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Evaluation of the frequency of *ADIPOQ* c.45 T>G and *ADIPOQ* c.276 G>T polymorphisms in adiponectin coding gene in girls with *anorexia nervosa*

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Abstract

Introduction: *Anorexia nervosa* (AN) is a serious chronic psychosomatic disorder, the essence of which are attempts by the sufferer to obtain a slim silhouette by deliberate weight loss (restrictive diet, strenuous physical exercise, provoking vomiting). The aetiology of this disorder is multifactorial. Genetic factors that influence the predisposition to AN have been sought. A broad meta-analysis points to a strong genetic correlation between AN and insulin resistance. Adiponectin (ADIPO) increases insulin sensitivity. In our pilot study we demonstrated that the TT genotype in locus *ADIPOQ* c.276 G>T of the ADIPO gene and a higher concentration of ADIPO in blood serum occurred significantly more frequently in 68 girls suffering from AN than in 38 healthy girls. The objective of this study was to evaluate the frequency of the occurrence of *ADIPOQ* c.45 T>G and *ADIPOQ* c.276 G>T in the ADIPO gene in a larger cohort of girls with AN and healthy girls, as well as an analysis of correlations between variants of the aforementioned polymorphisms and the levels of ADIPO in blood serum.

Material and methods: The study covered 472 girls (age: 11–19 years): 308 with the restrictive form of AN (AN) and 164 healthy girls (C). The level of ADIPO in blood serum was determined by means of the ELISA method on a Bio-Vendor, LLC (Asheville, North Carolina, USA). The DNA isolation was carried out by means of Genomic Mini AX BLOOD (SPIN). The PCR reaction was carried out in a Thermo-Cycle T100 thermocycler. 80–150 ng of the studied DNA and relevant F and R starters were added to the reaction mixture. The reaction products were subjected to digestion by restriction enzymes and separated on agarose gels (RFLP).

Results: The distribution of genotypes in the polymorphic site *ADP c.45* of the ADIPO gene and *ADP c.276* was similar in both groups. In both groups the T allele was most frequent in locus *ADIPOQ c.45* and the G allele in locus *ADIPOQ c.276*. In all the study subjects collectively (AN and C) a statistically significant negative correlation between the levels of ADIPO in blood serum on one hand and body weight ($r = –0.46$; $p < 0.0001$) and BMI ($r = –0.67$; $p < 0.0001$) on the other was demonstrated. Exclusively in the AN group a significant correlation between the level of ADIPO in blood and the distribution of TG, TT, and GG alleles in loci *ADIPOQ c.45* and *ADIPOQ c.276* was demonstrated ($p = 0.0052$ and $p < 0.0001$, respectively).

Conclusions: The genotype in loci *ADIPOQ* c.45 and *ADIPOQ* c.276 of the ADIPO gene seems to have no effect on the predisposition to AN. Girls suffering from AN with the TT genotype in loci *ADIPOQ* c.45 and *ADIPOQ* c. 276 may demonstrate higher insulin sensitivity because they have significantly higher levels of ADIPO than girls suffering from AN with other genotypes. This may be suggestive of their better adaptation to the state of malnutrition, and it has a potential effect on treatment results. **(Endokrynol Pol 2021; 72 (5): 520–528)**

Key words: *adiponectin; anorexia nervosa; polymorphism*

Introduction

Anorexia nervosa (AN) is a chronic medical condition of a psychosomatic nature, affecting ca. 1% of the population of girls and women aged 15–24 years [1]. The diagnostic criteria for AN are provided in Table 1 [2]. *Anorexia nervosa* is associated with a high risk of serious somatic complications, caused by chronic starvation,

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Table 1. *Diagnostic criteria for anorexia nervosa according to the American Psychiatric Society, DSM-5 (2013) [2]*

A.	Restriction of energy intake relative to requirements leading to a significantly low body weight in the context of age, sex, developmental trajectory, and physical health. Significantly low weight is defined as a weight that is less than minimally normal or, for children and adolescents, less than that minimally expected
B.	Intense fear of gaining weight or becoming fat, or persistent behaviour that interferes with weight gain, even though at a significantly low weight
C.	Disturbance in the way in which one's body weight or shape is experienced, undue influence of body weight or shape on self-evaluation, or persistent lack of recognition of the seriousness of the current low body weight

Specify current type:

Restricting Type: during the last three months, the person has not engaged in recurrent episodes of binge eating or purging behaviour (i.e. self-induced vomiting or the misuse of laxatives, diuretics, or enemas)

Binge-Eating/Purging Type: during the last three months, the person has engaged in recurrent episodes of binge eating or purging behaviour (i.e. self-induced vomiting or the misuse of laxatives, diuretics, or enemas)

deep malnutrition, adipose tissue atrophy, and cachexia. Furthermore, it is also comorbid with many psychiatric disorders, such as depression, anxiety, and personality disorders [3].

