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Functional polymorphisms of the leptin and leptin receptor genes are associated with longevity and with the risk of myocardial infarction and of type 2 diabetes mellitus

Funkcjonalne polimorfizmy genów leptyny i receptora leptyny korelują z długowiecznością oraz z ryzykiem zawału mięśnia serca i cukrzycy 2 typu

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Abstract

Introduction: Longevity is commonly associated with good health and with delayed onset of age-related diseases with usually benign course. Leptin (LEP) significantly affects metabolism and numerous functions of the organism. To find out if extreme longevity and its phenotype are associated with genetic variants of leptin and leptin receptor (LEPR) genes, we analysed the frequencies of the –2548 G/A and +19 G/A *LEP*, as well as the K109R, Q223R, and K656N *LEPR* polymorphisms in centenarians and in control groups.
Material and methods: The frequencies of the *LEP* and *LEPR* polymorphisms were tested by restriction fragment length polymorphism in 128 centenarians, 414 young controls (Y), 226 myocardial infarction (MI) patients, and 190 type 2 diabetes mellitus (DM2) patients.
Results: The GG genotype of the –2548 G/A *LEP* polymorphism was significantly more common in centenarians than in the Y, MI and DM2 groups ($p = 0.048$, $p = 0.003$, $p = 0.049$, respectively). In addition, the AA genotype of the K109R *LEPR* polymorphism was significantly less frequent in centenarians than in the Y, MI, and DM2 groups ($p = 0.026$, $p = 0.013$, and $p = 0.001$, respectively).
Conclusions: We suggest that the leptin pathway plays a role in the regulation of longevity, possibly by modulating the risk of development of MI and of DM2. **(Endokrynol Pol 2014; 65 (1): 11–16)**

Key words: *leptin gene (LEP); leptin receptor gene (LEPR); polymorphism; longevity; centenarians; myocardial infarction (MI); type 2 diabetes mellitus (DM2)*

Streszczenie

Wstęp: Długowieczności często towarzyszy dobry stan zdrowia, opóźnione zachorowanie na „choroby związane z wiekiem", których przebieg jest zwykle łagodny. Leptyna (LEP) znacząco wpływa na metabolizm oraz na inne funkcje organizmu. Aby sprawdzić, czy ekstremalna długowieczność i jej fenotyp są powiązane z odmianami genów leptyny i receptora leptyny (LEPR), przebadaliśmy częstość występowania polimorfizmów –2548 G/A i +19 G/A genu *LEP* oraz K109R, Q223R, i K656N genu *LEPR* u stulatków i w grupach kontrolnych.
Materiał i metody: Częstość występowania polimorfizmów genów *LEP* i *LEPR* badano metodą analizy długości fragmentów restrykcyjnych u 128 stulatków, 414 młodych kontroli (Y), 226 pacjentów z zawałem serca (MI) i u 190 pacjentów z cukrzycą 2 typu (DM2).
Wyniki: Genotyp GG polimorfizmu –2548 G/A genu *LEP* był znamiennie częstszy u stulatków niż w grupach Y, MI i DM2 (odpowiednio $p = 0{,}048$, $p = 0{,}003$ i $p = 0{,}049$). Genotyp AA polimorfizmu K109R genu *LEPR* był znamiennie rzadszy u stulatków niż w grupach Y, MI i DM2 (odpowiednio $p = 0{,}026$, $p = 0{,}013$ i $p = 0{,}001$).
Wnioski: Sugerujemy, że szlak oddziaływań leptyny bierze udział w regulowaniu długości życia, być może poprzez modulowanie ryzyka zachorowania na MI i DM2. **(Endokrynol Pol 2014; 65 (1): 11–16)**

Słowa kluczowe: *gen leptyny (LEP); gen receptora leptyny (LEPR); polimorfizm; długowieczność; stulatkowie; zawał serca (MI); cukrzyca 2 typu (DM2)*

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Introduction

Leptin (LEP) is a protein hormone with structural similarities to long-chain helical cytokines. It is secreted mainly by adipose tissue and, in small amounts, by other tissues [1]. LEP regulates appetite and energy balance and is associated with body mass and composition, and with BMI [2]. It also co-regulates other functions of the organism [3–11]. LEP acts on target tissues via ubiquitously expressed, membrane-localised receptors (LEPR) that display a structural similarity with the class I cytokine receptor family [12].

