Total and high molecular weight adiponectin and level--modifying polymorphisms of *ADIPOQ* in centenarians

Całkowita i wysokocząsteczkowa adiponektyna oraz modyfikujące stężenie adiponektyny polimorfizmy genu *ADIPOQ* u stulatków

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Abstract

Introduction: Adiponectin demonstrates a protective role against the development of obesity, type 2 diabetes mellitus, and cardiovascular disease. The –11377C > G, –11391G > A, and –11426A > G promoter polymorphisms of *ADIPOQ* gene influence the level of circulating adiponectin. We examined the level of total and high molecular weight (HMW) adiponectin in centenarians and associated it with biochemical parameters. We checked if the expression and concentration-modifying polymorphisms of *ADIPOQ* are associated with extreme longevity.
Material and methods: Total and HMW adiponectin were examined using ELISA in 40 female centenarians. The frequencies of the *ADIPOQ* polymorphisms were tested by restriction fragment length polymorphism in 148 centenarians, 414 young controls, in 207 myocardial infarction patients, and in 190 type 2 diabetes mellitus patients.
Results: The mean concentration of total adiponectin in centenarians was 13.19 ± 1.37 µg/mL and of HMW adiponectin it was 9.17 ± 1.15 µg/mL. They were positively correlated with HDL (r = 0.4696, p = 0.0025 and r = 0.3912, p = 0.015, respectively), and negatively with BMI (r = –0.3702, p = 0.034 and r = –0.3963, p = 0.025) and triglycerides (r = –0.346, p = 0.028 and r = –0.3227, p = 0.045). A very rare AA genotype of the –11391G > A polymorphism was significantly more common in centenarians than in young controls (p = 0.026) and, while compared to the GG genotype, it was associated with a 2.4-fold higher mean concentration of total adiponectin (26.53 ± 13.29 µg//mL *v.* 10.97 ± 4.28 µg/mL) and with an almost 3-fold higher mean HMW adiponectin (20.65 ± 12.72 µg/mL *v.* 7.36 ± 3.35 µg/mL).
Conclusions: Serum adiponectin concentration in female centenarians is associated with biochemical parameters that are favourable for cardiovascular risk. We suggest that adiponectin might be of importance for extreme longevity. **(Endokrynol Pol 2012; 63 (6): 439–446)**

Key words: total and high molecular weight (HMW) adiponectin, adiponectin gene (ADIPOQ), polymorphism, centenarians, myocardial infarction (MI), type 2 diabetes mellitus (DM2)

Streszczenie

Wstęp: Adiponektyna odgrywa ochronną rolę w patogenezie otyłości, cukrzycy typu 2 i chorób układu krążenia. Polimorfizmy –11377C > G, –11391G > A, i –11426A > G promotora *ADIPOQ* wpływają na stężenie adiponektyny. Autorzy zbadali stężenie całkowitej i wysokocząsteczkowej adiponektyny u stulatków i powiązali je z parametrami biochemicznymi. Sprawdzili, czy modyfikujące stężenie adiponektyny polimorfizmy *ADIPOQ* korelują z długowiecznością.
Materiał i metody: Stężenia całkowitej i wielkocząsteczkowej adiponektyny oznaczono metodą ELISA u 40 stulatków. Częstość występowania polimorfizmów *ADIPOQ* zbadano metodą analizy długości fragmentów restrykcyjnych u 148 stulatków, 414 młodych, zdrowych ochotników, 207 chorych po przebytym zawale serca i u 190 chorych z cukrzycą typu 2.
Wyniki: Średnie stężenie całkowitej adiponektyny u stulatków wynosiło 13,19 ± 1,37 *µg*/ml, a adiponektyny wielkocząsteczkowej 9,17 ± 1,15 *µg*/ml. Stężenia te pozytywnie korelowały ze stężeniem HDL (odpowiednio r = 0,4696, p = 0,0025 i r = 0,3912, p = 0,015), a negatywnie z BMI (r = –0,3702, p = 0,034 i r = –0,3963, p = 0,025) i stężeniem triglicerydów (r = –0,346, p = 0,028 i r = –0,3227, p = 0,045). Bardzo rzadki genotyp AA polimorfizmu –11391G > A występował znamiennie częściej u stulatków niż u młodych kontroli (p = 0,026) i, w porównaniu z genotypem GG, był związany z 2,4-krotnie wyższym stężeniem całkowitej adiponektyny (26,53 ± 13,29 *µg*/ml *v.* 10,97 ± 4,28 *µg*/ml) i prawie 3-krotnie wyższym stężeniem adiponektyny wielkocząsteczkowej (20,65 ± 12,72 *µg*/ml *v.* 7,36 ± 3,35 *µg*/ml).
Wnioski: Korelacja pomiędzy stężeniami całkowitej i wielkocząsteczkowej adiponektyny u stulatków a korzystnymi z punktu widzenia ryzyka sercowo-naczyniowego parametrami biochemicznymi może świadczyć o udziale adiponektyny w promowaniu długowieczności.
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Słowa kluczowe: całkowita i wielkocząsteczkowa (HMW) adiponektyna, gen adiponektyny (ADIPOQ), polimorfizm, stulatkowie, zawał mięśnia serca (MI), cukrzyca typu 2 (DM2)

