Research Article

The Relationship between the Transforming Growth Factor β1 T29C Gene Polymorphism and Left Ventricular Geometry and Function in Hypertensive Subjects

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The distribution of the T29C TGFβ1 gene polymorphism was analyzed in 198 hypertensives with left ventricular hypertrophy (LVH) and in 235 hypertensives without LVH. Circulating TGFβ1 levels, procollagen type III levels, microalbuminuria, and left ventricular geometry and function were evaluated in all the hypertensives with LVH subgrouped according to T29C TGFβ1 gene polymorphism. Circulating TGFβ1 was evaluated by ELISA technique, procollagen type III by a specific radioimmunoassay, microalbuminuria by radioimmunoassay, and left ventricular geometry and function by echocardiography. All groups were comparable for gender, age, and sex. Regarding T29C TGFβ1 gene polymorphism, prevalence of TC or CC genotypes was significantly (P<.05) higher in hypertensives with LVH than hypertensives without LVH TC and CC LVH hypertensives were characterized by a higher prevalence of subjects with microalbuminuria (P<.05 TC and CC versus TT), by increased levels of TGFβ1, procollagen type III, urinary albumin excretion, LVM, LVM/h2.7, and lower values of left ventricular ejection fraction (P<.05 TC and CC versus TT). Our data suggest that T29C TGFβ1 gene polymorphism was associated with clinical characteristics adequate to recognize a subset of LVH hypertensives with a higher severity of hypertension.

1. Introduction

Hypertension represents the most common powerful risk factor for cardiovascular morbidity and mortality [1]. High blood pressure is associated with adverse morphological and functional changes in the cardiovascular and renal system, including left ventricular hypertrophy (LVH), microalbuminuria and progressive renal and heart disease.

The disproportional accumulation of fibrous tissue is the major characteristic of the adverse structural remodelling of cardiac tissue in hypertensives promoting systolic and diastolic dysfunction [2–5].

Transforming growth factor β1 (TGFβ1) is a multifunctional cytokine and its gene has been found in position 19q13.2, 19q13.1 on chromosome 19 and it has 7 exons and the length of the whole gene is 17.52 kb.

TGFβ1 overproduction acts on cardiomyocytes as well as on cardiac fibroblasts inducing cardiac fibrosis and hypertrophy [6, 7]. In addition, the reduction in circulating TGFβ1 through a block of the renin-angiotensin system (RAS) was reported to be associated with an improvement of renal function and a reversion in LVH [8, 9]. Moreover, recent experimental data indicate that blockade of the TGFβ, through a novel orally specific inhibitor of the TGFβ receptor 1, results in significant improvement of deleterious cardiac remodelling after infarction [10].

Production of TGFβ1 is in part under genetic control [11]. Eight single nucleotide polymorphism (SNPs) have
Table 1: Clinical characteristics and distribution of Leu10 → Pro10 genotypes in hypertensives (Ht) with and without left ventricular hypertrophy (LVH).

