# Single Nucleotide Polymorphisms in exon 3 of the adiponectin gene in subjects with type 2 diabetes mellitus

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## Abstract

**Purpose:** Adiponectin (APM1) – a newly discovered adipocytokine secreted by fat tissue – was recently suggested to play a role in the genetic predisposition to type 2 diabetes, obesity and insulin resistance. Adiponectin gene is localized on chromosome 3q27 within the region which was identified as susceptibility locus for type 2 diabetes and metabolic syndrome. Till now genetic associations of two SNP in exon 2 (+45T/G) and intron 2 (+276G/T) of adiponectin gene with type 2 diabetes and adiponectin level were reported in Japanese population and with insulin resistance in some Caucasian populations (Italy, Germany). Moreover, in the proximal promoter region of the APM1 gene: SNP-11426A/G and –11391A/-11377G haplotype predicted the associations with fasting plasma glucose, type 2 diabetes and adiponectin levels. On the other hand the role of mutations in exon 3 of the adiponectin gene is not so well studied.

**Material and methods:** The aim of our study was the screening for rare mutation in exon 3 of adiponectin gene in the Polish subjects with type 2 diabetes as there is no data available about the frequency and role of these mutations in our population. The study was performed in the group of 187 Polish origin patients with type 2 diabetes (32 female and 155 male, mean age 54.1±8.6 yrs) and 102 age and sex matched healthy controls.

**Results:** The frequency of adiponectin gene mutations in exon 3 was 3.9%, while in the control group 0.98% and this difference was not statistically significant. We also observed that adiponectin level is significantly lower in patients with c.331 T→C mutation (Y111H) in comparison to subjects without this mutation (5.0 ug/ml vs 14.4 ug/ml, p=0.0148).

**Conclusions:** To our knowledge the present study is the first which shows that in Polish populations.

**Key words:** adiponectin, polymorphism, exon 3, type 2 diabetes.

## Introduction

Adiponectin (APM1) – a newly discovered adipocytokine secreted by fat tissue – was recently suggested to play a role in the genetic predisposition to type 2 diabetes, obesity and insulin resistance [1,2].

There is an increasing evidence that hypoadiponectinemia, observed in these different forms of metabolic syndrome, may have a role in the pathogenesis of insulin resistance [3-5]. It was shown that administration of adiponectin to animals with insulin resistance lowered free fatty acid by improving their oxidation in skeletal muscle and decreasing serum levels of glucose [6].

Adiponectin gene is localized on chromosome 3q27 within the region which was identified as susceptibility locus for type 2 diabetes and metabolic syndrome [7]. Till now genetic associations of two SNP in exon 2 (+45T/G) and intron 2 (+276G/T) of adiponectin gene with type 2 diabetes and adiponectin level were reported in Japanese population and with insulin resistance in some Caucasian populations (Italy, Germany) [8-11]. Moreover, in the proximal promoter region of the APM1 gene: SNP-11426A/G and -11391A/-11377G haplotype predicted the associations with fasting plasma glucose, type 2 diabetes and adiponectin levels [9,12].

On the other hand the role of mutations in exon 3 of the adiponectin gene is not so well studied. However, the association of these rare mutation with type 2 diabetes was recently shown in French Caucasians, but it was not confirmed in by the Swedish group [8,12].

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***Table 1.*** **Clinical chracteristics of the patients with the mutations detected in exon 3 of the adiponectin gene and subjects without these mutations**

| Subject ID | Age | Sex | DM T2 | CHD | Mutation in exon 3 | Amino acid substitution | Adiponectin serum level | BMI | Hypertension | Hyperlipdemia |
|---|---|---|---|---|---|---|---|---|---|---|
| CASE 024 | 49 | m | yes | yes | TAC→CAC | Tyr111His | 5.7 | 26.3 | 1 | 1 |
| CASE 037 | 49 | m | yes | yes | TAC→CAC | Tyr111His | 3.2 | 26.1 | 0 | 0 |
| CASE 173 | 54 | m | yes | yes | TAC→CAC | Tyr111His | 9.4 | 30.1 | 1 | 0 |
| CASE 067 | 62 | m | yes | yes | ACC→ACT | Thr83Thr | 26.0 | 25.1 | 1 | 0 |
| CONT 186 | 51 | m | no | yes | TAC→CAC | Tyr111His | 1.5 | 25.9 | 0 | 1 |
| CASEs without mutation | 53.8 | 32f/ 150m | 99/182 | 111/182 | no | no | 14.4 | 30.3 | 103/182 | 123/182 |

The aim of our study was the screening for rare mutation in exon 3 of adiponectin gene in the Polish subjects with type 2 diabetes as there is no data available about the frequency and role of these mutations in our population.

## Material and methods

The study was performed in the group of 187 Polish origin patients with type 2 diabetes (32 female and 155 male, mean age 54.1±8.6 yrs) and 102 age and sex matched healthy controls.

For the study of the adiponectin gene mutations: c.250 G→C(GGA→CGA) G84R (Glycine→Arginine); c.268 G→A (GGT→ATG) G90S (Glycine→Serine); c.331 T→C (TAC→CAC) Y111H (Tyrosine→Histidine); c.491 TAC (ATC→ACC) I164T (Isoleucine→Threonine) 476 bp fragment of exon 3 was directly sequenced using forward: 5'-GTGAGTGGGAGCCACAGGGATG-3' and rewers: 5'-GCCGGAGGCCTGGTCCACATTA-3' primers.