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Introduction

Adiponectin is a protein hormone secreted by adipocytes. It has a higher expression in lean than in obese individuals and in women than in men [1]. Adiponectin is encoded by the *ADIPOQ* gene consisting of three exons and two introns. Many common promoter-, exon- and intron-located single nucleotide polymorphisms of this gene have been reported [2].

Adiponectin inhibits the expression of gluconeogenic enzymes and the rate of glucose production. It also stimulates glucose utilisation and fatty acids oxidation [1]. Consequently, its level correlates positively with insulin sensitivity and with HDL level, and negatively with the levels of fasting glucose, insulin, total cholesterol, LDL, triglycerides, CRP, interleukin-6 and -18, and with TNF [3–6]. A high level of circulating adiponectin protects against the development of obesity, insulin resistance, glucose intolerance, type 2 diabetes mellitus (DM2), hypertension, metabolic syndrome, atherosclerosis, and cardiovascular disease (CVD) [4, 6–9]. A protective role of high molecular weight (HMW) adiponectin against these diseases and a negative role of low molecular weight adiponectin on DM2 and CVD have recently been underlined [10–13]. However, in patients with prevalent CVD, hyperadiponectinaemia is not protective, but represents a compensatory mechanism and is a predictor of all-cause and of CVD mortality [8]. With advancing age, the level of total adiponectin increases in both sexes. In very old individuals it is significantly higher than in younger subjects [5,14].

The –11377C > G, –11391G > A, and –1426A > G polymorphisms in the *ADIPOQ* promoter influence the circulating adiponectin level: the presence of the G allele of the –11377C > G, the G allele of the –11391G > A, and the G allele of the –11426A > G polymorphisms are associated with a lower level of this hormone [15–19]. This phenomenon is the result of modulation of transcription from the *ADIPOQ* promoter [16–19].

The aims of the study were to evaluate the level of total and HMW adiponectin and its associations with biochemical parameters in centenarians and to determine if the expression level-modifying polymorphisms of the *ADIPOQ* are associated with extreme longevity.

Material and methods

Study participants

All study subjects were ethnically homogenous Polish Caucasians. Analysis of the *ADIPOQ* genetic variants was performed in four groups: 148 centenarians (99.6–107.2 years old, mean ± SD 101.1 ± 1.2 years old, 129 females and 19 males), participants in the 'PolStu2001' study [20]; a young control group con-sisting of 414 blood donors and volunteers (18 to 45 years old, mean 27.1 ± 7.3, 233 females and 181 males, with only 79 individuals who were 35 years old or older) with no signs and symptoms of any disease; a myocardial infarction (MI) control group consisting of 207 patients (first MI at the age of 28-55, mean age at first MI 47.1 ± 5.2, 53 females and 154 males); and a type 2 diabetes mellitus (DM2) control group consisting of 190 patients (diagnosed at the age of 26-55, mean age at diagnosis 47.7 ± 5.6, 120 females and 70 males). Some of the MI and DM2 control patients were participants in the 'PolSenior' study [21].