<table>
<thead>
<tr>
<th></th>
<th>Ht with LVH</th>
<th>Ht without LVH</th>
<th>P=</th>
</tr>
</thead>
<tbody>
<tr>
<td>n.</td>
<td>198</td>
<td>235</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>50 ± 10</td>
<td>49 ± 10</td>
<td>.300</td>
</tr>
<tr>
<td>Gender (F/M)</td>
<td>90/108</td>
<td>124/111</td>
<td>.590</td>
</tr>
<tr>
<td>BMI (Kg/m²)</td>
<td>25 ± 6</td>
<td>24 ± 6</td>
<td>.085</td>
</tr>
<tr>
<td>WHR</td>
<td>0.86 ± 0.1</td>
<td>0.85 ± 0.1</td>
<td>.300</td>
</tr>
<tr>
<td>SBP (mm/Hg)</td>
<td>144 ± 15</td>
<td>143 ± 16</td>
<td>.505</td>
</tr>
<tr>
<td>DBP (mm/Hg)</td>
<td>88 ± 9</td>
<td>87 ± 10</td>
<td>.279</td>
</tr>
<tr>
<td>MBP (mm/Hg)</td>
<td>106 ± 9</td>
<td>105 ± 9</td>
<td>.250</td>
</tr>
<tr>
<td>Ht treated (%)</td>
<td>94.4% (187/198)</td>
<td>94.9% (223/235)</td>
<td>.987*</td>
</tr>
<tr>
<td>Diuretics</td>
<td>9% (17/187)</td>
<td>12.1% (27/223)</td>
<td>.394*</td>
</tr>
<tr>
<td>ACEi</td>
<td>58.8% (110/187)</td>
<td>61.8% (138/223)</td>
<td>.605*</td>
</tr>
<tr>
<td>ARB</td>
<td>42.8% (80/187)</td>
<td>40.3% (90/223)</td>
<td>.607*</td>
</tr>
<tr>
<td>CaCB</td>
<td>11.8% (22/187)</td>
<td>17.3% (30/223)</td>
<td>.199*</td>
</tr>
<tr>
<td>Duration of treatment (yrs)</td>
<td>6.5 ± 3.3</td>
<td>6.2 ± 3.9</td>
<td>.393</td>
</tr>
<tr>
<td>T29C (Leu10 → Pro)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>15.1% (30/198)</td>
<td>30.6% (72/235)</td>
<td>.0418</td>
</tr>
<tr>
<td>TC</td>
<td>64.7%* (128/198)</td>
<td>49.8% (117/235)</td>
<td>.0434</td>
</tr>
<tr>
<td>CC</td>
<td>20.2%* (40/198)</td>
<td>19.6% (46/235)</td>
<td>.0472</td>
</tr>
<tr>
<td>TC + CC</td>
<td>84.9%</td>
<td>69.4%</td>
<td>.0466</td>
</tr>
</tbody>
</table>

* z test.
F: Female; M: Male; BMI: Body mass index; WHR: Waist hip ratio; SBP: systolic blood pressure; DBP: diastolic blood pressure; MBP: mean blood pressure. ACEi: angiotensin converting enzyme inhibitors; ARB: Angiotensin receptor blockers; CaCB: calcium channel blockers. n.s. = not significant.
## Table 2: Distribution of clinical measurements in the hypertensives with left ventricular hypertrophy subgrouped according to Leu\(^{10}\) → Pro\(^{10}\) TGF\(\beta 1\) genotypes*.

<table>
<thead>
<tr>
<th></th>
<th>TT n. 30</th>
<th>TC n. 128</th>
<th>CC n. 40</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (F/M)</td>
<td>16/14</td>
<td>60/68</td>
<td>14/26</td>
</tr>
<tr>
<td>Age</td>
<td>55 ± 10</td>
<td>50 ± 9</td>
<td>50 ± 9</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>69 ± 11</td>
<td>72 ± 10</td>
<td>70 ± 9</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.67 ± 0.1</td>
<td>1.65 ± 0.1</td>
<td>1.68 ± 0.1</td>
</tr>
<tr>
<td>BMI (Kg/m(^2))</td>
<td>24 ± 2</td>
<td>25 ± 2</td>
<td>24 ± 3</td>
</tr>
<tr>
<td>WHR</td>
<td>0.85 ± 0.1</td>
<td>0.87 ± 0.1</td>
<td>0.87 ± 0.1</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>143 ± 16</td>
<td>146 ± 16</td>
<td>141 ± 16</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>87 ± 10</td>
<td>90 ± 11</td>
<td>89 ± 8</td>
</tr>
<tr>
<td>MBP (mmHg)</td>
<td>105 ± 11</td>
<td>108 ± 11</td>
<td>106 ± 9</td>
</tr>
</tbody>
</table>

### Antihypertensive drugs
- Diuretics: 3/30 (10%) 10/128 (7.9%) 4/40 (10%)
- ACEi: 16/30 (53.4%) 75/128 (58.6%) 19/40 (47.5%)
- ARB: 12/30 (40%) 58/128 (42.1%) 14/40 (35%)
- CaCB: 4/30 (13.3%) 12/128 (8.4%) 6/40 (15%)

F: Female; M: Male; BMI: Body mass index; WHR: waist hip ratio; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; MBP: Mean blood pressure; ACEi: angiotensin converting enzyme inhibitors; ARB: Angiotensin receptor blockers; CaCB: calcium channel blockers.