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The majority of published data indicate that ageing is associated with an increase in the LEP serum level [13, 14], although some reports suggest the opposite correlation [15]. Age-related hyperleptinemia correlates with resistance to LEP function on fat distribution and on insulin action [8, 16]. Hyperleptinemia and LEP resistance in elderly humans are risk factors for calcification of vascular cells [17], aggregation of platelets [18], increased oxidation of LDL particles [19], hypertension [20], obesity [21], impaired insulin signalling [22], coronary artery disease and metabolic syndrome [23], as well as stroke [24, 25], low bone density [6, 26], cancer [27] etc.

This prompted us to hypothesise that certain *LEP* and *LEPR* functional polymorphisms might extend life by lowering the risk of developing age-related diseases such as diabetes, cardiovascular disease and cancer, as well as by influencing the ageing process through modulation of metabolism and of insulin function.

To verify our hypothesis, we analysed the frequencies of the –2548 G/A, and +19 G/A *LEP*, as well as the K109R A/G, Q223R A/G, and K656N G/C *LEPR* polymorphisms in centenarians, in young persons, in myocardial infarction (MI) patients, and in type 2 diabetes (DM2) patients. The polymorphisms were selected on the basis of their proven functionality or association with the risk of development of diseases the frequency of which is increased in aged humans (see 'Discussion' for details).

We here present evidence that the frequency of certain *LEP* and *LEPR* polymorphisms is significantly different in centenarians and in young controls, in MI, and in DM2 patients, suggesting that the LEP axis may play a role in the regulation of longevity.

Material and methods

Study participants

A cross-sectional analysis of the *LEP* and *LEPR* genetic variants was performed in: 128 Polish centenarians never diagnosed with myocardial infarction (MI) or with type 2 diabetes mellitus (DM2) (C, 99.58-107.25 years, mean ± SD 101.11 ± 1.19 years, 112 females and 16 males) — participants in the 'PolStu2001' study [28]; in a young control group consisting of 414 blood donors and volunteers (Y, 18–45 years, mean 27.1 ± 7.3 years, 233 females and 181 males) with no signs and symptoms of any disease; in a myocardial infarction (MI) control group consisting of 226 patients, (first MI at the age of 28–55 years, mean 46.9 ± 5.3 years, 59 females, 167 males); and in a type 2 diabetes mellitus (DM2) control group consisting of 190 patients (diagnosed at the age of 26–55, mean 47.2 ± 5.3 years, 120 females, 70 males). Some of the MI and DM2 patients were participants of the 'PolSenior' study of the ageing population in Poland [29].

All participants gave written informed consent for participation in the study. The study protocol was approved by the Bioethical Committee of the Medical University of Warsaw.

DNA isolation and analysis of restriction fragment length polymorphisms

Four ml blood samples were used for the isolation of the genomic DNA; this was performed using the salting-out procedure [30]. Genotyping of the selected polymorphisms in *LEP* and in *LEPR* was done by PCR amplification followed by digestion with an appropriate restriction enzyme (restriction fragment length polymorphism method — RFLP). PCR-RFLP conditions used for the analysis are shown in Table I. The Q223R polymorphism of *LEPR* was analysed as previously described [31]. Conditions for the analysis of the remaining polymorphisms were developed in our laboratory. The restriction fragments obtained were visualised on 2–3% agarose gels.

Statistical analysis

All analyses were performed using the Statistica software package (StatSoft, Tulsa, OK, USA). Hardy-Weinberg equilibrium and differences in genotype frequencies in the introductory analysis, as well as between the analysed age/disease groups, were checked with the chi^2 test. The distribution of genotypes was analysed in three models of inheritance: dominant, recessive and codominant using 2×2, 2×2, 3×2 contingency tables, respectively. The differences in quantitative parameters between genotypes in the C group were assessed using ad-hoc test (Kruskal-Wallis ANOVA for cholesterol or ANOVA for other parameters) and post-hoc tests (Student's t-test for cholesterol or U Mann-Whitney test for other parameters). For all tests, the level of significance was established at 0.05.

Results

Distribution of the LEP and LEPR polymorphisms in centenarians vs. young individuals

Two polymorphisms in the leptin gene promoter (–2548 G/A, +19 G/A) and three amino acid sequence-changing polymorphisms in the leptin receptor-encoding gene (K109R A/G, Q223R A/G, K656N G/C) were studied. The frequencies of each genotype in centenarians and in young controls are shown in Table II.

