

ELŻBIERA HABER¹, MAŁGORZATA SŁOWIŃSKA-LISOWSKA¹, PAWEŁ JÓŻKOW¹,
ŁUKASZ ŁACZMAŃSKI², MAREK MĘDRAŚ^{1,2}

Relationships Between the G861C Polymorphism of the 5-HT_{1B} Serotonin Receptor Gene and the Physical Activity in Men

Związek między polimorfizmem genu receptora serotoniny 5-HT_{1B} a poziomem aktywności fizycznej mężczyzn

¹ Department of Sport Medicine, University of Physical Education, Wrocław, Poland

² Department of Clinical Endocrinology, Diabetology and Isotope Therapy, Wrocław Medical University, Poland

Abstract

Background. The serotonergic system is considered to be involved in exercise behaviour. There is no available data on the relationship between the polymorphism G861C of the serotonin receptor gene 5-HT_{1B} and the level of physical activity.

Objectives. The aim of the research was to estimate the association between the serotonin receptor gene polymorphism 5-HT_{1B} G861C and the level of physical activity.

Material and Methods. The experiments were carried out on 387 men aged 23–72, living in Lower Silesia (Poland). The level of physical activity was determined with use of International Physical Activity Questionnaire (IPAQ, last 7-day recall). Genotyping of the polymorphic locus G861C of serotonin receptor gene 5-HT_{1B} was made by the polymerase chain reaction (PCR) and minisequencing.

Results. In the examined group 3 types of genotypes were detected in the polymorphic locus: G/G, G/C and C/C. There was no association between the physical activity level and the G861C polymorphism of the serotonin receptor gene. However the authors have noticed a discordance of the genotypes' distribution and the Hardy-Weinberg equilibrium. C/C genotype was more frequent than expected in men characterized by low, but not moderate or high, level of physical activity.

Conclusion. Obtained results could suggest a possible role of the serotonin receptor gene variability and the physical activity in men (*Adv Clin Exp Med* 2010, 19, 4, 455–459).

Key words: polymorphism of the 5-HT_{1B} serotonin receptor gene, physical activity, men.

Streszczenie

Wprowadzenie. Udział genów w populacyjnej zmienności poziomu aktywności fizycznej skłania do poszukiwania jej specyficznego podłoża molekularnego. W piśmiennictwie nie ma danych dotyczących relacji między polimorfizmem G861C genu receptora serotoniny 5-HT_{1B} a poziomem aktywności fizycznej.

Cel pracy. Ocena związku poziomu aktywności fizycznej z polimorfizmem genu receptora serotoniny 5-HT_{1B} G861C.

Materiał i metody. Badania przeprowadzono na grupie 387 mężczyzn w wieku 23–72 lat, mieszkańców Dolnego Śląska. Poziom ich aktywności fizycznej oceniono na podstawie formy skróconej kwestionariusza IPAQ (IPAQ, last 7-day recall). Oznaczenia polimorfizmu genu receptora 5-HT_{1B} G861C dokonano za pomocą reakcji łańcuchowej polimerazy PCR i minisekwencjonowania.

Wyniki. Według kryteriów kwestionariusza IPAQ około 50% mężczyzn z terenu Dolnego Śląska wykazuje wysoki poziom aktywności fizycznej. W badanej grupie stwierdzono występowanie genotypów trzech rodzajów: G/G, G/C i C/C. Nie wykazano zależności między poziomem aktywności fizycznej a polimorfizmem genu receptora serotoniny 5-HT_{1B} G861C. Weryfikując otrzymany rozkład częstości genotypów z rozkładem zakładanym przez prawo Hardy'ego-Weinberga w grupie o niskim poziomie aktywności fizycznej i łącznie w całej populacji, stwierdzono brak zgodności, podczas gdy w grupie o średnim i wysokim poziomie aktywności fizycznej obserwowano

no rozkład zgodny z założeniami wyżej wymienionego prawa. Niezgodność polegała na częstszym występowaniu genotypu C/C w grupie o niskim poziomie aktywności fizycznej.