*All variables were not significantly different among the groups.

### 2.2. DNA Isolation and Genotyping.
Genotyping was performed by investigators blinded to clinical status.

Peripheral venous blood was collected in EDTA from all the patients and stored at −70°C.

The PCR approach was used to analyze SNP in the coding regions of TGF\(\beta 1\).

The study of the TGB\(\beta 1\) polymorphism was done by analyzing the sequence. The polymorphism is on exon 1 at +869 from the beginning of the transcription and generates an amino acid substitution in position 10 (Leu → Pro). This study analyzed the genotypes derived from this substitution.

PCR was performed on purified DNA obtained using the GenElute Blood Genomic DNA Kit by Sigma; which provided sequence-specific oligonucleotide primers T869C:

Forward 5′-TTCCCTCGAGGCCCTCCTCCTA-3′
Reverse 5′-GCGGCAGCTTGGACAGGAT-3′

Briefly, PCR reactions were carried out in a total volume of 50-μL containing approximately 5 μL of genomic DNA (0.1 μg/μL), 2 μL of forward and reverse primers (100 ng/μL), 5 μL of 10 × reaction buffer (160 mM (NH\(_4\))\(_2\)SO\(_4\), 670 mM Tris-HCl (pH 8.8 at 25°C), 15 mM MgCl\(_2\), 0, 1% Tween 20), 4 μL of 2 mM dNTPs (invitrogen), 4 μL of DMSO, 0, 1u of Taq Polymerase.

Amplification was carried out in a Robocycler using cycle parameters of 3 minutes and 30 seconds at 95°C (initial denaturation), 35 cycles of 95°C for 45 seconds (denaturation), 62°C for 30 seconds (primer annealing) and a final extension for 10 minutes at 72°C.

T869C: the PCR generated amplicons with a fragment size of 294 Bp.

All PCR products were resolved on 2% agarose gel with 3 μL of ethidium bromide (Figure 1).

### 2.3. Sequencing.
PCR products were sequenced to genotype in all the subjects. PCR reactions in 50 μL were directly sequenced by MWG (the full name is Eurofins MWG Operon, see http://www.eurofinsdna.com/home.html) and from sequence electropherograms was analysed the presence of single-nucleotide polymorphisms T869C in all the subjects (Figure 2). The distribution of SNP TGF\(\beta 1\) gene between both hypertensive groups were reported in Table 1.

### 2.4. Biochemical Measurements.
Patients underwent a general analytical laboratory parameters profile including BUN, creatinine and clearance, glycaemia, electrolytes (serum sodium, potassium, chloride), cholesterol by routine laboratory methods.

Peripheral venous blood was obtained from each patient and the sera were isolated and stored at −70°C. TGF\(\beta 1\) levels were determined by using a solid-phase specific sandwich ELISA technique (R&D Systems, Inc. Minneapolis, USA) as previously described [17]. The interassay and in-assay variations for determining TGF\(\beta 1\) were 8% and 6%,
Figure 2: Localization scheme of the polymorphism at +869 C/T, situated inside exon 1, positions of the forward and reverse primers of the 294 Bp fragment are indicated.

respectively. The sensitivity, hence minum level of detection of TGFβ1 by sandwich ELISA, was 5 pg/mL.

To determine amino-terminal PIIPP, blood samples were taken from each patient and stored at 40°C until manipulation. PIIPP were determined by using a specific radioimmunoassay (Orion Diagnostic Finland), as previously described [18]. The sensitivity of PIIPP was 1 ng/mL, the intra-assay variations ranged from 1.7% to 4.3% and interassay variations from 3.2% to 5.3%.