The aetiology of AN remains unclear; however, it is broadly believed that the origins of this condition are multifactorial. Scholars point to the co-existence of certain individual factors predisposing to AN: genetic factors, as well as specific personality traits and environmental features: sociocultural, family-related, and socioeconomic.

Studies on twins and families suggest that the development of AN is fostered by genetic factors [4]. The concordance rate of AN in monozygotic twins is estimated at 55–56% *vs*. 5–7% in dizygotic twins [5, 6]. A large, controlled study of families (1831 relatives, 504 probands with AN) demonstrated that first-degree female relatives exhibit an at least 10-fold higher risk of AN development than the general population [7]. *Anorexia nervosa* heritability is estimated at 48% to 88% [7], which warrants the search of genes predisposing to development of the disorder [7–10]. Linkage studies in eating disorders demonstrated that locus D1S3721 in the region 1p34.2 might be associated with the restrictive type of AN [44]. Signals for AN were discovered on chromosomes 2, 3, 4, and 13. A detailed mapping of regions located on chromosome 1 allowed the selection of genes of the receptor for serotonin *HTR1D* and the receptor for opioids *OPRD1* as potential genes of AN susceptibility [11].

Many studies have been carried out in search of AN candidate genes [12–15]; they do not, however, have sufficient statistical power due to small numbers of subjects, methodological differences, and population heterogeneity [12]. For example, one analysis covered genes of oestrogen receptors 1 and 2 (ESR1; ESR2 = ER), essential for female puberty, because AN is much more frequent in girls than in boys, most often manifesting itself in adolescence [12]. The analysis focused on genes participating in the dopaminergic and serotoninergic regulation, because deviations in these systems had been demonstrated in the acute stage of the disease and after recovery [16–19], and serotonin and dopamine affect eating habits, physical activity, and mechanisms of reward [12]. Molecular studies on the genes of leptin, leptin receptor, downstream protein coding genes towards the signal pathway of leptin — AGRP, ghrelin, GOAT, or studies focusing on polymorphisms of the gene of the brain-derived neurotrophic factor (BDNF), similarly to other studies, have not provided firm evidence of their effect on eating disorders [20, 21].

Genome-wide association studies (GWAS), based on investigating the entire genome with no preliminary hypotheses posed, suggest that AN is a polygenetic condition, with several dozen gene variants involved in its pathogenesis, each of which has a minor effect modifying the susceptibility to AN [11].

A meta-analysis conducted by Duncan et al. [22] on the largest cohort of AN patients investigated thus far (3495 subjects) and in a control group (10,982 subjects), by means of linkage disequilibrium score regression, demonstrated that the strongest genetic correlation among all the metabolic and mental phenotypes examined by these authors was a negative correlation between *anorexia nervosa* and insulin resistance. Adiponectin (ADIPO) is mentioned among hormones playing a part in shaping insulin sensitivity [23–25]. The adiponectin coding gene *ADIPOQ* (*APM1*), mapped in the region 3q27.3 and containing 4 exons, has high expression in mature, differentiated adipocytes of the white, mostly visceral adipose tissue [26]. AdipoR1 and AdipoR2 demonstrate high expression, among others, in the paraventricular nucleus of the hypothalamus, associated with the regulation of the feeling of hunger and satiety [27, 28]. ADIPO takes part in the regulation of the energy homeostasis of the body, demonstrating activity that increases insulin sensitivity and prevents diabetes, atherosclerosis, and inflammation, as well as the antiapoptotic, antifibrotic, and proangiogenic activity [29–32].

Previous studies concerning the frequency of polymorphisms in ADIPO genes in AN patients are scarce. Křížova et al. [33] compared the frequency of polymorphic variants +45T>G and +276G>T in the Czech population, investigating their influence on the level of adiponectin circulating in blood and on metabolic phenotypes. Nevertheless, the studied groups were small (28 females with AN and 38 healthy females).

Our first attempt at assessing the frequency of polymorphisms in the adiponectin gene in the Polish population was undertaken in 2017. We performed a pilot study of 68 girls suffering from AN and 38 healthy control females [34], showing statistically significant differences in the frequency of polymorphisms of the ADIPO gene between AN patients and controls. We observed that the TT genotype in locus *ADIPOQ* c.276G>T occurs significantly more frequently in AN patients. Therefore, it seemed justified to continue the research in a larger cohort. We assumed that it may allow us to find the genetic variant predisposing to AN and help to understand the pathogenesis of the disorder.

The objective of the study is to assess the frequency of polymorphisms of *ADIPOQ* c.45T>G and *ADIPOQ* c.276G>T in the adiponectin gene in girls suffering from anorexia nervosa and in healthy subjects and to analyse correlations between variants of these polymorphisms and levels of adiponectin in blood serum.