Due to a limited availability of plasma, total and HMW adiponectin concentrations were measured only in 40 pre-selected centenarian women (100 to 104.7 years old, mean 100.9 ± 0.96). The selection was based on the availability of the biological material and on the genotype of the *ADIPOQ* polymorphisms they carried; special attention was paid to ensure that as many individuals as possible with rare variants of the studied polymorphisms were included in this study. Following this procedure, all but one (GG of the –11426A > G) genotypes of the tested *ADIPOQ* polymorphisms were represented in this group. Five out of 40 centenarian women did not take any medications. Seventeen were treated with one to three medications, usually sedatives, drugs improving brain blood flow, vitamins, laxatives, and painkillers. The remaining 18 women were treated with four or more medications; in addition to the supporting drugs (the same as mentioned above), these were various drugs used to treat hypertension and cardiovascular disease.

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.

Basic biochemical parameters
Basic biochemical parameters in serum/plasma of the 40 abovementioned centenarian women were examined in the diagnostic laboratory of the Infant Jesus Teaching Hospital of the Medical University of Warsaw, according to a routine procedure.

Analysis of total and high molecular weight adiponectin concentrations
Concentrations of total and HMW adiponectin were measured with an Adiponectin (Multimeric) ELISA kit (ALPCO Diagnostics, Salem, NH, USA) according to the manufacturer's protocol.

DNA isolation and analysis of restriction fragment length polymorphisms
Blood samples of 4 ml were used to isolate the genomic DNA; this was performed by a salting-out

Table I. *PCR-RFLP conditions used for the analysis of the –11377C>G, –11391G>A and –11426A > G polymorphisms in the* ADIPOQ *gene*

Tabela I. *Warunki PCR-RFLP stosowane podczas analizy polimorfizmów –11377C>G, –11391G>A i –11426A > G w genie* ADIPOQ

Polymorphism	PCR Primers	T_m	PCR product	Restriction	Alleles
–11377C > G	F: 5'GCTCTGTGTGGACTGTGGAG3'	63°C	302 bp	*Hhal*, 37°C, 3 h	C: 302 bp
	R: 5'AGAAGCAGCCTGGAGAACTG3'				G: 182, 120 bp
–11391G > A	F: 5'GCTCTGTGTGGACTGTGGAG3'	63°C	278 bp	*MspI*, 37°C, 3 h	G: 166, 112 bp
	R: 5'CTGCCACCCACTTAGGTGTT3'				A: 278 bp
–11426A > G	F: 5'CATCTCAACGGCCTAATGTG3'	56°C	273 bp	*TasI*, 65°C, 3 h	A: 185, 62, 26 bp
	R: 5'AAGCCACACATTCTGATGAACTAA3'				G: 185, 88 bp

T_m — PCR melting temperature, F — forward, R — reverse, bp — base pairs

procedure [22]. The concentration and purity of DNA were assessed by UV spectroscopy. Genotyping of the selected polymorphisms in the *ADIPOQ* was done by PCR amplification followed by digestion with an appropriate restriction enzyme (restriction fragment length polymorphism method, RFLP). PCR conditions were: initial denaturation at 94°C for 5 min followed by 40 cycles of 94°C for 30 s, 56°C or 63°C for 30 s, 72°C for 30 s, final elongation at 72°C for 5 min. Each 12.5 μl reaction contained 50 ng of the genomic DNA, 2.0 mM MgCl$_2$, 10 pmol of each primer, 0.25 mM of each dNTP, and 1 unit of *Taq* polymerase in a buffer supplied by the manufacturer (Invitrogen, Carlsbad, CA, USA). Other details of the PCR-RFLP conditions are shown in Table I. The restriction fragments obtained were visualised on 2–3% agarose gels.