Introductory analysis was performed in Y subjects. No significant sex-dependent differences in the frequencies of these polymorphisms were detected. Similarly, combined analysis of Y subjects and of C subjects showed that the distribution of the analysed polymorphisms did not differ significantly between sexes.

Table I. *PCR-RFLP conditions used for the analysis of the selected polymorphisms in the LEP and in the LEPR genes*
Tabela I. *Warunki PCR-RFLP użyte do analizy wybranych polimorfizmów genów LEP i LEPR*

Gene	Polymorphism	Primers	T_m (°C)	PCR product (bp)	Restriction enzyme	Alleles (bp)
LEP	–2548 G/A	F: 5'TGGGTACTTATACAACAAGAATAAACA3'	54	325	*Hha*I	A: 325
		R: 5'AAAGCAAAGACAGGCATAAAAA3'				G: 180, 145
	+19 G/A	F: 5'ATGGAGCCCCGTAGGAATC3'	59	220	*Taa*I	G: 220
		R: 5'CAGCTCCCGGTAACCTTCTA3'				A: 188, 32
LEPR	K109R A/G	F: 5'CTTTTGCCTGCTGGACTCTC3'	59	217	*Bsu*RI	A: 217
		R: 5'AAACTAAAGAAT TTACTGTTGAAACAAATGGC3'				G: 186, 31
	Q223R A/G	F: 5'ACCCTTTAAGCTGGGTGTCCCAAATAG3'	56	416	*Bse*NI	A: 221, 125, 71
		R: 5'AGCTAGCAAATATTTTTGTAAGCAATT3'				G: 186, 31
	K656N G/C	F: 5'ACTAGATGGACTGGGATATTGGAGTAAT3'	56	251	*Bst*UI	G: 251
		R: 5'CTTCCAAAGTAAAGTGACATTTTTCGC3'				C: 231, 20

T_m — melting temperature; F — forward; R — reverse; bp — base pairs

Table II. *Frequencies of the examined genotypes in centenarians, young study subjects, myocardial infarction (MI), and type 2 diabetes mellitus (DM2) patients (%)*
Tabela II. *Częstość badanych genotypów u stulatków, młodych kontroli, pacjentów z zawałem serca (MI), pacjentów z cukrzycą typu 2 (DM2) (%)*

	LEP –2548 G/A		*LEP* +19 G/A		*LEPR* K109R A/G		*LEPR* Q223R A/G		*LEPR* K656N G/C	
Centenarians	GG	43.75	GG	32.00	AA	41.40	AA	24.22	GG	78.91
(n = 128)	GA	42.19	GA	42.40	AG	50.00	AG	46.87	GC	17.97
	AA	14.06	AA	25.60	GG	8.60	GG	28.91	CC	3.12
Young controls	GG	33.57	GG	33.65	AA	52.66	AA	28.02	GG	71.74
(n = 414)	GA	49.03	GA	48.56	AG	41.30	AG	49.76	GC	26.57
	AA	17.40	AA	17.79	GG	6.04	GG	22.22	CC	1.69
MI	GG	29.64	GG	31.41	AA	52.11	AA	26.11	GG	72.57
(n = 226)	GA	53.10	GA	53.10	AG	38.16	AG	47.78	GC	25.22
	AA	17.26	AA	15.49	GG	9.73	GG	26.11	CC	2.21
DM2	GG	37.37	GG	29.47	AA	58.42	AA	25.26	GG	75.79
(n = 190)	GA	51.05	GA	50.00	AG	33.68	AG	51.58	GC	20.53
	AA	11.58	AA	20.53	GG	7.90	GG	23.16	CC	3.68

Next, the *LEP* and *LEPR* polymorphisms were compared in the two age groups. We found that the GG genotype of the –2548 G/A *LEP* polymorphism was significantly more frequent in centenarians than in young controls (43.75% *v.* 33.57%, p = 0.048, OR = 1.539 [95% CI: 1.006–2.352]). The +19 G/A *LEP* polymorphism was similarly distributed.

The K109R *LEPR* polymorphism was also differently distributed among the two groups: the AA genotype was significantly less frequent in the C group than in the Y group (41.40% *v.* 52.66%, p = 0.026, OR = 0.635 [95% CI: 0.417–0.967]). For the Q223R and K656N *LEPR* polymorphisms, no significant differences in the distribution of genotypes were observed between the groups.