Wnioski. Wyniki badań mogą sugerować udział genu receptora serotoniny 5-HT_{1B} w populacyjnej zmienności poziomu aktywności fizycznej (*Adv Clin Exp Med* 2010, 19, 4, 455–459).

Słowa kluczowe: polimorfizm genu receptora serotoniny 5-HT_{1B}, aktywność fizyczna, mężczyźni.

Recent literature confirms the importance of genetic factors for undertaking exercise, especially in males [1, 2]. One of the elements that influences physical activity is the serotonergic system [3]. The dysfunction of the serotonergic system facilitates alcoholism, narcotic drugs addiction, depression as well as aggression [4–6]. There is data indicating presence of relationships between above mentioned disorders and the G861C polymorphism of the 5-HT_{1B} serotonin receptor gene [7, 8].

It has been shown that stimulation of the 5-HT_{1B} receptor increases locomotor activity of laboratory animals [9]. However there is no information on relationships between the function of the 5-HT_{1B} receptor and physical activity in humans.

The aim of this work was to assess associations between the G861C polymorphism of the 5-HT_{1B} serotonin receptor gene and the level of physical activity in healthy males.

Material and Methods

The authors invited 900 male adults to participate in own investigation. They were randomly chosen from the database of the Regional Statistic Bureau of The Lower Silesian Province. Eventually the authors could enroll 387, ethnically homogenous, men for the study (43% of the target group).

All the investigated persons were physically and anamnesticly examined. The mean age was 47 ± 12.8 years, body mass 84.22 ± 13.62 kg, height 175 ± 6.8 cm, BMI 27.30 ± 4.1 , 43% of the study subjects accomplished higher education. 25% declared tobacco smoking, 21% – hypertension, 19% – low back pain, 5% – benign prostatic hyperplasia and 4% – diabetes.

The anthropometric characteristics of the investigated group is shown in Table 1.

The authors evaluated the level of physical activity of the investigated persons with the IPAQ questionnaire (International Physical Activity Questionnaire) [10].

The authors calculated the energy expenditure associated with the physical effort and thus distinguished three groups of subjects characterized by: low (group I), medium (group II) and high level of physical activity (group III).

According to the IPAQ criteria persons in group III should make at least 12 500 steps daily or the equivalent activity of high or medium intensity. It means that they have daily at least one hour of medium activity more as compared with baseline level (estimated as an equivalent of 5000 steps). Medium activity level is the smallest amount of physical activity that positively affects the human health status.

The blood samples were taken from the elbow vein to obtain the DNA for the genotype polymorphism estimation. Full blood with EDTA was stored at -20°C till the moment of DNA isolation by the standard method usage. G861C polymorphism of 5-HT_{1B} serotonin receptor gene estimation was made by the polymerase (PCR) chain reaction and minisequencing (SnaPshot kit, Applied Biosystems, USA). The first step was the amplification of the gene fragment with the 548 alkaline pairs.

Amplification was made by use the PCR Core Kit (Qiagen) as well as the following reaction mixture: Starter 1 : 5' - GAAACAGACGCCCAA-CAGGAC - 3', Starter 2 : 5' - CCAGAAACCGC-GAAAGAAGAT - 3', 1 x PCR buffer.

Reaction of amplification was led in following conditions: initial denaturation lasting 3 minutes in 95°C , then 35 cycles of denaturation in 95°C lasting 30 s, addition in 55°C lasting 45 s, elonga-

Table 1. The anthropometric characteristics of the investigated group of men. SD – standard deviation

Tabela 1. Charakterystyka antropometryczna badanej grupy mężczyzn. SD – odchylenie standardowe

	Mean (Średnia)	SD	Min-max. (min.-maks.)
Age – years (Wiek – lata)	47.00	± 12.81	23÷72
Body mass (Masa ciała) [kg]	84.22	± 13.62	54÷132
Height (Wysokość ciała) [cm]	175.61	± 6.8	159÷196
BMI [kg/m ²]	27.30	± 4.1	16÷42.91

tion in 72°C lasting 30 s as well as the final elongation in 72°C lasting 5 minutes.

Then, the amplification product was purified from the free nucleotides and oligonucleotidal fragments by use Exo and SAP enzymes (Fermentas, USA).