Statistical analysis

The genotype distribution was analysed using the Web-Assotest programme (available at: http://www.ekstroem.com/assotest/assotest.html) assuming dominant and recessive models of inheritance. Under each model, the odds ratio (OR) with a 95% confidence interval (CI) and the p value for an association were calculated. The Hardy-Weinberg equilibrium was assessed by a χ^2 test.

The impact of genotypes on total and HMW adiponectin concentrations was calculated using the Statistica software package (StatSoft, Tulsa, OK, USA). Normality of distribution and homogeneity of the variance were checked with the Shapiro-Wilk and Levene's tests, respectively. Where these conditions were not met, analyses were performed with non-parametric Kruskal-Wallis ANOVA (ad-hoc) and U Mann-Whitney (post hoc) tests.

Correlations between quantitative values were performed with the Spearman correlation test (Statistica software package).

For all tests, the level of significance was established at 0.05.

Results

Concentrations of total and high molecular weight adiponectin and their associations with biochemical parameters in centenarians

Adiponectin concentrations were measured in 40 female centenarians. The ranges and the median values of BMI, fasting glucose, insulin, total cholesterol, HDL, and triglycerides in these centenarians are shown in Table II. The mean concentration of total adiponectin was 13.19 ± 1.37 μg/mL (median 10.82 μg/mL, 1st quartile 8.86, 3rd quartile 16.17), and the mean concentration of HMW adiponectin was 9.17 ± 1.15 μg/mL (median 7.47 μg/mL, 1st quartile 5.36, 3rd quartile 11.54). Total and HMW adiponectin were strongly correlated (r = 0.8956, p < 0.0001). The mean HMW to total adiponectin ratio was 0.695.

Total and HMW adiponectin were positively correlated with the level of HDL (r = 0.4696, p = 0.0025 and r = 0.3912, p = 0.015, respectively) and negatively with BMI (r = –0.3702, p = 0.034 and r = –0.3963, p = 0.025) and with the level of triglycerides (r = –0.346, p = 0.028 and r = –0.3227, p = 0.045, respectively). No other significant correlations with clinical parameters were found. No correlation with the number or type of medications was noticed.

Distribution of the ADIPOQ –11377C > G, –11391G > A, and –11426A > G polymorphisms in centenarians and in young controls

Genotype frequencies of the *ADIPOQ* polymorphisms in centenarians and in young controls are shown in Table III.

Table II. *Values of the basic metabolic parameters (fasting) in 40 female centenarians who underwent testing for total and HMW adiponectin*

Tabela II. *Wartości podstawowych parametrów metabolicznych oznaczone na czczo u 40 kobiet z grupy stulatków, u których badano stężenia całkowitej i wielkocząsteczkowej adiponektyny*

	Range	Median (1st quartile, 3rd quartile)	Mean ± SD
BMI* [kg/m²]	15.75–33.20	22.96 (20.92, 26.25)	23.72 ± 4.02
Glucose [mg/dL]	34–315	87 (80.75, 95.75)	95.30 ± 42.42
Insulin [pmol/L]	9.86–435	32.15 (23.17, 68.95)	57.21 ± 72.10
Total cholesterol [mg/dL]	127–287	201 (165.75, 242)	201.65 ± 44.82
HDL [mg/dL]	32–117	67 (48, 82)	64.41 ± 20.66
Triglycerides [mg/dL]	47–247	104.5 (74, 135)	109.62 ± 44.49

*calculated for 33 centenarians

Table III. *Frequencies of the examined genotypes in centenarians, young controls, MI patients, and in DM2 patients*

Tabela III. *Częstości badanych genotypów u stulatków, zdrowych, młodych kontroli, chorych po przebytym zawale serca i chorych z cukrzycą typu 2*

