



The age of developing diabetes and FTO polymorphisms (rs9939609, rs1421085, and rs9930506)

Wiek w momencie ujawnienia się cukrzycy a polimorfizmy genu FTO (rs9939609, rs1421085 i rs9930506)

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Abstract

Introduction: Type 2 diabetes (T2DM) is a common complex metabolic disorder that has a strong genetic predisposition. Fat mass and obesity-associated protein (FTO) is one of the genes of interest to us. Hypomethylation of a CpG site in the *FTO* gene was significantly associated with the risk of T2DM. The aim of the study was to find the answer to the question of whether the polymorphism changes of the *FTO* gene in the pathogenesis of type 2 diabetes are comparable in young, middle aged, and elderly people.

Material and methods: The study involved 282 consecutive patients with type 2 diabetes, who attended a primary healthcare clinic in Southern Poland. The study subjects were divided into three groups according to the age at which type 2 diabetes mellitus was diagnosed (> 40 years old, 40–60 years old, and > 60 years old). The genotyping of rs9939609, rs1421085, and rs9930506 FTO polymorphisms was conducted using TaqManPre-designed SNP Genotyping Assay.

Results: No statistically significant difference was shown between the examined FTO polymorphism (rs9939609, rs1421085, and rs9930506) distribution between the subjects diagnosed with diabetes < 40 years, 40–60 years, and > 60 years old.

Conclusions: There were no statistically significant relationships between the different analysed anthropometric and other parameters and distribution of examined FTO polymorphisms (rs9939609, rs1421085, and rs9930506). The age of diabetes was not affected by the tested FTO polymorphisms (rs9939609, rs1421085, and rs9930506). (*Endokrynol Pol* 2017; 68 (4): 402–406)

Key words: diabetes type 2; FTO gene; age

Streszczenie

Wstęp: Cukrzyca typu 2 (T2DM, *type 2 diabetes*) jest często występującym złożonym zaburzeniem metabolicznym o silnych uwarunkowaniach genetycznych. Jednym z genów budzących zainteresowanie badaczy jest gen *FTO* (*fat mass and obesity-associated gene*). Hipometylacja w pozycji CpG genu *FTO* wiązała się istotnie z ryzykiem rozwoju T2DM. Celem badania było znalezienie odpowiedzi na pytanie, czy polimorfizmy genu *FTO* w patogenezie cukrzycy typu 2 są podobne u osób w młodym, średnim i podeszłym wieku.

Materiały i metody: Do badania włączono 282 kolejnych chorych na cukrzycę typu 2, którzy zgłaszali się do placówki podstawowej opieki zdrowotnej w Polsce południowej. Uczestników badania podzielono na trzy grupy w zależności od wieku w momencie rozpoznania cukrzycy typu 2 (> 40 lat, 40–60 lat i > 60 lat). Przeprowadzono genotypowanie polimorfizmów rs9939609, rs1421085 i rs9930506 genu *FTO*, używając zestawu TaqMan do genotypowania SNP.

Wyniki: Nie wykazano statystycznie istotnych różnic w rozkładzie badanych polimorfizmów genu *FTO* (rs9939609, rs1421085 i rs9930506) między osobami, które w momencie rozpoznania cukrzycy miały < 40 lat, 40–60 lat lub > 60 lat.

Wnioski: Nie stwierdzono statystycznie istotnych związków między różnymi analizowanymi parametrami (np. parametrami antropometrycznymi) a rozkładem badanych polimorfizmów genu *FTO* (rs9939609, rs1421085 i rs9930506). Oceniane polimorfizmy genu *FTO* (rs9939609, rs1421085 i rs9930506) nie wpływały na wiek chorych w momencie rozpoznania cukrzycy. (*Endokrynol Pol* 2017; 68 (4): 402–406)

Słowa kluczowe: cukrzyca typu 2; gen *FTO*; wiek

Introduction

According to the International Diabetes Federation's Diabetes Atlas sixth edition, it was estimated that 382 million people had diabetes in the year 2013 worldwide, and this figure is expected to increase to 592 million in 2035 [1]. In 2014, 4.9 million deaths were attributable to

diabetes. It is also the leading cause of blindness, chronic kidney disease, and amputation. A substantial effort to understand the pathophysiology of type 2 diabetes (T2DM) and to develop novel preventive and therapeutic measures for T2DM has been made in recent years [2].