2.5. Urinary Albumin Excretion (UAE). To eliminate the intra-individual day-to-day variability of UAE, three consecutive 24-hour urine collections were used. In addition, to assess the completeness of a 24 hours urine collection, measurements of urinary rate of clearance of creatinine were evaluated. UAE was measured by radioimmuno-assay (limit of detection, 0.1 mg/dL; Inter-assay coefficient 3.5%). Microalbuminuric patients were defined as UAE ≥ 20 and <300 mg/24 hours.

2.6. Echocardiographic Measurements. All patients underwent an echocardiographic examination M and B-mode, by a computerized echocardiography (ESAOTE, Italy) for the determination of following parameters: left ventricular telediastolic internal diameter (LVIDd), interventricular septum (IVSTd), and posterior wall thickness (PWTd). The Penn convention was used to calculate left ventricular diastolic internal diameter (LVIDd), interventricular septum (IVSTd), and posterior wall thickness (PWTd). The Penn convention was used to calculate left ventricular mass (LVM). LVM was normalized for height to the 2.7 power [19]. The relative wall thickness (RWT) by formula 

\[ \text{RWT} = \frac{2 \times \text{PWTd} + \text{IVSTd}}{2 \times (\text{PWTd} + \text{IVSTd})} \]

The distribution of T29C genotypes With and Without LVH. The distribution of T29C genotypes in both hypertensive groups has been reported in Table 1. The prevalence of TC (P = .0434), and TC plus CC (P = .0466) genotypes, was significantly higher in hypertensives with LVH than hypertensives without LVH (64.7% versus 49.8%, and 84.9% versus 61.4%, respectively).

Genotype frequency distribution in the two groups of hypertensives occurred according to Hardy-Weinberg proportions.

Since genotype frequency distribution was not significant different in hypertensives without LVH, only hypertensives
with LVH were further subdivided into three groups, according to T/C genotypes.

3.2. Distribution of Clinical Characteristics in Hypertensives With LVH Subgrouped According to T29C TGFβ1 Genotypes. The association between genotypes and clinical characteristics in the three groups of hypertensives with LVH have been reported in Table 4. All the groups were homogeneous regarding to age, BMI, WHR and blood pressure. LVH hypertensives with TC or CC genotype were characterized by a significant higher prevalence of subjects with microalbuminuria (P < .05 TC and CC versus TT).

No significant difference in the prevalence and duration of antihypertensive drugs utilized was observed among the groups (Tables 1 and 2). In addition no significant difference also in statin administration was observed among the groups.

3.3. Circulating TGFβ1, Type III Collagen, Urinary Albumin Excretion and Echocardiographic Parameters. Urinary albumin excretion, circulating TGFβ1, PIIIP, LVM and LVM/h² levels were significantly (P < .05) higher and left ventricular ejection fraction values were significantly (P < .05) lower in LVH hypertensives with TC or CC genotype than those detectable in LVH hypertensives homozygous for allele T (Tables 3 and 4).

4. Logistic Regression Analysis

This analysis indicated an association between higher levels of PIIIP and TC or CC genotypes, even if adjusted for LVM/h² and urinary albumin excretion values (Table 5).

5. Discussion and Conclusion

To our knowledge this is the first study to investigate the impact of TGFβ1 Leu → Pro at codon 10 polymorphism (rs1800470) on left ventricular geometry and function in hypertensive patients. Our data indicate a higher prevalence of subjects with microalbuminuria, higher value of LVM and lower left ventricular ejection fraction. In our opinion, this association doesn’t reflect unknown differences in population ancestry between the two hypertensive groups. We consider the probability of false positive inference attributable to population studied rather small because the two hypertensive groups were recruited from an ethnically homogeneous population. In this context recent results from Xu et al. [22] revealed a genetic association of TGFβ1 + 915 Arg → Pro at codon 25 polymorphism with LVH in a Chinese hypertensive population, while the codon 10 genotypes did not show any association to LVH. Even if in this study we have analyzed only TGFβ1 Leu → Pro at codon 10 polymorphism, our recent unpublished data indicated no association between TGFβ1 + 915 Arg → Pro at codon 25 polymorphism and hypertension in a Caucasian hypertensive population. These contrasting data may be explained by ethnicity of two hypertensive populations considered (Caucasian and Chinese).