Material and methods

The total of 472 girls (aged 11–19) from the region of Silesia were recruited to the study: 308 girls suffering from *anorexia nervosa* (AN) hospitalised in Independent Public Clinical Hospital No. 1 in Zabrze, Medical University of Silesia in Katowice, and 164 healthy girls (C), secondary school students from Zabrze and Gliwice. The criterion qualifying to the study for the AN group was a diagnosis of the restrictive form of AN (according to ICD-10 [35] and DSM 5 [2]) and informed consent to take part in the study given by the subject's parents/legal guardians and by the subject herself if aged > 16 years. The criteria qualifying to the study for the girls in the control group were as follows: normal body weight, no chronic illnesses, no use of medications over the previous month, no slimming diets and other methods of losing weight over the previous 3 months, and informed consent to take part in the study given by the subject's parents/legal guardians and by the subject herself if aged > 16 years.

The Bioethics Committee of the Medical University of Silesia in Katowice issued permission to conduct the study (Decision No. KNW/0022/KB1/108/1/11 dated 20 September 2011).

Measurements of body weight and height and calculations of BMI and BMI-SDS were performed for all the subjects, based on percentile grids applicable to the Polish population for the relevant sex and age [36].

The molecular tests were carried out in the Department of Genetics (Institute of Psychiatry and Neurology, Warsaw) with the use of DNA isolated from frozen samples of peripheral blood. The isolation was obtained by means of a commercial Genomic Mini Ax Blood Spin kit (A&A Biotechnology, Gdynia, Poland).

The analysis of selected polymorphisms in the gene of adiponectin (*ADIPOQ*) was performed according to the following schedule:

— amplification in the PCR reaction of a section of the gene containing a polymorphic locus. The PCR reaction was conducted in the Thermo Cycle thermal cycler by Bio-Rad with the application of the NXT Taq PCR kit by EURx, containing the following: hot start NXT TaqDNA Polymerase, a reaction buffer, $MgCl_2$, and dNTPs, and with the application of relevant F and R starters. 80–150 ng of the DNA tested was added to the reaction mixture. The reaction was conducted in 10 μL in the following conditions: 3 min. at 95°C, and then 35 cycles: 30 seconds at 95°C, 30 seconds at 58°C, and 1 minute at 72°C. The final stage was a 5-minute incubation of the product of the PCR reaction at 72°C;
— digesting the product of the PCR reaction with a restriction enzyme. The reaction was conducted for 1 hour at a temperature of 30°C for *SmaI*, and at 37°C for *Mva1269I*;
— electrophoretic division of the digestion products obtained in the agarose gel containing ethidium bromide;
— preparation of photographic documentation;
— statistical analysis of the obtained results.

The starters applied and the selected restriction enzymes identifying the polymorphic loci examined are listed below:

ADIPOQ gene polymorphism c.45 T > G
Starter F 5′– GAA GTA GAC TCT GCT GAG ATG G – 3′
Starter R 5′ – TAT CAG TGT AGG AGG TCT GTG ATG – 3′
Restrictive enzyme *SmaI*

ADIPOQ gene polymorphism c.276 G > T
Starter F 5′– GGC CTC TTT CAT CAC AGA CC – 3′
Starter R 5′ – AGA TGC AGC AAA GCC AAA GT – 3′
Restrictive enzyme *Mva1269I*

Statistical analysis

The database was drawn up in an Microsoft Excel spreadsheet (Redmond, Washington, USA). Statistical calculations were performed using MedCalc software ver. 19.1.3 (MedCalc, Ostend, Belgium). The level of $p = 0.05$ was considered significant. The following parameters of the descriptive statistics were determined: arithmetic mean, median, minimum and maximum value, lower and upper quartile, standard deviation (SD), standard error (SE), and 95% confidence interval around the mean value. For all parameters the conformity of their distribution with the normal distribution was verified. The conformity assessment was made using the Shapiro-Wilk test. The assessment of differences between the means was performed using Student's t-test with a separate evaluation of variances and the U Mann-Whitney test. A one-way analysis of variances ANOVA was also performed. Homogeneity of variances was evaluated by means of Levene's test.

The tests results were presented graphically as a box plot with a mean value and a 95% confidence interval for the mean value marked as an error bar. An analysis of contingent tables was performed for qualitative variables. Descriptive statistics were calculated: Pearson's chi-square, and the significance level p, C contingency coefficient. The results are presented in graphic form by means of categorised histograms.

Results

The average age of the girls in groups AN and C was similar (15.06 ± 1.57 years and 15.16 ± 2.12 years, respectively). The average body weight in the AN group was significantly lower ($p < 0.05$) than in the C group (40.21 ± 6.01 kg and 49.87 ± 7.93 kg, respectively). The average BMI and BMI-SDS in girls suffering from AN was significantly lower ($p < 0.05$) than in healthy girls (BMI: 15.19 ± 1.67 kg/m^2 *vs.* 20.12 ± 2.46 kg/m^2, BMI-SDS: –2.53 ± 0.96 *vs.* 0.12 ± 1.06, respectively) (Tab. 2).