Type 2 diabetes (T2DM) is a common complex metabolic disorder that has a strong genetic predisposition.

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During the past decade, progress in genetic association studies has enabled the identification of at least 75 independent genetic loci for T2DM, thus allowing a better understanding of the genetic architecture of T2DM [2]. Among genes we think about of TCF7L2, PPARG, KCNJ11 and FTO, and identified novel loci for an association in the intergenic region [3–5].

Fat mass and obesity-associated protein (FTO) is one of the genes of interest to us. Hypomethylation of a CpG site in the *FTO* gene was significantly associated with the risk of T2DM [6]. Fat mass and obesity-associated protein, also known as alpha-ketoglutarate-dependent dioxygenase (FTO), is an enzyme that in humans is encoded by the *FTO* gene located on chromosome 16. As one homolog in the AlkB family of proteins, it is the first mRNA demethylase to be identified. The *FTO* gene is widely expressed in both foetal and adult tissues. This gene is a nuclear protein of the AlkB-related non-haem iron and 2-oxoglutarate-dependent oxygenase superfamily, but the exact physiological function of this gene is not known. Other non-heme iron enzymes function to reverse alkylated DNA and RNA damage by oxidative demethylation. Studies in mice and humans indicate a role in nervous and cardiovascular systems and a strong association with body mass index, obesity risk, and type 2 diabetes. The presence of the FTO rs9939609 A allele was found to be positively correlated with symptoms of metabolic syndrome, including higher fasting insulin, glucose, and triglycerides, and lower HDL cholesterol. The researchers identified 10 different FTO SNPs in the first intron of the gene, which associated with both BMI and type-2 diabetes.

The development of type 2 diabetes occurs at different ages. In some patients the disease develops at a young age, in some middle age, and in some over 60 years old. In the pathogenesis of diabetes participate environmental as well as genetic factors. It has previously been shown that the polymorphisms in the gene *FTO* play an important role in the development of diabetes. An interesting seem to find an answer to the question of whether the genetic effect is the same in the young and in the elderly.

Aim

The aim of the study was to find the answer to the question of whether the polymorphism changes of the *FTO* gene in the pathogenesis of type 2 diabetes are comparable in young, middle aged, and elderly people. To find the answer to the above questions we estimated three FTO polymorphisms located in the first intron of this gene in people diagnosed with type 2 diabetes in age < 40 years, between 40 and 60 years, and > 60 years.

Material and methods

The study involved 282 consecutive patients with type 2 diabetes, who attended a primary healthcare clinic in Southern Poland. Study subjects were divided into three groups according to the age at which type 2 diabetes mellitus was diagnosed (> 40 years old, 40–60 years old, and > 60 years old). The genotyping of rs9939609, rs1421085, and rs9930506 FTO polymorphisms was conducted with fluorescent-labelled probes using pre-designed single nucleotide polymorphisms assessment sets (TaqManPre-designed SNP Genotyping Assay, Applied Biosystems). The distribution of the abovementioned polymorphisms are in Hardy-Weinberg equilibrium [7].

Statistical analysis

The data obtained were presented as mean \pm standard deviation. Normality of data distribution was tested with the Shapiro-Wilk test. In the case of non-normal distribution, data were normalised with logarithmic transformation. Analysis of variances with Tukey post-hoc test and t-Student test were used in groups for mean comparison. Homogeneity of variances was tested with the Levene test. Correlations were calculated with the Spearman correlation coefficient. All calculations were performed with Statistica 9.0 software, and $p < 0.05$ was set as statistically significant.