Although changes in the heart caused by hypertension are well known, the effective mechanisms are not entirely clarified. Despite the role of hemodynamic effects and growth factors have been largely reported, other metabolic and inflammatory factors have to be also considered [23].

Literature data and the results of our previous studies indicate an overproduction of circulating TGFβ1 in hypertensives. Moreover, hypertensives with target organ damage (TOD) have higher circulating levels of TGFβ1 than hypertensives without TOD. This finding seems to attribute an important role to TGFβ1 overproduction in the pathophysiology of essential hypertension and its sequelae.
The TGFβ1 overproduction in hypertension may be explained by the effects of various factors, such as elevated angiotensin II, increased fluid shear stress and a differential expression of TGFβ1 linked to DNA polymorphisms in the promoter [11]. Plasma concentrations of active and also of acid-activable TGFβ1 are predominantly under genetic control (heritability estimate 0.54). Up regulation of TGFβ1 system in monocytes of hypertensive patients and association of TGFβ1 gene polymorphism with risk of hypertension suggests that quantitative difference in TGFβ1 production may determine the intensity of the process of vascular remodelling and therefore influence overall susceptibility to the development of hypertension [26]. On the other hand experimental data indicate that abnormalities in responsiveness to TGFβ1 overproduction, as evidenced by collagen formation, may represent a pathophysiological molecular mechanism in hypertension [27, 28]. The results from ECTIM study [12] suggest that TGFβ1 Arg25-Pro polymorphism might be associated with hypertension but does not address the issue of quantitative phenotypes related to TGFβ1 production in relation to hypertension. On the contrary no study has been addressed the evaluation of the role of Leu-to-Pro polymorphism at codon 10 to explain whether the substitution has functional and biological importance, or it could affect protein transport. In view of this we have now shown that the serum concentration of TGFβ1 was significantly different in LVH hypertensives subgrouped according to T29 → C polymorphism. It is possible that the T29 → C polymorphism of the TGFβ1 gene is linked to some other genes that are actually responsible for the development of hypertension.

Moreover, the significant change in circulating TGFβ1 values among LVH hypertensive groups indicated that TC or CC genotypes could be able to induce quantitative change in the production of the cytokine and it might affect also the function of signal peptide, perhaps influencing intracellular trafficking or export efficiency of preprotein [15].

In addition, our data suggest some clinical considerations. First, LVH hypertensive subjects with TC or CC genotypes were characterized by a higher value of microalbuminuria, LVM and by a systolic left ventricular dysfunction. This might indicate that hypertension in these subjects has to be considered more severe. Another aspect of our study that seems to be interesting is related to the higher collagen production in LVH hypertensive subjects with TC or CC genotype than in those homozygous for allele T. This finding is further supported by analysis of logistic regression indicating that PIPIP may be considered the most important marker associated to T and C alleles. In fact, analysis of odd ratio indicates that the risk of association with T or C allele increases eight fold for each PIPIP quintile variation. In our opinion this finding might further support our previous results [5,23] indicating that overproduction in circulating TGFβ1 may contribute to the progression of renal and cardiovascular damage in obese and/or hypertensive subjects. In particular, the present study emphasizes the unfavourable effects of TGFβ1 overproduction on left ventricular geometry and function that might be mediated by an higher collagen production [5, 24, 29]. In view of this the association of TC or CC with a higher clinical severity of hypertension seems to indicate that TGFβ1 is a susceptibility locus for hypertension. A potential limit of this study was to have computed no a priori evaluation of the β error and consequently the power of our statistical analysis. However this involves considerations only about the sample size. Accordingly the negative results of our study has needed further evaluations.

In conclusion our data are attractive to indicate that LVH hypertensive subjects with TC or CC genotypes (Leu10 → Pro polymorphism, rs 1800470) might be considered a particular subset of LVH hypertensives with a more severity of hypertension. However, the present results require verifications in other populations, since it is well known that hypertension is under the control of many genes that contribute modest individual effects, and TGFβ1 may act in concert with other hypertension susceptibility loci.

Acknowledgments

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References