Distribution of the LEP and LEPR polymorphisms in centenarians vs. myocardial infarction patients

The frequencies of the same polymorphisms in myocardial infarction patients (MI) are shown in Table II. We found no significant differences in the distribution of all analysed *LEP* polymorphisms between the MI

and Y groups. However, comparison of the C and MI groups revealed that polymorphisms of the *LEP* gene promoter were differentially distributed: the GG genotype of the –2548 G/A polymorphism was more frequent in centenarians than in MI patients (43.75% *v*. 29.64%, p = 0.003, OR = 1.85 [95% CI: 1.18–2.89]).

No significant differences in frequencies of the K109R, Q223R and K656N *LEPR* polymorphisms were detected between the MI and Y groups. The AA genotype of the K109R was significantly less frequent in the C group than in the MI group (41.40% *v*. 52.11%, p = 0.013, OR = 0.65 [95% CI: 0.42–1.00]). The differences in distribution of the remaining two *LEPR* polymorphisms in the C *vs*. MI groups were insignificant.

Distribution of the LEP and LEPR polymorphisms in centenarians v. type 2 diabetes mellitus patients

Analysis of the frequencies of the polymorphisms under study in type 2 diabetes mellitus patients (Table II) gave the following results.

The AA genotype of the –2548 G/A *LEP* polymorphism was significantly less frequent in the DM2 group than in the Y group (11.58% *v*. 17.40%, p = 0.018, OR = 1.60 [95% CI: 0.96–2.67]). The genotypes of the +19 G/A *LEP* polymorphism were not differently distributed among these groups. Comparison of the C and DM2 groups showed that the GG genotype of the –2548 G/A *LEP* polymorphism was significantly more common in centenarians (43.75% *v*. 37.37%, p = 0.049, OR = 1.3 [95% CI: 0.83–2.05]). The +19 G/A polymorphism was distributed equally in the C and DM2 groups.

Analysis of the *LEPR* polymorphisms indicated that the AA genotype of the K109R polymorphism and the GG genotype of the K656N polymorphism were less frequent in the Y group than in the DM2 group (K109R: 52.66% *v*. 58.42%, p = 0.029, OR = 0.79 [95% CI: 0.561.12]; K656N: 71.74% *v*. 75.79%, p = 0.046, OR = 0.81 [95% CI: 0.54–1.20]), while the frequencies of the Q223R genotypes did not differ between these groups. The AA genotype of the K109R polymorphism was also significantly less frequent in the C group than in the DM2 group (41.40% *v*. 58.42%, p = 0.001, OR = 0.50 [95% CI: 0.32–0.79], respectively). The frequencies of the remaining *LEPR* genotypes did not differ between these groups.

Correlations of the LEP and LEPR polymorphisms with metabolic variables in centenarians

The metabolic variables of the C group were as follows: body mass index (BMI) 15.3–37.4 kg/m^2, mean ± SD 23.6 ± 4.4 kg/m^2; fasting glucose 26–315 mg/dL, mean ± SD 86.7 ± 31.8 mg/dL; insulin 5.82-586 pmol/L, mean ± SD 58.73 ± 81.18 pmol/L; total cholesterol 100–316 mg/dL, mean ± SD 195.3 ± 45.4 mg/dL; HDL cholesterol 26–136 mg/dL, mean ± SD 60.0 ± 17.7 mg/dL; triglycerides 45–436 mg/dL, mean ± SD 108.8 ± 51.4 mg/dL. Association analysis of genotypes of the *LEP* and *LEPR* polymorphisms with these metabolic measures was performed.

The Q223R *LEPR* polymorphism correlated with fasting glucose: carriers of the AA genotype of this polymorphism had a significantly lower mean fasting glucose level than GG homozygotes (80.12 ± 12.21 mg/dL *v*. 95.75 ± 39.42 mg/dL, p = 0.002). They also had a significantly lower mean fasting insulin level than carriers of at least one G allele (44.59 ± 78.92 pmol/L *v*. 64.52 ± ± 87.53 pmol/L, p = 0.013), and GG homozygotes (44.59 ± ± 78.92 pmol/L *v*. 52.74 ± 46.91 pmol/L, p = 0.06).

We found no significant associations between the G-2548A and G+19A *LEP*, as well as the K109R and K656N *LEPR* genotypes and BMI, fasting glucose, total cholesterol, HDL cholesterol, and triglycerides levels.