Results

All patients were divided into three groups. Table I presents the main results conducted among subjects.

In the analysed group patients differed in the age of diagnosis of diabetes, and diabetes duration. In subjects with diabetes diagnosed and BMI > 60 it was significantly lower than in the other groups. It was also shown that subjects with diabetes diagnosed at > 60 years old significantly less frequently used insulin than people in the other groups, and significantly more often used oral treatment than those with diabetes diagnose at age 40–60 years. A similar analysis was performed for subjects who were carriers of all kinds of FTO polymorphisms. There were no significant differences between these groups.

Tables II A, B, show the results of determinations of FTO polymorphisms rs9939609, rs1421085, and rs9930506.

No statistically significant difference was shown between the examined FTO polymorphism (rs9939609, rs1421085, and rs9930506) distribution between the subjects with diabetes diagnosed at < 40, 40–60, and > 60 years.

There were no statistically significant relationships between the different analysed anthropometric

Table I. Basic results obtained in the subjects after the division depending on the age of diagnosis of diabetes**Tabela I.** Podstawowe wyniki w zależności od wieku chorych w momencie rozpoznania cukrzycy

	Diabetes diagnosis at < 40 years old	Diabetes diagnosis at 40–60 years old	Diabetes diagnosis at > 60 years old	Statistical significance
	A	B	C	
N	82	100	100	
Age [years]	54.2 ± 11.5	62.5 ± 8.6	73.8 ± 7.3	p < 0.05 between each group
Age at diagnosis of diabetes [years]	36.9 ± 4.5	51.0 ± 4.7	67.5 ± 6.1	p < 0.05 between each group
Duration of diabetes [years]	17.4 ± 11.0	11.6 ± 8.0	6.3 ± 5.6	p < 0.05 between each group
BMI [kg/m ²]	31.5 ± 5.2	30.7 ± 5.0	29.0 ± 4.4	p < 0.05 between groups C and A and B
Hypertension [%]	59 (72%)	75 (75%)	74 (74%)	NS
Dyslipidaemia [%]	39 (48%)	61 (61%)	56 (56%)	NS
Oral hypoglycaemic drugs [%]	82.9	76.0	89.0	p < 0.05 between groups C and B
Insulin therapy [%]	68.3	55.0	29.0	p < 0.05 between groups C and A and B

Table IIA. Distribution of FTO polymorphism rs9939609 in the study groups**Tabela IIA.** Rozkład polimorfizmu rs9939609 genu FTO w badanych grupach

	AA	AT	TT
Diabetes diagnosis at < 40 years	20 (24.30%)	34(41.46%)	28 (34.15%)
Diabetes diagnosis at 40–60 years	24 (24%)	52 (52%)	24 (24%)
Diabetes diagnosis at > 60 years	22 (22%)	43 (43%)	35 (35%)

Table IIB. Distribution of FTO polymorphism rs1421085 in the study groups**Tabela IIB.** Rozkład polimorfizmu rs1421085 genu FTO w badanych grupach

	CC	CT	TT
Diabetes diagnosis at < 40 years	21 (25.61%)	38 (46.34%)	23 (28.05%)
Diabetes diagnosis at 40–60 years	28 (28%)	50 (50%)	22 (22%)
Diabetes diagnosis at > 60 years	25 (25%)	48 (48%)	27 (27%)

Table IIC. Distribution of FTO polymorphism rs9930506 in the study groups**Tabela IIC.** Rozkład polimorfizmu rs9930506 genu FTO w badanych grupach

	AA	AG	GG
Diabetes diagnosis at < 40 years	28 (34.15%)	32 (39.02%)	22 (26.83%)
Diabetes diagnosis at 40–60 years	21 (21%)	47 (47%)	32 (32%)
Diabetes diagnosis at > 60 years	29 (29%)	47(47%)	24 (24%)

and other parameters and distribution of FTO examined polymorphisms (rs9939609, rs1421085, and rs9930506).