Detection of the polymorphic locus in purified products of amplification was made by minisequencing method. In comparison to the starter, the result of this reaction was a product elongated by one nucleotide, marked by particular fluorophor. Detection of the product were made by use ABI 310 Analyser (Applied Biosystems, USA), and the identification was made by GeneScan ver. 3.1.2 software (Applied Biosystems, USA).

Evaluation of the distribution of genotypes with Hardy-Weinberg equilibrium in groups with different activity level was made by a non-parametric χ^2 test. The statistical importance was defined as $p < 0.05$.

Results

Obtained results are presented in Tables 2 and 3.

Almost 50% of the investigated men declared high level of physical activity level, 30% – medium and 20% – low.

Genotyping of the 861 polymorphic locus of the 5-HT_{1B} serotonin receptor gene was performed in 347 men (because of the presence of endogenous polymerase chain reaction inhibitors and several cases of hemolysis in the biological material the authors could not obtain results for all the investigated persons).

Majority of them had G/G genotype – 61.7%, while G/C was present in 30.6% and C/C in 7.8%.

In group I, 68% of the studied subjects had the G/G genotype, 22% – G/C and 10% – C/C. In group II, almost 59% had the G/G genotype, 34% – G/C and 8% – C/C. In group III the distribution of the genotypes was respectively: 61% – G/G, 32% – G/C and 7% – C/C.

There was no statistically significant relationship ($\chi^2 = 3.04$, $p = 0.55$) between the physical activity and the frequencies of the investigated genotypes.

The distribution of the investigated genotypes was compared to the Hardy-Weinberg principle (Table 3).

The authors found that the distribution of genotypes was in H-W disequilibrium in group I ($p = 0.006$). The frequency of the C/C genotype (9.9%) was higher than expected (4.5%). In groups II and III there were no significant differences of the genotypes frequencies.

In the whole studied sample, the C/C genotype frequency was 7.8%, whereas its theoretical distribution according to the Hardy-Weinberg principle was 5.3% ($p = 0.001$).

Discussion

The serotonergic system plays an important role in human behavior.

Although the 5-HTR 1 b receptor has been identified in many regions of the central nervous system, its role in psychopathology is still being determined. The G861C polymorphism of 5-HT_{1B} serotonin receptor is linked to suicidal tendencies [11, 12], depression [13], aggression [14], alcohol-

Table 2. Frequencies of the particular genotypes in the 861 polymorphic locus of the 5-HT_{1B} serotonin receptor gene in groups of different physical activity level

Tabela 2. Częstość występowania poszczególnych rodzajów genotypów w miejscu polimorficznym 861 genu receptora serotoniny 5-HT_{1B} w grupach z różnym poziomem aktywności fizycznej

Physical activity level (Poziom aktywności fizycznej)	Genotypes (Rodzaje genotypów)						Total (Razem)	
	G/G		G/C		C/C			
	N	%	N	%	N	%	N	%
Low (Niski)	48	67.6	16	22.5	7	9.9	71	20.4
Medium (Średni)	61	58.6	35	33.6	8	7.7	104	30.0
High (Wysoki)	105	61.0	55	32.0	12	7.0	172	49.6
Total (Łącznie)	214	61.7	106	30.6	27	7.8	347	100

Table 3. Genotype frequencies compared to the Hardy-Weinberg principle [H-W]**Tabela 3.** Częstości występowania genotypów G i C oraz porównanie z prawem Hardy'ego-Weinberga (H-W)

The physical activity level (Poziom aktywności fizycznej)	Alleles frequency (Częstość alleli) [%]		Genotypes type (Genotypy) [%]						p value (Wartość p)
	PG	PC	G/G		G/C		C/C		
				H-W		H-W		H-W	
Low (Niski)	78.9	21.1	67.6	62.2	22.5	33.3	9.9	4.5	0.006**
Medium (Średni)	75.5	24.5	58.6	57.9	33.6	37.0	7.7	5.3	0.35
High (Wysoki)	77.0	23.0	61.0	59.3	32.0	35.4	7.0	5.3	0.21
Total (Łącznie)	76.9	23.1	61.7	59.2	30.6	35.5	7.8	5.3	0.001***

** $p < 0.05$.*** $p < 0.001$.

ism [8] and drug addiction [15]. The G861C polymorphism of 5-HT_{1B} serotonin receptor gene has been also found to be associated with the attention deficit hyperactivity disorder (ADHD) [16, 17].

