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Preliminary results of the investigations regarding the association of transforming growth factor- beta1 (*TGFB1)* gene polymorphism to metabolic syndrome in a Romanian patients group

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ABSTRACT

Genetic factors have a variable impact in predisposition for common chronic diseases, such as those grouped as metabolic syndrome (MS). MS, obesity, type 2 diabetes and hypertension have a common factor, represented by the inflammatory processes. There are numerous susceptibility genes associated with inflammation and these diseases; one gene of them is transforming growth factor –beta1 (*TGFB1*) gene. The promoter SNP TGFb1 -509C>T (rs1800469) has been reported as the risk factor associated with diabetes, kidney and heart diseases in European population. This work describes the distribution of this SNP in the MS and healthy groups from Romanian population. Our preliminary data could not confirm an association between MS and TGFb1 -509C>T.

Keywords: *genetic factors, diabetes, hypertension, gnene, growth factor, TGFB1, metabolic syndrome, Romanian.*

1. INTRODUCTION

Inflammation processes involve high levels of transforming growth factor -beta 1 (*TGF-β1 or TGFB1*). This observation represented the idea that motivated initiation of this work in which we tested the association of a SNP in this gene and metabolic syndrome (MS) and its components.

The biology of TGFB1. *TGFB1* gene codes for a family of proteins with immunoregulatory and cell signaling properties in various tissues [1], under normal or pathological conditions. There are two classes of TGF identified: (i) TGF-alpha- linked with a malignant cell phenotype and (ii) TGF-beta (TGFB)-expressed in normal cells; they have diverse roles in embryonic development, cytodifferentiation during proliferation, maintenance of pluripotency or immunoregulatory effects. TGFB isoforms, TGFB1, TGFB2 and TGFB3, [2], [3] share sequences and structural similarities [4-6].

The expression of TGF-beta1 has been detected in cells participating in inflammatory response and chronic inflammatory conditions [7],[8].

Signaling network controlled by TGFB involve transcription factors (coactivators or corepressors) like SMAD (term derived from MAD, Mothers against decapentaplegic homolog -Mad and the *C. elegans* gene Sma) and non-SMAD proteins; the roles of this signaling path are: (i) to control the transcription of TGF beta dependent genes, which affect cell growth and proliferation processes (ii) to link extracellular signals from TGF beta ligands with intracellular signaling factors [9-11].

Genetic factors controlling the expression and function of TGFB1 protein. The *TGF-β1* gene is located 19q13.1-13.3. There have been investigated more than six polymorphic loci in this gene: -C988A (rs1800820), -G800A (rs1800468), -C509T (rs1800469), T869C (rs1982073; Leu10/Pro10; T29->C), G915C (rs1800471), and C11929T (Thr263Ile; rs1800472) (Fig.1) [12], [13] for association with different diseases. The SNP C-to-T at position -509 relative to the first major transcription start site (-509C>T SNP; rs1800469) was found to be involved in transcription of *TGFB1* and TGFB1 plasma concentration. These polymorphisms have been linked with altered immune processes, inflammation in certain pathological states such as arthrosis, nephrite, myocardial infarction, allergies, asthma, polycystic ovary syndrome, cancer, polycystic ovary syndrome, diabetes type 2 mellitus (DTM) and hypertension [1], [2] [8], [14-22]. Some studies described the potential association of *TGFB*1 gene polymorphism with metabolic syndrome [23], [24].

The present study aimed to determine whether the C-509T (rs1800469) polymorphism of *TGFB1*gene was associated with metabolic syndrome risk in a group of Romanian patients.

2. MATERIALS AND METHODS

Sample characterization and inclusion criteria. This approach was based on classical clinical case-control system organized during 2016-2017, when they accessed the Emergency Giurgiu County Hospital. Patients with metabolic syndrome (MS) (n=47) and clinically healthy persons (n=80) were selected

Clinical evaluation included body mass index or BMI, blood pressure, cholesterol- LDL level: (i) **blood pressure** (as SBP or systolic blood pressure >130mmHg; and DBP or diastolic blood pressure >85 mmHg, with enrolment in a hypotensive treatment program; (ii) BMI calculated based on waist measure >94 cm for men; waist measure for women >80 cm; (iii) Particular habits: alcohol consumption; (iv) **Biochemistry** investigations included: Hyperglycemia (glycemia à jejun >100 mg/dl or diagnosis of type 2 diabetes (Table 1).