## Results

In the studied group with type 2 diabetes we have found four mutations in exon 3 of the adiponectin gene (three missence Y111H and one silent mutation at the position c.249 C→T, and only one mutation (Y111H) in subject without type 2 diabetes (but with the history of CHD and hyperlipidemia).

In our population we didn't find any mutation which were earlier observed in Japanese population (including c.491 T→C (I164T) and in the previous studies in French Caucasians: at the positions c.250 G→C (G84R) or c.268 G→A (G90S) [8,13].

The frequency of adiponectin gene mutations in exon 3 was 3.9%, while in the control group 0.98% and this difference was not statistically significant. We also observed that adiponectin level is significantly lower in patients with c.331 T→C mutation (Y111H) in comparison to subjects without this mutation (5.0 ug/ml vs 14.4 ug/ml, p=0.0148) (*Tab. 1*).

## Discussion

To our knowledge the present study is the first which shows that in Polish populations. Tyr111His mutation in exon 3 of adiponectin gene could contribute to the lower level of adiponectin in the peripheral blood. Similar observation about the association of at least one of G84R, G90S or Y111H variants with adiponectin level and type 2 diabetes was previously described in the group of 1373 French Caucasian subjects with obesity and type 2 diabetes [8]. Contrary to our and Vasseur and col. observations these three SNPs were at a very low frequency in Swedish patients and were not associated with type 2 diabetes [12].

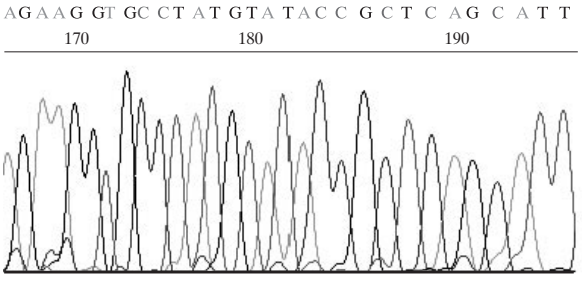
At present it is difficult to explain how the observed mutation could influence the adiponectin level or function. As Y111H missense mutation is located in the highly conserved region of the adiponectin gene – at the hinge between the collagen and globular domains, it was previously suggested that this mutation may partially hinder the complexation of collagenous homotrimers in boundles and alter the spatial organization of protein, interfere in post-translational modifications or influence the interactions of proteins with their receptor [8].

In our study the frequency of adiponectin gene mutations in exon 3 was three times higher than in the control group (3.9% vs 0.98%) but this difference didn't reach the statistical significance. We think that the main reason for the lack of statistical differences between the groups is that the frequency of the studied mutation is relatively low and to prove the difference one would have to study much larger group of subjects.

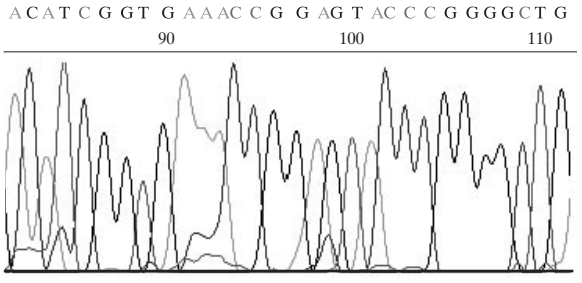
In summary our study suggests that Y111H missense mutation is associated with the lower levels of adiponectin in the peripheral blood and its potential association with type 2 diabe-

*Figure 1.* **Fragments of DNA sequences of exon 3 of adiponectin gene with or without studied mutations**

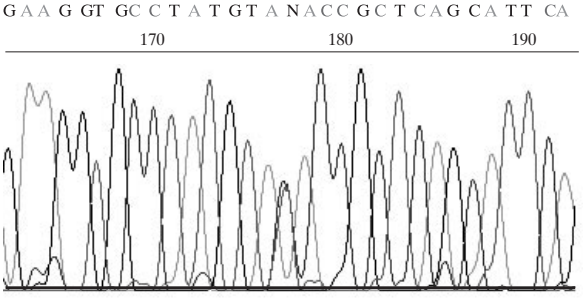
A) DNA sequence without c.331 T→C mutation (Y111H)



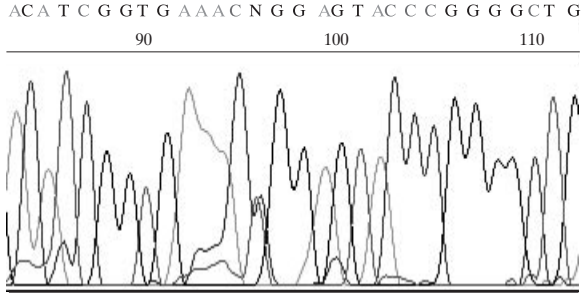

C) DNA sequence without c.249 C→T mutation (Thr83Thr)




B) DNA sequence with c.331 T→C mutation (Y111H)




D) DNA sequence with c.249 C→T mutation (Thr83Thr)




tes, coronary artery disease and maybe some other disorders of metabolic syndrome need to be studied in the larger population of subjects.

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