Animal studies have suggested the involvement of the 5-HT_{1B} serotonin receptor in the locomotor behaviour. Studies carried out in laboratory animals proved that stimulation of the 5-HT_{1B} receptor with use of an active antagonist (CP 94253) may increase the locomotor activity [9]. Other authors noticed that injecting an antagonist of the 5-HT_{1B} receptor (RU 24969) into the substantia nigra of a rat causes the rotary movements of the animal [18].

In studied sample the frequencies of 861G and 861C alleles were respectively: 77% and 23%. These results stand in line with a previous survey in which the 861C allele frequency was 22% in Caucasians, 20% in Afro-Americans, 36% in Latin Americans and 38% in Asians [19].

The authors did not observe any statistically significant relationship between G861C polymorphism of the 5-HT_{1B} serotonin receptor gene and the activity level in the studied subjects. However in men presenting the lowest level of physical activ-

ity the distribution of genotypes was different than the expected by the Hardy-Weinberg equilibrium.

In the whole investigated sample, the C/C genotype frequency was 7.8%, whereas its theoretical distribution was 5.3% ($p < 0.001$).

The incompatibility of the obtained genotype frequencies in the low activity group suggests a role of the 5-HT_{1B} receptor polymorphism in the regulation of locomotor activity.

It is necessary to mention that own results may be biased by a few factors. Almost 50% of the investigated men were classified as highly active while only 20% of them declared a low level of daily physical effort. To the contrary, other authors indicated that sedentary lifestyle prevails in Poland [20]. In yet another study it was noticed that the declared level of physical activity is higher if the IPAQ questionnaire is filled individually rather than in presence of a qualified pollster [21]. It is also worth mentioning that the IPAQ questionnaire may overestimate the level of physical activity [22].

The authors concluded that the level of physical activity in men may be influenced by the genetic variance of the 5-HT_{1B} serotonin receptor.

References

- [1] **Maia JA, Thomis M, Beunen GJ:** Genetic factors in physical activity levels: a twin study. *Am J Prev Med* 2002, 23 (2 Suppl), 87–91.
- [2] **Fernstrom JD, Fernstrom MH:** Exercise, serum free tryptophan, and central fatigue. *J Nutr* 2006, 136 (2), 553–559.
- [3] **Blomstrand E, Perett D, Parry-Billings M, Newsholme EA:** Effect of sustained exercise on plasma amino acid concentrations and on 5-hydroxytryptamine metabolism in six different brain regions in the rat. *Acta Physiol Scand* 1989, 36 (3), 473–481.