	−11377C > G			−11391G > A			−11426A > G		
	gt	%	n	gt	%	n	gt	%	n
Centenarians	CC	48.65	72	GG	81.89	122	AA	82.99	122
	CG	43.92	65	GA	16.10	24	AG	16.32	24
	GG	7.43	11	AA	2.01	3	GG	0.69	1
Young controls	CC	51.69	214	GG	85.51	354	AA	84.30	349
	CG	38.65	160	GA	14.25	59	AG	14.97	62
	GG	9.66	40	AA	0.24	1	GG	0.73	3
MI patients	CC	50.48	105	GG	86.90	179	AA	81.82	171
	CG	41.82	87	GA	12.62	26	AG	17.22	36
	GG	7.70	16	AA	0.48	1	GG	0.96	2
DM2 patients	CC	49.21	93	GG	88.89	168	AA	81.08	150
	CG	43.92	83	GA	9.52	18	AG	18.38	34
	GG	6.88	13	AA	1.59	3	GG	0.54	1

gt: genotype, %: percentage, n: number of examined samples

The initial analysis was aimed at establishing the pattern of their distribution in both sexes. Upon analysis of the young controls group, we found that the tested polymorphisms were distributed equally in males and in females.

Next, we established genotype frequencies of these polymorphisms in both age groups. We found that the AA genotype of the −11391G > A polymorphism was significantly more common in centenarians than in young controls (p = 0.026, 2.01% v. 0.24%, OR = 8.48 [95% CI 1.20–59.60]), and in centenarians without MI and/or DM2 (n = 130) than in young controls (p = 0.031, 2.29% v. 0.24%, OR = 9.68 [95% CI 1–93.87]). The −11377C > G and −11426A > G polymorphisms were distributed similarly in all tested groups.

Associations of the ADIPOQ −11377C > G, −11391G > A, and −11426A > G polymorphisms with the mean concentrations of total and high molecular weight adiponectin in centenarians

Almost all genotypes of the −11377C > G, −11391G > A, and −11426A > G polymorphisms were associated with the similar mean concentrations of total and HMW adiponectin in the tested group of 40 female centenarians (Table IV). However, we noticed that a very rare AA genotype of the −11391G > A polymorphism was associated with a 2.4-fold higher mean concentration of total adiponectin and with an almost 3-fold higher mean HMW adiponectin compared to the values associated with the GG genotype (26.53 ± 13.29 μg/mL v. 10.97 ±

Table IV. *Association of the ADIPOQ genotypes with total and HMW adiponectin in female centenarians*

Tabela IV. *Związek genotypów genu ADIPOQ ze stężeniem całkowitej i wielkocząsteczkowej adiponektyny u kobiet-stulatek*

		Range	Median (1st quartile, 3rd quartile)	Mean ± SD	p value
Total adiponectin [µg/mL]					
–11377C > G					ns
gt	**n**				
CC	13	5.30–35.90	17.10 (10.3, 19.06)	17.19 ± 9.86	
CG	23	5.61–19.98	10.2 (7.62, 14.06)	11.17 ± 4.48	
GG	4	10.51–15.24	10.71 (10.51, 12.0)	11.79 ± 2.31	
–11391G > A					ns
gt	**n**				
GG	26	5.30–19.98	10.51 (7.64, 12.68)	10.97 ± 4.25	
GA	12	5.46–34.67	14.96 (10.03, 18.83)	15.78 ± 8.20	
AA	2	17.10–35.90	26.50 (21.80, 31.20)	26.50 ± 13.29	
–11426A > G					ns
gt	**n**				
AA	31	5.61–35.90	10.92 (8.99, 15.86)	13.49 ± 7.44	
AG	9	5.30–19.52	10.71 (9.01, 19.06)	12.14 ± 5.69	
HMW adiponectin [µg/mL]					
–11377C > G					ns
gt	**n**				
CC	13	3.04–29.67	11.74 (5.68, 14.68)	12.67 ± 8.90	
CG	23	3.09–15.14	6.90 (4.92, 9.84)	7.58 ± 3.32	
GG	4	5.72–10.97	6.87 (6.14, 8.34)	7.61 ± 2.35	
–11391G > A					ns
gt	**n**				
GG	26	3.04–15.14	6.44 (4.94, 8.60)	7.38 ± 3.37	
GA	12	3.81–28.89	11.07 (6.49, 12.36)	11.26 ± 7.10	
AA	2	11.64–29.67	20.65 (16.15, 25.16)	20.65 ± 12.75	
–11426A > G					ns
gt	**n**				
AA	31	3.09–29.67	7.83 (5.12, 11.36)	9.45 ± 6.41	
AG	9	3.04–13.75	6.33 (5.68, 11.55)	7.98 ± 3.79	

gt: genotype, ns: not significant, n: number of examined samples

± 4.28 µg/mL and 20.65 ± 12.72 µg/mL *v.* 7.36 ± 3.35 µg//mL for total and HMW adiponectin, respectively).