Discussion and conclusions

Ageing is accompanied by an increased risk of developing cardiovascular disease, cancer, diabetes, osteoporosis, sarcopenia and diseases related to immune system dysfunction. All these conditions negatively affect longevity. It is well documented that age progression is associated with an increase in the level of LEP [13, 14]; however, this rule does not seem to apply to centenarians [32]. To ascertain whether LEP signalling has an impact on extreme longevity, which is usually associated with good health and delayed occurrence of typical age-related diseases, we analysed the frequencies of the –2548 G/A and +19 G/A *LEP*, as well as the K109R A/G, Q223R A/G, and K656N G/C *LEPR* polymorphisms in centenarians and compared them to their frequencies in young individuals, as well as in MI and in DM2 patients who were diagnosed before the age of 55. We purposely selected young MI and DM2 patients, since a genetic predisposition towards development of various diseases plays a more important role in young than in old persons and, therefore, can be more easily detected.

We found that the GG genotype of the –2548 G/A *LEP* promoter polymorphism is significantly more common in centenarians never diagnosed with MI or with DM2 than in young controls, and in the MI and DM2 groups. This finding seems to contrast with previously published data showing that the G allele of this polymorphism is associated with obesity [33, 34]. There are at least three potential explanations for such a discrepancy: first, obesity at a younger age might be of low importance for extreme longevity; second, this polymorphism might be a low-importance regulatory factor of longevity; third, a protective role of this geno-

type against development of MI or DM2 might prevail over its influence on obesity. This third hypothesis seems to be the most probable, since, as mentioned above, we also found that this genotype was significantly more common in the C group than in the MI and DM2 groups. In addition, overrepresentation of this genotype in the C group remains in agreement with previous findings that it is the A allele which increases the risk of development of non-small cell lung cancer, oral, prostate and breast cancers, as well as non-Hodgkin's lymphoma [35–41]. Indeed, our centenarian study subjects rarely suffered from neoplastic disease: only two of them had cancers (uterus or thyroid), and six others had non-melanoma-type skin cancer of benign clinical course in sun-exposed skin areas.

The +19 G/A *LEP* promoter polymorphism, had not been extensively studied; however, available reports show that its GG and GA genotypes correlate with the risk of various cancers [35, 42, 43]. We found that it was related neither to longevity, nor to MI or DM2.

The K109R *LEPR* polymorphism is the next one that is associated with extreme longevity. We showed that its AA genotype is significantly less common in the C group than in the Y, MI and DM2 groups. This remains in agreement with previous findings that this genotype is associated with obesity [44] and with increased DM2 risk [45].

The presence of the Q223R *LEPR* G allele was previously associated with obesity [46–48]. However, the findings regarding its association with altered glucose and lipid metabolism are conflicting [48–51]. In addition, the GG genotype of this polymorphism correlates with increased risk of breast carcinoma and of oral cancer [38, 39, 41]. We showed in this work that this polymorphism is in fact not associated with longevity. Inconsistency of the available data regarding its role in morbidity makes plausible the idea that this polymorphism does not play a role in longevity regulation. Be that as it may, we should remember that the C group might not be sufficiently large to show a correlation.

While correlating metabolic parameters' values with the *LEP* and *LEPR* genotypes, we found that centenarian carriers of the Q223R *LEPR* AA genotype paradoxically had a significantly lower mean fasting glucose and insulin levels compared to carriers of other genotypes.

This finding is of great importance since it has been previously found that low-normal fasting glucose levels are associated with lower risk of DM2 compared to high-normal values [52, 54]. Our results remain in agreement with those of Chin et al. and of Takahashi-Yasuno et al. [49, 50].

This study presents only preliminary data and has several limitations. First, the *LEP* and *LEPR* polymorphism frequencies were analysed only in young and in centenarian age groups, while it would have been more informative to test an additional group of intermediate age. Second, the number of centenarians, although high if compared to centenarian groups analysed in the majority of other publications, amounted to only 128; therefore, our results should be confirmed on a much larger group of centenarians of various ethnic backgrounds. Third, it could be objected that our study groups were uneven with respect to sex distribution. This is, however, of little or no significance since our introductory analysis showed no association of the analysed polymorphisms with sex.

To sum up, we suggest that the leptin pathway could impact upon human longevity. We also suggest that this might be so, at least in part, because of the modulation of the risk of developing cardiovascular disease and type 2 diabetes mellitus.

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