to experience anxiety dreams, and they woke up tired or exhausted; on the contrary, carriers of the C/C genotype were much less likely to experience anxiety dreams.

4. The prevalence of polymorphic variants of the candidate gene *rs934945* of the *PER2* gene was as follows—genotype A/A—4.47%, genotype A/G—30.17%, and genotype G/G—65.36%. Among individuals with the A/A genotype of the *PER2* gene, there was a tendency of seeing a larger number of anxiety dreams for a total of 4–7 days per month (12.5%), compared with carriers of the A/G genotypes (7.4%) and carriers of the G/G genotype (12%). G/G carriers of the *PER2* genotype were significantly less likely to wake up at night (51.9%), and men, carriers of the genotype A/A, on the contrary, woke up more often (25%) twice or more times per night, in general, from four to seven times a week. Individuals carrying the genotype A/A of the *PER2* gene tended to have a shorter sleep duration of 5 hours or less (62.5%), compared to carriers of the genotypes A/G (57.4%) and G/G.

5. The prevalence of polymorphic variants of the candidate gene *NPAS2* rs4851377 was as follows—C/C genotype was found in 13.3%, C/T genotype—in 53.3%, and T/T genotype—in 33.3%. In the population, the C/C genotype of the *NPAS2* rs4851377 gene was significantly more common in those who slept at least 8 hours a day (33.3%) and 9 hours (33.3%), and the C/T and T/T genotypes were found in people with 7 hours of sleep (50% and 53.3%, respectively). It was found that 6-hour sleep was 4.5 times significantly more often observed among the carriers of the T allele than among the carriers of the C allele, who had 9-hour sleep. Moreover, 7-hour sleep versus 9-hour sleep was four times more often found in carriers of the T allele than in carriers of the C allele.

6. In the male population, homozygous genotype 4/4 of the dopamine receptor of subtype 4 *DRD4* gene is the most represented (57.9%). With the frequency distribution of VNTR genotypes of *DAT* gene polymorphism, it was found that

the homozygous genotype 10/10 is more common (54.8%), whereas the heterozygous genotype 9/10 was less common (36.6%). Genotype 9/9 was observed in 3.7%. The incidence of other genotypes was 1.7% or less. Genotype 4/6 of the *DRD4* gene and genotype 9/9 of the *DAT* gene were significantly associated with sleep disorders.

7. The G/G genotype of the *TNF-α-308* gene was found in 79.1% of individuals, the A/G genotype in 19% of cases, and the A/A genotype was found in 1.9% of men. In the population, 88.6% of men had the G allele and 11.4% had the A allele. Carriers of the G/G genotype of the *TNF-α-308* gene compared to carriers of all other genotypes assessed their sleep as "good" (98.3%) much more often than as "satisfactory" (67.5%) or "poor" (84.2%). Carriers of the heterozygous A/G genotype of the *TNF-α-308* gene, compared with carriers of all other genotypes, more often rated sleep as "satisfactory" (30%) than "good" (15.2%).

8. The frequency of the homozygous C/C genotype of the neuropeptide S gene (*NPSR1* rs324981 gene) was 19.4%, T/T was less frequent (27.8%), and the most common was the heterozygous C/T genotype (52.8%). A tendency of growing dissatisfaction with the quality of their sleep among men with T/T genotype carriers has been revealed—70%. Male T-allele carriers were more likely to report sleep disturbances than C-allele carriers.

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## Conflict of interest

No conflict of interest.

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