Tables III A, B, and C, D, E, F show the results of determination of allele distribution for FTO polymorphisms rs9939609, rs1421085, and rs9930506.

No statistically significant difference was shown between the FTO allele (rs9939609, rs1421085, and rs9930506)

distribution between the subjects with diabetes diagnosed at < 40, 40–60, and > 60 years. We did not find statistically significant difference in the FTO allele (rs9939609, rs1421085, and rs9930506) distribution between the subjects with diabetes diagnosed at < 40, 40–60, and > 60 years, taken together.

There were no statistically significant relationships between the different analysed parameters and

Table IIIA. Allele distribution of FTO polymorphism rs9939609 in the study groups**Tabela IIIA. Rozkład alleli z polimorfizmem rs9939609 genu FTO w badanych grupach**

	Allele A (%)	Allele T (%)
Diabetes diagnosis at < 40 years	74(44%)	90 (56%)
Diabetes diagnosis at 40–60 years	100 (50%)	100 (50%)
Diabetes diagnosis at > 60 years	87 (43.5%)	113 (56.5%)

Table IIIC. Allele distribution of FTO polymorphism rs9930506 in the study groups**Tabela IIIC. Rozkład alleli z polimorfizmem rs9930506 genu FTO w badanych grupach**

	Allele A (%)	Allele G (%)
Diabetes diagnosis at < 40 years	88 (52.4%)	76 (47.6%)
Diabetes diagnosis at 40–60 years	89 (44.5%)	111 (55.5%)
Diabetes diagnosis at > 60 years	105 (52.5%)	95 (47.5%)

Table IIIE. Allele distribution of FTO polymorphism rs1421085 in the study groups**Tabela IIIE. Rozkład alleli z polimorfizmem rs1421085 genu FTO w badanych grupach**

	Allele C (%)	Allele T (%)
Diabetes diagnosis at < 40 years	80 (47.6%)	84 (53.25%)
Diabetes diagnosis at 40–60 years and diabetes diagnosis at > 60 years, together	204 (51%)	196 (49%)

distribution of examined FTO alleles (rs9939609, rs1421085, and rs9930506).

Discussion

The Fat Mass and Obesity (FTO) gene is considered as leading obesity and type 2 diabetes [8]. The FTO rs9939609 minor allele, the risk allele for obesity, increased the risk of type 2 diabetes, this association remaining statistically significant even after adjustment for BMI [9]. Similar results were reported in a Scandinavian population [10]. In contrast, other studies, despite finding a higher type 2 diabetes risk in carriers of the risk-allele for obesity, concluded that this association disappears when adjusting for BMI and diabetes [11].

A CpG site in the first intron of the FTO gene showed small (3.35%) but significant ($P = 0.000021$) hypomethylation of cases relative to controls. The effect was independent of the sequence polymorphism in the region and persisted among individuals carrying the sequence-risk alleles. The odds of belonging to the

Table IIIB. Allele distribution of FTO polymorphism rs1421085 in the study groups**Tabela IIIB. Rozkład alleli z polimorfizmem rs1421085 genu FTO w badanych grupach**

	Allele C (%)	Allele T (%)
Diabetes diagnosis at < 40 years	80 (47.6%)	84 (53.25%)
Diabetes diagnosis at 40–60 years	106 (53%)	94 (47%)
Diabetes diagnosis at > 60 years	98 (49%)	102 (51%)

Table IIID. Allele distribution of FTO polymorphism rs9939609 in the study groups**Tabela IIID. Rozkład alleli z polimorfizmem rs9939609 genu FTO w badanych grupach**

	Allele A (%)	Allele T (%)
Diabetes diagnosis at < 40 years	74 (44%)	90 (56%)
Diabetes diagnosis at 40–60 years and diabetes diagnosis at > 60 years, together	187 (46.25%)	213 (53.25%)