Table 2. *Characteristics of examined girls with anorexia nervosa and healthy girls and the results of serum adiponectin assessment*

	AN (n = 308)	C (n = 164)
	Mean ± SD (range)	Mean ± SD (range)
Age [years]	15.06 ± 1.57 (11–19)	15.16 ± 2.12 (11 - 19)
Height [cm]	162.47 ± 6.47 (143.0–177.0)	160.31 ± 7.06 (142.0 - 175.0)
Body weight [kg]	40.21 ± 6.01 (18.7–54.8)	49.87* ± 7.93 (30.2 - 65.0)
BMI [kg/m²]	15.19 ± 1.67 (9.11–18.6)	20.12* ± 2.46 (13.9 - 26.13)
BMI-SDS	–2.53 ± 0.96 (–5.49– –1.94)	0.12* ± 1.06 (–2.17–2.0)
Adiponectin [μg/mL]	40.24 ± 6.76 (27.86–66.78)	15.02** ± 5.87 (4.19–29.83)

AN — girls with anorexia nervosa; C — healthy controls; SD — standard deviation; BMI — body mass index; BMI- SDS — body mass index standard deviation score (subject's BMI from the mean BMI for age and gender); AN *vs.* C* $p < 0.05$; AN *vs.* C ** $p < 0.0001$

The average level of ADIPO in blood serum in girls suffering from AN was significantly higher ($p < 0.0001$) than in the group of healthy girls (40.24 ± 6.76 μg/mL *vs.* 15.02 ± 5.87 μg/mL) (Tab. 2; Fig. 1).

The allele frequency in the polymorphic site *ADIPOQ c.45* and *ADIPOQ c.276* of the adiponectin gene (AN and C) is provided in Table 3.

The most common allele arrangement in the polymorphic site *ADIPOQ c.45* of the adiponectin gene in girls suffering from AN was the TT genotype, similarly to the C group. The TG genotype occurred in this gene with a similar frequency in both groups (Tab. 3, Fig. 2). The allele arrangement in the polymorphic site *ADIPOQ c.276* of the adiponectin gene occurred with a similar frequency in both groups (Tab. 3, Fig. 3).

The genotype distribution in the polymorphic site *ADIPOQ c.45* of the adiponectin gene in both groups did not differ statistically significantly ($p = 0.2628$), similarly to the polymorphic site *ADIPOQ c.276* ($p = 0.6645$).

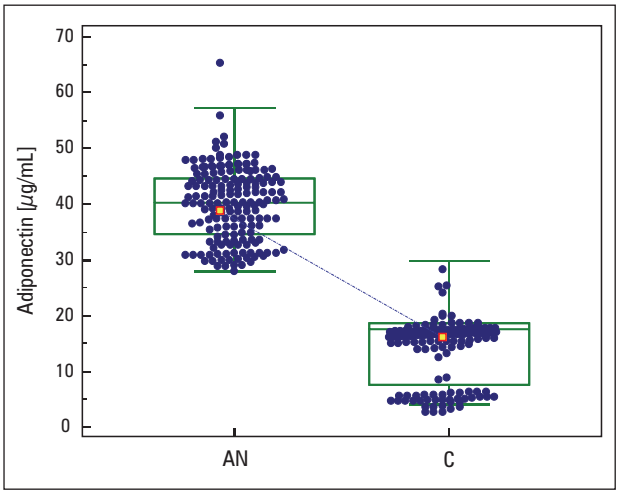

Figure 1. *Serum adiponectin concentrations [μg/mL] in girls with anorexia nervosa (AN) and healthy controls (C). AN vs. C p < 0.0001*

The frequency of individual alleles in polymorphic sites of *ADIPOQ c.45* and *ADIPOQ c.276* in both groups was similar and no statistically significant differences were observed in this respect. The T allele was more frequent in the polymorphic site *ADIPOQ c.45* and the G allele in the polymorphic site *ADIPOQ c.276* (Tab. 4, Fig. 4 and 5).

In all the studied girls collectively (AN and C) a statistically significant negative correlation between the levels of ADIPO in blood serum on the one hand and the body weight ($r = –0.46$; $p < 0.0001$) and BMI ($r = –0.67$; $p < 0.0001$) on the other was observed. No statistically significant relationships between these parameters in individual studied groups (AN and C) were observed.