- [4] **Mann JJ**: Role of the serotonergic system in the pathogenesis of major depression and suicidal behavior. *Neuropsychopharmacology* 1999, 21(2 Suppl), 99–105.
- [5] **Ballenger JC, Goodwin FK, Major LF, Brown GL**: Alcohol and central serotonin metabolism in man. *Arch Gen Psychiatry*. 1979, 36, 224–227.
- [6] **Virkkunen M, Rawlings R, Tokola R, Poland RE, Guidotti A, Nemeroff C, Bissette G, Kalogeras K, Karonen SL, Linnoila M**: CSF biochemistries, glucose metabolism, and diurnal activity rhythms in alcoholic, violent offenders, fire setters, and healthy volunteers. *Arch Gen Psychiatry* 1994, 51(1), 20–27.
- [7] **Huang Y, Grailhe R, Arango V, Hen R, Mann JJ**: Relationship of psychopathology to the human serotonin 1b genotype and receptor binding kinetics in postmortem brain tissue. *Neuropsychopharmacology* 1999, 21, 238–246.
- [8] **Crabbe JC, Philip TJ, Feller DJ, Hen R, Wenger CD, Lessov CN, Schafer GL**: Elevated alcohol consumption in null mutant mice lacking 5-HT_{1b} receptors. *Nat Genet* 1996, 14, 98–101.
- [9] **Tatarczyńska E, Antkiewicz-Michaluk L, Kłodzińska A, Stachowicz K, Chojnacka-Wójcik**: Antidepressant-like effect of the selective 5-HT_{1B} receptor agonist CP 94253: a possible mechanism of action. *Eur J Pharmacol* 2005, 23, 516(1), 46–50.
- [10] **Craig CL, Marshall AL, Sjöström M, Bauman AE, Booth ML, Ainsworth BE, Pratt M, Ekelund U, Yngve A, Sallis JF, Oja P**: International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* 2003, 35(8), 1381–1395.
- [11] **Arranz B, Eriksson A, Mellerup E, Plenge P, Marcusson J**: Brain 5-HT_{1a}, 5-HT_{1d} 5-HT₂ receptors in suicide victims. *Biol Psychiatry* 1994, 35, 457–463.
- [12] **Lowther S, Katona CLE, Crompton MR, Horton RW**: 5-HT_{1d} I 5-HT_{1b/1f} binding sites in depressed suicides: increased 5-HT_{1d} binding in globus pallidus but not cortex. *Mol Psychiatry* 1997, 2, 314–321.
- [13] **Huang Y, Grailhe R, Arango V, Hen R, Mann JJ**: Relationship of psychopathology to the human serotonin 1b genotype and receptor binding kinetics in postmortem brain tissue. *Neuropsychopharmacology* 1999, 21, 238–246.
- [14] **Saudou F, Aimara DA, Dierich A, LeMeur M, Raboz S, Segu L, Buhot M, Hen R**: Enhanced aggressive behavior in mice lacking 5-HT_{1b} receptor. *Science* 1994, 265, 1875–1878.
- [15] **Rocha BA, Searce-Levie K, Lucas JJ, Hiroi N, Castanon N, Crabbe JC, Nestler EJ, Hen R**: Increased vulnerability to cocaine in mice lacking the serotonin-1b receptor. *Nature* 1998, 393, 175–178.
- [16] **Smoller JW, Biederman J, Arbeitman L, Doyle AE, Fagerness J, Perlis RH, Sklar P, Faraone SV**: Association between the 5-HTR_{1b} receptor gene and inattentive subtype of ADHD. *Biol Psychiatry* 2006, 59(50), 460–467.
- [17] **Quist JF, Barr CL, Schachar R, Roberts W, Malone M, Tannock R, Basile VS, Beitchman J, Kennedy JL**: The serotonin 5-HT_{1B} receptor gene and attention deficit hyperactivity disorder. *Mol Psychiatry* 2003, 8 (1), 98–102.
- [18] **Oberlander C, Hunt PF, Dumont C, Boissier JR**: Dopamine independent rotational response to unilateral intranigral injection of serotonin. *Life Sci* 1981, 28 (23), 2595–2601.
- [19] **Ramboz S, Saudou F, Amara DA, Belzung C, Segu L, Misslin R, Buhot MC, Hen R**: 5-HT_{1b} receptor knockout: behavioural consequences. *Behav Brain Res* 1996, 73, 305–312.
- [20] **Kaleta D, Makowiec-Dąbrowska T, Jegier A**: Occupational and leisure-time energy expenditure and body mass index. *Int J Occup Med Environ Health* 2007, 20(1), 9–16.
- [21] **Biernat E, Stupnicki R, Lebedziński B, Janczewska L**: Assessment of physical activity by applying IPAQ questionnaire. *Phys Educ Sport* 2008, 52(2), 83–89.
- [22] **Rzewnicki R, Vanden Auweele Y, De Bourdeaudhuij I**: Addressing overreporting on the International Physical Activity Questionnaire (IPAQ) telephone survey with a population sample. *Public Health Nutr* 2003, 6 (3), 299–305.

Address for correspondence:

Małgorzata Słowińska-Lisowska
Department of Sport Medicine
University of Physical Education
Jana Paderewskiego 35
51-612 Wrocław
Poland
E-mail: slowi@gmail.com
Tel.: +48 71 347 3553, 501 143 753 20

Conflict of interest: None declared

Received: 17.11.2009

Revised: 16.06.2010

Accepted: 27.07.2010