Distribution of the ADIPOQ –11377C > G, –11391G > A, and –11426A > G polymorphisms in myocardial infarction patients and in type 2 diabetes mellitus patients

Genotype frequencies of the *ADIPOQ* polymorphisms in MI patients and DM2 patients are shown in Table III. There was a trend toward significant difference in the frequency of the AA genotype of the –11391G > A

polymorphism between DM2 patients and young controls (p = 0.072, 1.59% *v.* 0.24%, OR = 6.66 [95% CI 0.69–64.46]). The difference between DM2 patients and centenarians was not significant. The –11377C > G and –11426A > G polymorphisms were equally distributed in DM2 patients, young controls, centenarians, and centenarians without DM2.

No significant differences were found in the distribution of the tested polymorphisms between MI patients, young controls, centenarians, and centenarians without MI.

Discussion and conclusions

Previous reports concerning adiponectin in centenarians have established this level approximately at $17\,\mu g$/mL and at $20\,\mu g$/mL [14, 23]. In this study, we found that the mean total adiponectin in female centenarians is approximately $13\,\mu g$/mL; even though lower than previously reported, this is still higher than in all younger age groups: the mean concentration of this hormone in healthy humans has been reported to be approximately $5–11\,\mu g$/mL at 20–45 years old [12, 14], $6.5–10\,\mu g$/mL at 55–70 years old [14, 24], and $8–11\,\mu g$/mL at 80-90 years old [12]. The mean concentration of HMW adiponectin in healthy humans was reported to be $3–7\,\mu g$/mL in young and elderly and $3–9\,\mu g$/mL in old humans [10, 12], with its level being higher in females than in males. To date, HMW adiponectin had not been measured in centenarians; we report here that its mean concentration in females of this age group is approximately $9\,\mu g$/mL. The mean ratio of HMW to total adiponectin in centenarians is 0.695, which is higher than that of younger age groups [11, 12, 25].

Altogether, these figures show that extreme longevity is associated with the highest concentrations of total and HMW adiponectin and with the highest HMW/ /total adiponectin ratio. This phenomenon might have a number of explanations.

Firstly, high concentrations of total and HMW adiponectin truly promote longevity. It should be noted though, that the mechanisms of such action of this hormone are not completely clear. As described in the Introduction, it decreases the risk of development of life-shortening diseases, but we should keep in mind that total and HMW adiponectin were consistently higher in centenarians than in individuals younger than 90 years old, who were free of such diseases (healthy ageing). Therefore, other, yet unknown, functions might be also involved in the longevity-promoting action of adiponectin.

Secondly, posttranslational modifications, such as proteolytic cleavage, glycosylation, and hydroxylation that affect the conformation and stability of adiponectin might be increasingly inadequate during ageing [26–28]. This might 'force' adipose cells to overexpress adiponectin in order to compensate for its ageing-associated decrease in activity [29, 30].

Thirdly, not adiponectin itself, but expression and activity of its receptors might be more important in ageing. Indeed, it has been shown that expression of adiponectin receptors is inadequate in aged humans and animals [30, 31]; this could result in a compensatory increase of expression of the ligand.

It has still not been established whether HMW adiponectin testing should be used routinely in clinical practice. It clearly has a great cognitive value and helps to elucidate the mechanisms connecting obesity and obesity-related diseases. Available data strongly suggests that HMW adiponectin is a better predictor than total adiponectin in assessing the risk of metabolic and cardiovascular diseases [10–12]. However, before an HMW adiponectin test is recommended for routine use, these observations require validation using larger groups of healthy and diseased individuals. This is especially important because even well-characterised total adiponectin is not an element of routine diagnostics.