An analysis of correlations between the levels of ADIPO in blood serum and the genotype distribution

Table 3. *The genotype frequency in the polymorphic sites of the adiponectin gene (ADIPOQ c.45 and ADIPOQ c.276) in the examined girls with anorexia nervosa (AN) and healthy controls (C)*

Examined gene Genotype	AN Number (%)	C Number (%)
	n = 306	n = 161
	p = 0.2628; c = .075	
***ADIPOQ* c.45**		
TG	33 (10.8%)	25 (15.5%)
TT	272 (88.9%)	136 (84.5%)
GG	1 (0.3%)	0 (0%)
	n = 305	n = 160
	p = 0.6645; c = 0.042	
***ADIPOQ* c.276**		
TG	124 (40.7%)	72 (45.0%)
TT	46 (15.1%)	22 (13.8%)
GG	135 (44.2%)	66 (41.2%)

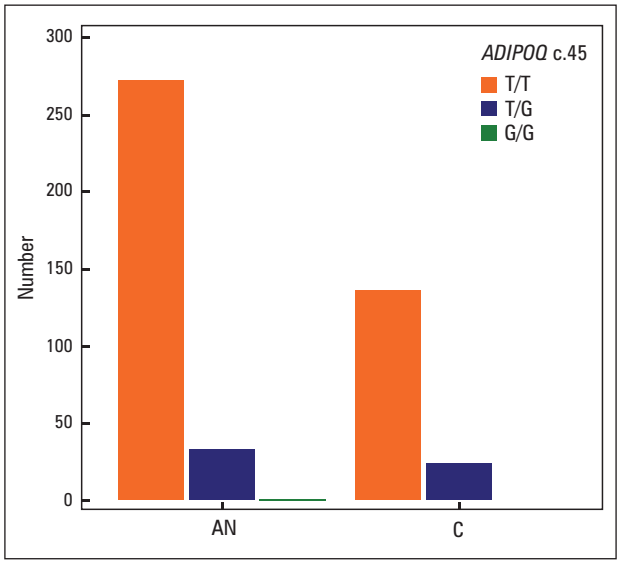

Figure 2. *Distribution of genotypes in locus ADIPOQ c.45 in girls with anorexia nervosa (AN) and healthy controls (C)*

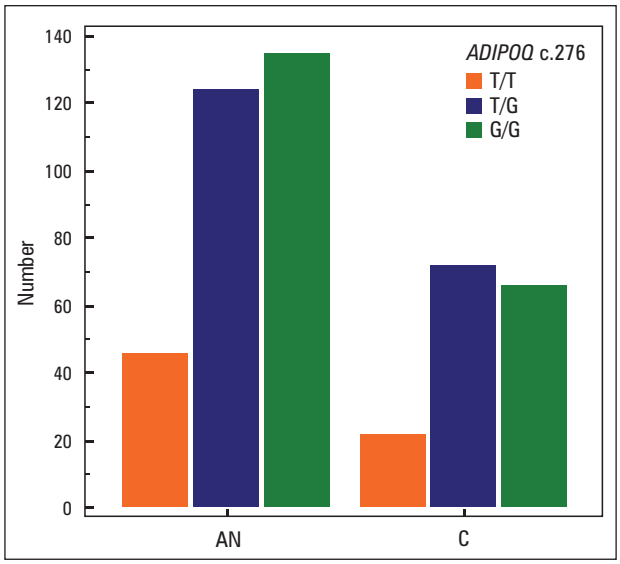

Figure 3. *Distribution of genotypes in locus ADIPOQ c.276 in girls with anorexia nervosa (AN) and healthy controls (C)*

Table 4. ***The allele frequency in the polymorphic sites of the adiponectin gene (****ADIPOQ c.45* **and** *ADIPOQ c.276****) in the examined girls with*** *anorexia nervosa* ***(AN) and healthy controls (C)***

Examined gene Allele	AN Number (%)	C Number (%)
	n = 306	n = 161
	p = 0.2259; c = 0.04	
***ADIPOQ* c.45**		
T	577 (94.3%)	297 (92.2%)
G	35 (5.7%)	25 (7.8%)
	n = 305	n = 161
	p = 0.779; c = 0.01	
***ADIPOQ* c.276**		
T	216 (35.4%)	117 (36.3%)
G	394 (64.6%)	205 (63.7%)

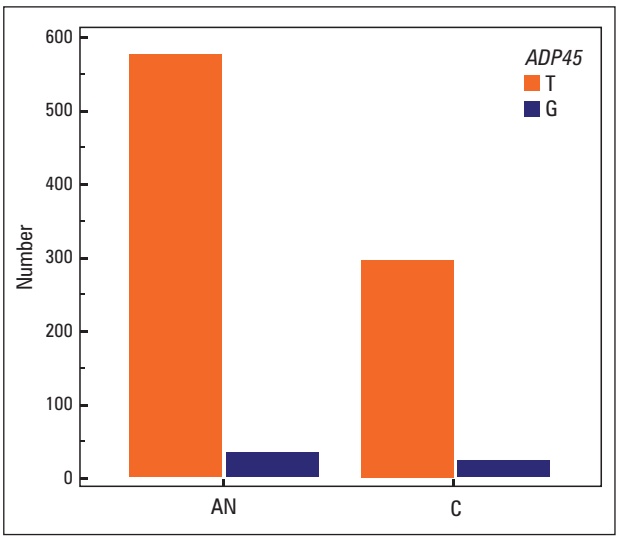

Figure 4. *Distribution of T and G alleles in ADIPOQ c.45 polymorphic site in girls with anorexia nervosa (AN) and healthy controls (C)*