Genotyping. *Collection of blood samples* were performed with vacutainers on sodium EDTA for the DNA

extraction and genetic tests, and without sodium EDTA when biochemical analyses were envisaged. Blood samples were stored by freezing till the extraction of DNA. *Extraction of DNA* was performed with Qiagen QiAmp kits for blood, according to the manufacturer's instructions. Samples of DNA were used further for the genetic test**.** *Genotyping TGFb1 C-509T* was performed by the RFLP-PCR method described by [25]. The PCR conditions were as follows: 50ng of genomic DNA, 20 picomole of each PCR primer in a total volume of 25μL containing 10mM Tris-HCl, pH 8.3, 50mMKCl, 2.0mM $MgCl_2$, 0.2mM each deoxyribonucleotide triphosphate, and 1 unit of AmpliTaq DNA polymerase (Applied Biosystems).. For PCR amplification two primers (from 5' to 3'end) were used: the upstream primer: GGAGAGCAATTCTTACAGGTG and the downstream primer: TAGGAGAAGGAGGGTCTGTC. The PCR conditions were as follows: 35 cycles at 95°C for 30 s, 60°C for 30 s, and 72°C for 30 s. The polymorphism analysis included *DdeI* enzyme restriction (5 unit *DdeI* in 10μL buffer for 30 min. at 37°C). Restriction products were analyzed by electrophoresis on 3% agarose gel. Each allele was recognized according to its size as compared with a molecular weight marker.

Genotyping. The RFLP-PCR technique resulted in the following products depending on the presence of T or C allele of the TGFB1 gene -C509T locus (Fig. 1): a single fragment of 120bp for the T allele (undigested by *DdeI*) or two fragments of 74 and 46bp for the C allele (digested by *DdeI*) (Fig. 2). PCR-RFLP resulted in different amplicons depending on their molecular weight that were obtained for T or C alleles.

Statistical analysis. Allelic frequencies are expressed as a percentage of the total number of alleles. The SAS system with $\chi 2$ test was utilized for statistical analyses, and a CI (confidence interval) of 95% and the p values < 0.05 were considered statistically significant.

3. RESULTS

This work aimed to demonstrate the association of the TGFB1 coding gene SNP C-509T with MS. This case-control study included MS and healthy control (C) groups. Subjects were included in one of the groups based on clinical and paraclinical data. The samples were genotyped for the *TGFB1* gene -C509T SNP and the distribution of the CC, CT and TT genotypes was recorded.

Clinical evaluation. The most important results of the clinical and biochemical investigations are presented in Table 1. These data were compared between MS and control (C) groups. From clinical characteristics of persons diagnosed with MS were determined frequency of hyperglycemia (85.81%), high systolic blood pressure (HSBP) (72.34%) and BMI >25 (53.20%).

Statistical analysis. Genotypes (common TT homozygotes, TC heterozygotes and minor CC homozygotes) and allelic T/C frequencies for TGF-B1 polymorphism in both groups were compared and statistically analyzed.

The distribution of the genotypes in the selected sample (total N=128 subjects) was as follows **(**diagram represented in Fig.2)**:** for MS group the number of patients was as follows: CC= 16, CT=20, TT= 11 (total N-MS = 47), for C healthy control group, total N-C=80) number of persons according to the genotypes were: CC=38, CT=33, TT=9 (Table 2).

Hardy - Weinberg equation. Genotypes of *TGFb1*– C-509T are distributed according to Hardy-Weinberg Equilibrium.

Discussions. This investigation aimed to test the association of the TGF beta1 polymorphism in C509T locus with the metabolic syndrome.

The results showed that the minor T allele in the *TGF-beta1* gene polymorphic region -C509T is distributed only slightly more frequent (44.68%) in MS patient group than in the control, healthy one (31.87%). Also, the statistical analysis did not show significant association between the -C509T gene polymorphisms and MS.

Table 1. Frequency (based on subject number) of the clinical characteristics for the MS and Control (healthy) state as inclusion criteria of the selected sample of individuals. Number of persons with MS clinical standard values

Diagnosis	Alcohol consumption number of persons/ percentage	BMI number of persons/ percentage	SBP number of persons/ percentage	DBP number of persons/ percentage	Glycemia number of persons/ percentage
MS	10 (21.27%)	25 (31.20%)	34* (72.34%)	34* (72.,34%)	40* (85.81%)
C	16(20%)	0	0	0	0

*Considering persons with MS clinical standard values and under specific treatment.

The data did not provide strong statistical evidence of an association between polymorphisms in the TGF-β1 gene with clinical parameters used as including criteria for MS (e.g. hypertension, diabetes, obesity). Results of similar investigations on European population are variable in terms of disease risk association for this geographical region. A negative correlation was reported on a Dutch population group [26]. A general result regards however to transcription factor binding to the *TGFB1* gene promoter, transcriptional activity of *TGFB1* gene, and TGFB1 protein plasma concentration in European population [12],[13], have been reported. Numerous positive correlations have been reported for German and Italian patients especially of vascular

diseases (stroke, myocardial infarction) [27-29]. Some reports refer to other polymorphic loci in their investigations regarding the genetic risk for myocardial infarction [30].

