Introduction

The predisposition of obese subjects to type 2 diabetes mellitus (T2D) is well known. However, many overweight subjects do not become diabetics, while non-overweight subjects can experience diabetes mellitus. It is likely that both genetic and environmental conditions influence the development of diabetes in overweight subjects.

We have recently reported that p53 codon 72 influences the relationship between overweight and diabetes [1]. P53 codon 72 is a polymorphic site within p53 gene [2-5]. The polymorphism is due to a single nucleotide substitution that changes arginine to proline in the protein. The arginine variant is a stronger apoptosis inducer while the proline variant is a stronger transcriptional activator [2]. The development of diabetes in overweight subjects is more frequent in *Arg/*Arg subjects than in carriers of *Pro variant [1].

In the present paper we have studied the effect of cigarette smoking on the relationship between p53 codon 72 polymorphism and susceptibility to type 2 diabetes mellitus in overweight subjects.

Material and Methods

We have reexamined the data on 281 subjects admitted to the Hospital for cardiovascular disease. The subjects gave informed consent to participate in the study that was approved by the Council of Department. P53 codon 72 genotype was determined by DNA analysis. Three way contingency table analysis was performed by a log linear model.

Results

(Table 1) shows the effect of *Arg/*Arg on susceptibility to T2D in overweight non-smoking subjects.
(Table 2) shows the effect of *Arg/*Arg on susceptibility to T2D in overweight smoking subjects.

In non-smoking subjects no statistically significant interaction among p53 codon 72 genotype, BMI and diabetes is observed. However the presence of a statistically significant Odds Ratio indicates that susceptibility to diabetes in overweight non-smoking subjects is greater in *Arg/*Arg genotype than in carriers of *Pro allele.

In smoking subjects there is a statistically significant interaction pointing to a strong effect of p53 codon 72 genotype on the relationship between BMI and diabetes. Indeed in smoking subjects carrying the *Arg/*Arg genotype the Odds Ratio is much greater as compared to that of non-smokers (15.15 vs 6.70) with the same genotype (Tables 2,3). This difference between smokers and non-smokers suggests that cigarette smoking cooperates with *Arg/*Arg genotype increasing the difference between this genotype and carriers of *Pro allele concerning the effect on the association between BMI and diabetes.

(Figure 1) depicts the difference between smokers and non-smokers.

Discussion

The present data suggest that the effect of *Arg/*Arg genotype on the relationship between BMI and diabetes is enhanced by cigarette smoking.

An association between p53 codon 72 and T2D has been observed [5] and we have reported a protective effect of *Pro/*Pro genotype [1]. P53 expression in adipose tissue seems to have a crucial role in the regulation of insulin resistance: up regulation of p53 activity increases insulin resistance [6]. On the other hand it is known that cigarette smoking is a predisposing factor for T2D. Both *Arg/*Arg genotype [2] and cigarette smoking [7-11] are apoptosis inducers: apoptosis in adipose tissue could have an important role in the increase of risk for T2D in obese subjects. Association study, however, cannot explain mechanism but may suggest productive experimental studies.

From the practical point of view our observation suggests that overweight subjects with *Arg/*Arg genotype and smoking habit may have a high risk to become diabetic.

The limitation of the present study is represented by the fact that it has been carried out in subjects with cardiovascular diseases.

Smoking is not only a risk factor for T2D but also cardiovascular disease. Many smokers have multiple smoking-related pathologies [12].

Table 1: Demographic characteristics.

| PARAMETER            | PROPORTION % |
|----------------------|--------------|
| Female               | 47.4%        |
| Hypertension         | 80.9%        |
| Cardiac hypertrophy  | 54.2%        |
| Cardiac arrhythmia   | 53.9%        |
| High total cholesterol| 67.3%       |
| High triglycerides   | 45.2%        |
| MEDIAN ±SD           |              |
| Age, years           | 66.7 ± 11.6  |
| BMI                  | 27.36 ± 5.2  |
| Systolic blood pressure, mmHg | 136.6 ± 15.6 |
| Diastolic blood pressure, mmHg | 84.8 ± 1.3  |

Table 2: p53 codon 72 and susceptibility to T2D in overweight subjects (Non-smoking subjects).

|                  | % frequency of *Arg/*Arg genotype | Absolute frequencies | Carriers of *Pro allele |
|------------------|-----------------------------------|----------------------|-------------------------|
|                  | % frequency                        | Absolute frequencies | % frequency             |

| DIABETICS       |                                |                      |                         |
|-----------------|---------------------------------|----------------------|-------------------------|
| BMI ≤ 25        | 14.3%                           | 3/21                 | 20.0%                   |
| BMI > 25        | 85.7%                           | 18/21                | 80.0%                   |
| NON DIABETICS   |                                |                      |                         |
| BMI ≤ 25        | 53.1%                           | 43/81                | 40.4%                   |
| BMI > 25        | 46.9%                           | 38/81                | 59.6%                   |

Three way contingency table analysis by a log linear model x=p53 y=BMI z=diabetes G df p xyz interaction 1.026 1 0.320

Odds ratio analysis (diabetics vs non-diabetics/ BMI≤25 vs BMI≥25) O.R. 95% C.I. *Arg/*Arg genotype 15.15 1.76-105.25 Carriers of *Pro allele 1.31 0.28-6.41

Table 3: P53 codon 72 and susceptibility to T2D in overweight subjects. (The effect of cigarette smoking).

|                  | % frequency of *Arg/*Arg genotype | Absolute frequencies | Carriers of *Pro allele |
|------------------|-----------------------------------|----------------------|-------------------------|
|                  | % frequency                        | Absolute frequencies | % frequency             |

| DIABETICS       |                                |                      |                         |
|-----------------|---------------------------------|----------------------|-------------------------|
| BMI ≤ 25        | 5.9%                            | 1/17                 | 36.4%                   |
| BMI > 25        | 94.1%                           | 16/17                | 63.6%                   |
| NON DIABETICS   |                                |                      |                         |
| BMI ≤ 25        | 48.6%                           | 18/37                | 42.9%                   |
| BMI > 25        | 51.4%                           | 19/37                | 57.1%                   |

Three way contingency table analysis by a log linear model x=p53 y=BMI z=diabetes G df p xyz interaction 4.378 1 0.035

Odds ratio analysis (diabetics vs non-diabetics/ BMI≤25 vs BMI≥25) O.R. 95% C.I. *Arg/*Arg genotype 6.70 1.69-24.36 Carriers of *Pro allele 2.70 0.71-11.22

Figure 1: Odds Ratio (diabetics vs non-diabetics/BMI≥25 vs BMI≤25) in relation to cigarette smoking and P53 codon 72 genotype.
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