demonstrated a statistically significant relationship between the level of this hormone in blood and the arrangement of TG, TT, and GG alleles in the polymorphic site *ADIPOQ c.45* and the polymorphic site *ADIPOQ c.276* of the adiponectin gene ($p = 0.0052$ and $p < 0.0001$, respectively) exclusively in girls with *anorexia nervosa* (Tab. 5, Fig. 6 and 7).

Discussion

In the Czech population the authors [33] compared the frequency of individual alleles in polymorphic sites *ADIPOQ* c.45 T>G and *ADIPOQ* c.276 G>T of the adiponectin gene in obese subjects (BMI: 43.48 ± 1.12 kg/m^2), in AN patients (BMI: 15.72 ± 0.36 kg/m^2), and in healthy females (BMI: 22.32 ± 0.40 kg/m^2). In subjects suffering from *anorexia nervosa* they demonstrated, similarly to our study, that the T allele is more frequent in locus *ADIPOQ* c.45 T>G (100% and 94.3%, respectively), the G allele is more frequent in locus *ADIPOQ* c.276 G>T (71% and 64.6%, respectively). We studied the frequency of not only individual alleles in these polymorphic sites but we also analysed the frequency of the genotypes.

The genotype distribution in the polymorphic sites *ADIPOQ* c.45 T>G and *ADIPOQ* c.276 G>T of the adiponectin gene in the studied girls with AN was

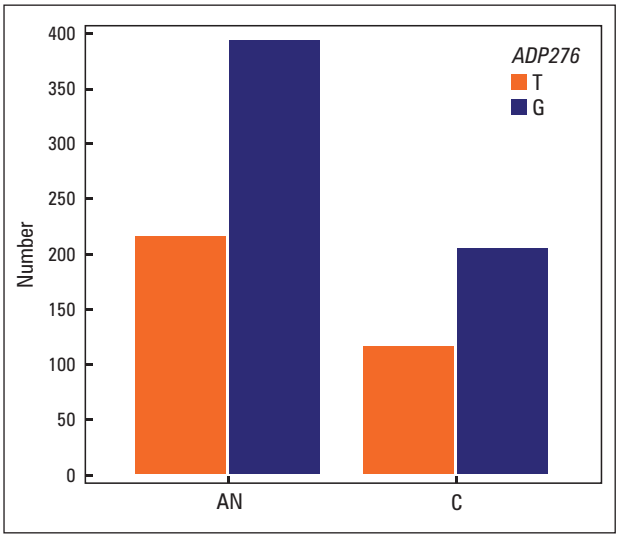

Figure 5. *Distribution of T and G alleles in ADIPOQ c.276 polymorphic site in girls with anorexia nervosa (AN) and healthy controls (C)*

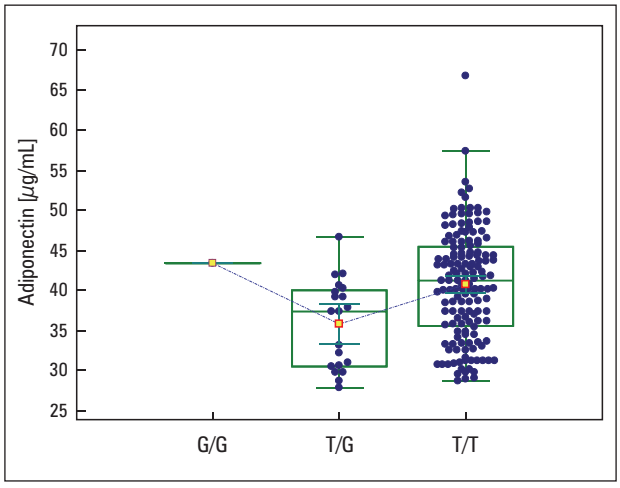

Figure 6. *Correlation between serum adiponectin concentration and genotype distribution in ADIPOQ c.45 polymorphic site of adiponectin gene in girls with anorexia nervosa (AN). p = 0.0052*