Table IIIF. Allele distribution of FTO polymorphism rs9930506 in the study groups**Tabela IIIF. Rozkład alleli z polimorfizmem rs9930506 genu FTO w badanych grupach**

	Allele A (%)	Allele G (%)
Diabetes diagnosis at < 40 years	88 (52.4%)	76 (47.6%)
Diabetes diagnosis at 40–60 years and diabetes diagnosis at > 60 years, together	194 (48.5%)	206 (51.5%)

T2DM group increased by 6.1% for every 1% decrease in methylation (OR 1.061, 95% CI: 1.032–1.090); the odds ratio for decrease of 1 standard deviation of methylation (adjusted to gender) was 1.5856 (95% CI: 1.2824–1.9606), and the sensitivity (area under the curve 0.638, 95% CI: 0.586–0.690; males 0.675, females 0.609) was better than that of the strongest known sequence variant [6].

In the pathogenesis of diabetes both environmental genetic factors take part. In the studies conducted to date, 75 independent genetic loci have been identified involved in the development of type 2 diabetes [2]. Type 2 diabetes develops at different ages, with some people developing this before the age of 40 years, others between 40–60 years of age, and some at over 60 years old. We asked ourselves whether the FTO polymorphism plays an important role in this process.

To find an answer to the above question, we examined a group of 282 patients with type 2 diabetes, divided into three groups taking into account the age at which they were diagnosed with the disease. Patients were assigned to the following groups: people with diabetes diagnosed at

< 40 years of age (n = 82), those with diabetes diagnosed at between 40 and 60 years of age (n = 100), and patients with diabetes diagnosed at > 60 years of age (n = 100). In all patients, we defined the basic anthropometric parameters analysed and used for treatment. We have shown that there are significant differences between the two groups. These differences are presented in Table I. In addition, we proved that people with previously diagnosed diabetes at the time of the study were younger, diabetes lasted longer with them, and their treatment more often included insulin. The results are no surprise; the age of diagnosis was the inclusion parameter for different groups. More frequent use of insulin in patients with diabetes diagnosed before the age of 40 years was due to the fact that diabetes lasted significantly longer with them. Diabetes mellitus is a progressive disease and it leads to systematic damage to the beta cells, eventually destroying them, which leads to a need for insulin therapy.

In our work, we examined three polymorphisms of the *FTO* gene. We compared the distribution of these polymorphisms in patients with diabetes and on this basis we have shown that the treatment groups did not differ in a statistically significant way from each other in relation to the distribution of polymorphisms rs9939609, rs1521085, and rs9930506 (Table IIA, 2B, 2C). At the same time, we found no statistically significant difference in the distributions of allele *FTO* polymorphisms studied between the two groups (Table IIIA, 3B, 3C).

Comparing the group of people with diabetes diagnosed between the ages of 40 and 60 years old and the group in which diabetes was diagnosed at 60 years of age, we also noted significant differences in the distribution of polymorphisms of the *FTO* gene, as well as alleles of these polymorphisms (Table IIID, 3E, 3F).

In our work we do not have considered, however, whether the *FTO* gene is involved in the pathogenesis of type 2 diabetes, but only over whether the importance of this gene in the pathogenesis of type 2 diabetes is higher in patients whose diabetes was diagnosed at a younger age (< 40 years old) as compared to diabetic patients diagnosed at age 40–60 years or over 60 years. The survey showed that the studied *FTO* polymorphisms have no impact on the age at which diabetes develops.

Conclusions

1. No significant relationships between the different analysed anthropometric and other parameters and distribution of examined *FTO* polymorphisms (rs9939609, rs1421085, and rs9930506).
2. The age of diabetes was not affected by the tested *FTO* polymorphisms (rs9939609, rs1421085, and rs9930506).

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