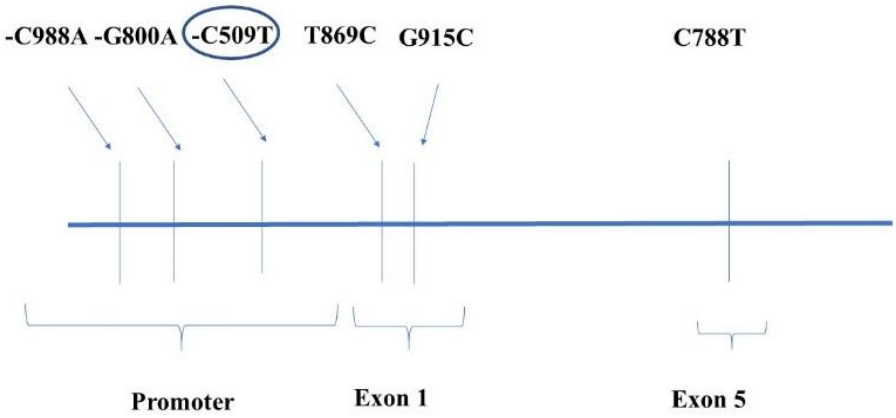

Figure 1. Diagram representing polymorphic loci on *TGFB1* gene. The most known six polymorphic loci with their corresponding SNPs are represented: three in the promoter region (-C988A (rs1800820), -G800A (rs1800468), - and -C509T (rs1800469)), two in the exon 1 (+T869C (rs1982073; Leu10/Pro10; T29->C) and +G915C (rs1800471)) and one in exon 5 (+C788T) (after [12]).

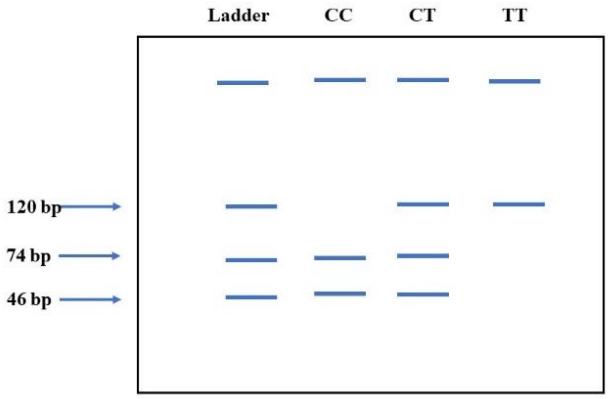

Figure 2. Typical electrophoregram obtained after fractionation of RFLP-PCR amplicons obtained for TGFB1 gene -C509T polymorphic locus (after [25]).

Table 2. Distribution of the *TGFB1* genotypes (CC/TT/TC) among the two clinically characterized groups MS/C.

TGFB1 genotype	CC	CT	TT	*Statistical value HWE*
MS (persons number)	16	20	11	X^2 **= 0.91**
C (persons number)	38	33	9	X^2 **= 0.2**
TOTAL(persons number	54	53	20	X^2 **= 1.29**

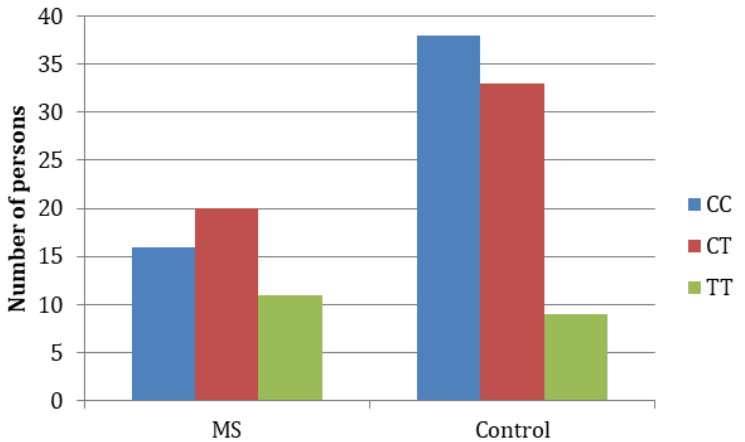

Figure 3. Distribution of *TGFB* C-509T genotypes in the sample group of MS patients and healthy (control) group.

4. CONCLUSIONS

This work investigated the association between the *TGFB1* gene polymorphism in -C509T locus with the MS in a Romanian population which attended a hospital service. The results of the statistical analysis did not provide sufficient data to demonstrate that the TT genotype confers a risk to development of the disease. The -C509T polymorphism may be considered as a risk factor for MS in the Romanian population based only on the already reported results in European population. These results should be confirmed by further studies, with larger samples, that would enable obtaining more association information and biological details regarding the underlying pathological mechanism relating the investigated polymorphism and metabolic syndrome risk. Blood TGFB1 protein level may be added to such studies together with other gene polymorphisms to a new, more relevant genetic profile in order this to be more informative and result in values with statistical significance.

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