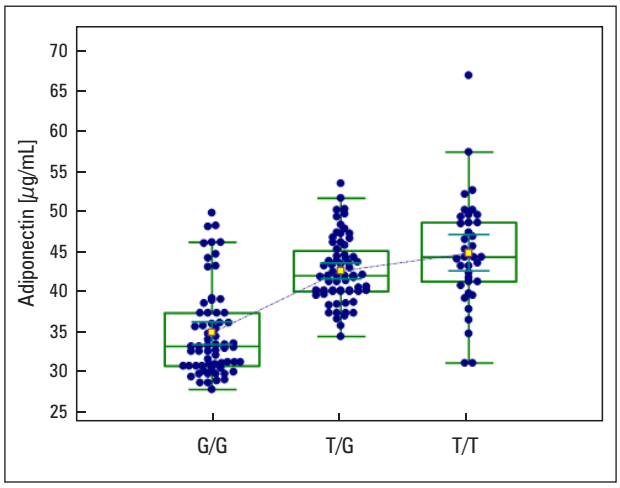

Figure 7. *Correlation between adiponectin serum concentration and distribution of genotypes in ADIPOQ c.276 polymorphic site in girls with anorexia nervosa (AN). p < 0.0001*

Table 5. ***Mean concentration of adiponectin in the serum depending on the genotype in the polymorphic site of the adiponectin gene (****ADIPOQ c.45* **and** *ADIPOQ c.276****) in examined girls with*** *anorexia nervosa* ***(AN) and healthy controls (C)***

Examined gene Genotype	**Adiponectin in serum [μg/mL]**	
	AN (n = 170)	C (n = 128)
	p = 0.0052	p = 0.15
***ADIPOQ* c.45**		
TG	37.41*	16.75
TT	41.27*	17.92
GG	43.44*	–
	AN (n = 170)	C (n = 130)
	p < 0.0001	p = 0.404
***ADIPOQ* c.276**		
TG	42.07**	17.65
TT	44.38**	16.79
GG	33.26**	18.06

not different from the distribution of these genotypes in healthy subjects. This could be suggestive of the fact that the allele arrangement in these polymorphic sites has no influence on the predisposition to *anorexia nervosa*.

We demonstrated significantly higher levels of ADIPO in blood serum in girls suffering from AN as compared to healthy subjects (p < 0.0001). The concentration of ADIPO in blood correlates negatively with the body weight and BMI in all the study subjects collectively (p < 0.0001). Similar observations have been made by other authors [37–39] and by us in our pilot study [34]. Ziora et al. [39] demonstrated that the group of girls suffering from AN (87 patients) was characterised by significantly (p < 0.0001) elevated levels of adiponectin (> 3.38 μg/mL) as compared to the healthy subjects (61 girls).

Delporte et al. [40] observed a 40% higher level of insulin sensitivity in a group of 26 females suffering from AN than in a group of 24 healthy females. In both groups they demonstrated a positive correlation between the level of ADIPO in blood and insulin sensitivity (p < 0.05) and a negative correlation (p < 0.05) with the level of triglycerides, total cholesterol, LDL, and HDL. They speculated that hyperadiponectinae-

mia in subjects suffering from AN might contribute to maintaining the state of increased insulin sensitivity as one of adaptation mechanisms to deep malnutrition.

Housova et al. [4] demonstrated that ADIPO levels in blood serum correlate negatively with BMI and are ca. 96% higher in the restrictive form of AN as compared to the control group. They did not observe any correlation in the group of subjects suffering from *bulimia nervosa*. They suggested that increased levels of ADIPO in blood could reflect the reduced fat content in the bodies of AN patients.

We based our research assumptions on study results obtained by previous authors [42–44] who analysed the loci in the ADIPO gene that contain functional polymorphisms influencing the modulation of insulin resistance.

Japanese authors [42], who studied 480 healthy subjects and 384 subjects suffering from type 2 diabetes, observed that there is a relationship between polymorphisms of an individual nucleotide in positions 45 ($p = 0.003$) and 276 ($p = 0.002$) in the ADIPO gene and type 2 diabetes. Patients with the GG genotype in position 45 or 276 exhibited a much higher risk of type 2 diabetes than subjects with the TT genotype. Subjects with the GG genotype in position 276 had a higher insulin resistance score than subjects with the TT genotype (1.61 ± 0.05 *vs*. 1.19 ± 0.12; $p = 0.001$). The G allele in position 276 was linearly related to lower levels of adiponectin in blood serum (G/G: 10.4 ± 0.85 μg/mL, G/T: 13.7 ± 0.87 μg/mL, T/T: 16.6 ± 2.24 μg/mL; $p = 0.01$) in individuals with a higher BMI. Individuals with the GG genotype in polymorphic site *ADIPOQ* c.276 (the putative risk gene) had lower levels of adiponectin in blood and higher insulin resistance than individuals with the TT genotypes. The authors concluded that this polymorphism, located in the intron of the *ADIPOQ* gene, influences the change in the level of expression of adiponectin, and thereby the change of its concentration in blood serum. They believe that in order to confirm it, a functional study should be carried out or levels of the expression of ADIPO should be measured directly in the white adipose tissue from patients with different genotypes in position *ADIPOQ* c.276.