The –11377C > G, –11391G > A, and v11426A > G promoter polymorphisms of the adiponectin-encoding *ADIPOQ* gene have been shown to influence the level of circulating adiponectin [15, 19] and to be associated with impaired glucose metabolism, metabolic syndrome, and with CVD, diseases that negatively influence longevity. For example, it has been shown that the G allele of the –11377C > G polymorphism is correlated with an increased risk of DM2 [15], increased carotid artery intima-media thickness [18], a higher extent of coronary stenosis, and with an increased risk of vascular events and of CVD [32]. The G allele of the –11391G > A polymorphism is correlated with obesity, a higher fasting insulin level, insulin resistance, homeostatic model assessment (HOMA) index, and with DM2 [15]. Finally, the G allele of the –11426A > G polymorphism showed association with a higher fasting glucose level and with DM2 [33].

We found that the AA genotype of the –11391G > A polymorphism was more common in centenarians (2.01%) than in young controls (0.24%). At first, this finding seemed to be unimportant due to the low frequency of this genotype in the analysed population. However, it gains significance when interpreted together with the findings of other authors. The frequency of the AA genotype of the –11391G > A polymorphism was 1% in a cohort of French and Italian children and adolescents [34], 1% in non-Hispanic white teenage US citizens [35], 0% in teenage African Americans [35], 0.7% in elderly French Caucasians without DM2 [15], and 0.08% in elderly British Caucasians without cardiovascular disease [32]. Clearly, the frequency of this variant seems to be highest in centenarians. In addition, we found that in centenarians it co-existed with higher total adiponectin concentrations compared to the GG genotype. This corroborates previous observations performed in younger individuals, in whom such a correlation was found and was significant [15, 32, 34, 35]. However, our finding that the AA genotype of the –11391G > A polymorphism co-exists with a higher concentration of HMW adiponectin has never previously been reported and should be evaluated in other populations. Finally, we observed that the AA

genotype of the –11391G > A polymorphism is more common in DM2 patients (1.59%) than in healthy controls (0.24%). This corroborates the findings of Vasseur et al., who showed that in elderly DM2 patients its frequency was 1.5% *v.* 0.7% in age-matched controls [15].

Nevertheless, this work should be viewed as preliminary. First, the analysed groups are relatively small; the analysis of large groups would have produced results with more statistical power. A Bonferroni correction is typically applied to decrease the risk of obtaining false-positive results due to the small size of the groups and to multiple testing; however, the value of this correction is under criticism [36, 37]; it is considered too restrictive, and its application lowers the chance of publication of negative or marginally significant results, causing a publication bias. Therefore, we chose not to apply it and to present both marginally positive and negative results, since this will undoubtedly be useful for further meta-analyses. Second, sex distribution in the groups analysed for the frequency of polymorphisms was unequal; this is however only a theoretical limitation, since we checked the sex-related distribution of the *ADIPOQ* polymorphisms in the Y group composed of healthy individuals and found that they were equally distributed in females and in males. Also, HMW adiponectin was analysed only in female centenarians; it is unclear whether its concentration follows the same pattern in male centenarians. This might not be so, because there are suggestions that HMW adiponectin is lower in males than in females due to the inhibitory action of testosterone on the HMW form release from adipocytes [38]. Finally, due to a low number of individuals with rare genetic variants of *ADIPOQ* (discussed above), correlations between these genotypes and total and HMW adiponectin have low statistical power and demand confirmation using a much larger group of individuals.

Taken together, our data shows that in females extreme longevity is associated with high concentrations of total and HMW adiponectin, as well as with a ratio of HMW to total adiponectin higher than in all younger age groups. Serum adiponectin in female centenarians is associated with biochemical parameters that lower cardiovascular risk. We also showed that only a very rare AA genotype of the 11391G > A *ADIPOQ* promoter polymorphism, but not the genotypes of the –11377C > G and –11426A > G polymorphisms, might be associated with longevity, and we speculate that this is so regardless of its association with an increased risk of DM2.

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