Such a study was carried out by Friedriksson et al. [43], evaluating the expression of mRNA for adiponectin and the polymorphism *ADIPOQ* c.276 G>T in 36 obese subjects without diabetes (BMI: 41.5 ± 4.9 kg/m^2). mRNA expression for adiponectin in visceral fat was positively correlated with the level of adiponectin in blood serum ($r = 0.54$; $p = 0.012$). Carriers of the T allele (GT and TT) had a significantly higher content of the adipose tissue as compared to carriers of the GG genotype (65 ± 6 *vs*. $56 \pm 10\%$; $p = 0.011$). Carriers of the GG genotype in locus *ADIPOQ* c.276 had reduced glucose tolerance, and carriers of the GT and TT had a 38% higher level of mRNA of adiponectin in the visceral adipose tissue (0.91 ± 0.06 for GT and TT *vs*. 0.66 ± 0.09 for carriers of the GG genotype; $p = 0.013$). The authors believe that the genetic variation in the ADIPO gene may influence the expression of the gene in the visceral adipose tissue, and they suggest a potential role of such a variation in the regulation of fat accumulation in the body of obese people.

Other authors [44] evaluated haplotypes for two polymorphisms, 45T –> G and 276G –> T, and their relationship with components of the metabolic syndrome and the level of ADIPO in blood in 413 subjects — representatives of the Caucasian race without diabetes. Each individual polymorphism was positively correlated with insulin resistance. On the other hand, the haplotype defined by these two co-existing polymorphisms was strongly associated with components of the metabolic syndrome. Homozygotes for the risk haplotype (carriers of the TT genotype in polymorphic site 45 and the GG genotype in polymorphic site 276) had a higher body weight ($p = 0.03$), waist circumference ($p = 0.004$), systolic ($p = 0.01$) and diastolic blood pressure ($p = 0.003$), fasting glycaemia ($p = 0.02$), insulin ($p = 0.005$), insulin resistance score (HOMA) ($p = 0.003$), and total-cholesterol-to-HDL ratio ($p = 0.01$). Homozygotes had significantly lower levels of adiponectin in blood serum ($p = 0.03$) irrespective of their sex, age, and body weight. In an independent study conducted in a group of 614 Caucasian subjects, including 310 subjects with type 2 diabetes, it was confirmed that the risk haplotype is associated with an increased body weight ($p = 0.03$) but not with type 2 diabetes itself. The authors concluded that the aforementioned variants in loci of adiponectin are associated with obesity and other features of insulin resistance syndrome. However, considering the nature of the two SNPs, the risk haplotype is probably a marker for linkage disequilibrium with a polymorphism so far unidentified, which influences serum levels of adiponectin and insulin sensitivity [44].

We demonstrated a significant relationship in AN patients between the ADIPO level in blood and the TG, TT, and GG allele arrangement in the polymorphic sites *ADIPOQ* c.45 and *ADIPOQ* c.276. The lowest levels of adiponectin in blood are associated with the TG arrangement in position *ADIPOQ* c.45 and the GG arrangement in position *ADIPOQ* c. 276. The highest levels of adiponectin in blood are associated with the TT genotype in both the studied loci. This may be indicative of the fact that girls suffering from *anorexia nervosa* and having the homozygotic TT arrangement of these loci of the adiponectin gene may demonstrate higher insulin sensitivity because they have significantly higher levels of ADIPO than other girls with AN.

Our study has several limitations. Association studies are usually planned to be carried out in large populations to assure adequate power to the study. It is not an easy task, however, to recruit individuals with conditions that are rare (and *anorexia nervosa* can be regarded as such) to take part in any research. Because the availability of such patients is low, our study lasted several years. The actual size of the group was decided having considered previous association studies and adapting our technical and financial capacities. Another limitation to the study could also be the fact that we determined the level of adiponectin in blood serum exclusively in the acute phase of the illness, and not in all the subjects. Nevertheless, it seems that in this phase of the illness numerous adaptive mechanisms to extreme malnutrition can be fully observed. On the other hand, the number of subjects for whom we determined adiponectin levels in blood is sufficient from the statistical point of view.

Our study suggests that the genotype in the polymorphic sites *ADIPOQ* c.45 and *ADIPOQ* c.276 of the adiponectin gene seems to have no effect on the predisposition to *anorexia nervosa*.

We conclude that girls suffering from *anorexia nervosa* with the TT genotype in loci *ADIPOQ* c.45 and *ADIPOQ* c. 276 of the adiponectin gene may demonstrate higher insulin sensitivity because they have significantly higher levels of adiponectin as compared to girls with *anorexia nervosa* having different genotypes. It may be suggestive of their better adaptation to the state of malnutrition, possibly making them more treatment resistant. This, however, calls for further research in that matter.

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