

Transforming Growth Factor β_1 Genotype and Change in Left Ventricular Mass during Antihypertensive Treatment—Results from the Swedish Irbesartan Left Ventricular Hypertrophy Investigation versus Atenolol (SILVHIA)

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Summary

Background: Angiotensin II, via the angiotensin II type 1 (AT_1) receptor, may mediate myocardial fibrosis and myocyte hypertrophy seen in hypertensive left ventricular (LV) hypertrophy through production of transforming growth factor β_1 (TGF- β_1); AT_1 -receptor antagonists reverse these changes. The TGF- β_1 G + 915C polymorphism is associated with interindividual variation in TGF- β_1 production. No study has yet determined the impact of this polymorphism on the response to antihypertensive treatment.

Hypothesis: We aimed to determine whether the TGF- β_1 G + 915C polymorphism was related to change in LV mass during antihypertensive treatment with either an AT_1 -receptor

antagonists or a beta $_1$ -adrenoceptor blocker. The polymorphism was hypothesized to have an impact mainly on the irbesartan group.

Methods: We determined the association between the TGF- β_1 genotype and regression of LV mass in 90 patients with essential hypertension and echocardiographically diagnosed LV hypertrophy, randomized in a double-blind study to receive treatment for 48 weeks with either the AT_1 -receptor antagonist irbesartan or the beta $_1$ -adrenoceptor blocker atenolol.

Results: Irbesartan-treated patients who were carriers of the C-allele, which is associated with low expression of TGF- β_1 , responded with a markedly greater decrease in LV mass index (LVMI) than subjects with the G/G genotype (adjusted mean change in LVMI -44.7 g/m 2 vs. -22.2 g/m 2 , $p = 0.007$), independent of blood pressure reduction. No association between genotype and change in LVMI was observed in the atenolol group.

Conclusions: The TGF- β_1 G + 915C polymorphism is related to the change in LVMI in response to antihypertensive treatment with the AT_1 -receptor antagonist irbesartan.

Key words: transforming growth factor, angiotensin, hypertension, polymorphism, left ventricular hypertrophy, irbesartan

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Introduction

Hypertension is associated with a number of adverse morphologic and functional changes in the cardiovascular system, including an increased left ventricular (LV) mass, characterized by an increased extracellular matrix production (myocardial fibrosis) as well as myocyte size (true hypertrophy).^{1,2} This, in turn, may lead to systolic dysfunction and congestive heart failure.¹ Selecting the best pharmacologic treatment for hypertensive patients is difficult since the response of the indi-

vidual patient, even when treated with the same antihypertensive drug, varies in an unpredictable way. To be able to predict the clinical response to a given antihypertensive drug is therefore of great importance for the individual patient.

Activation of the renin-angiotensin system may contribute to the increase in LV mass seen during hypertension. Studies have shown that angiotensin II, the primary effector molecule of the renin-angiotensin system,³ via its interaction with the angiotensin II type 1 (AT₁) receptor, induces hypertrophy of cardiac myocytes and causes cardiac fibroblasts to accumulate collagen, leading to an increased LV mass.²⁻⁵ These effects are independent of the peptides' effect on blood pressure.

Angiotensin II type 1-receptor antagonists interfere with the promotion by angiotensin II of vasoconstriction,⁶ cell growth,⁴ and fibrosis.⁵ Both angiotensin-converting enzyme (ACE) inhibitors and AT₁-receptor antagonists have been shown to prevent and reverse hypertension-induced LV hypertrophy and collagen deposition in a variety of murine models of hypertension.^{2-5, 7, 8} Meta-analyses suggest that reducing the renin-angiotensin system activity by an ACE inhibitor may be a more effective method for reversing hypertensive LV hypertrophy than diuretics, beta-receptor blockers, or calcium-channel blockers.^{9, 10} More recent studies¹¹⁻¹³ suggest that AT₁-receptor antagonists are equally potent in this respect.

Transforming growth factor β_1 (TGF- β_1) regulates extracellular matrix production, and overproduction has been associated with cardiac hypertrophy. For instance, marked increases in TGF- β_1 mRNA levels have been shown to precede cardiac hypertrophy and histopathologic changes such as accumulation of extracellular matrix protein in the heart.¹⁴⁻¹⁷ Porreca *et al.*¹⁸ identified TGF- β_1 hyperexpression in monocytes from hypertensive patients with myocardial hypertrophy compared with monocytes from normotensive control subjects. In addition, a recent study showed that patients with essential hypertension have higher plasma levels of TGF- β_1 than normotensive controls.¹⁹ In the same study, hypertensive patients with target organ damage such as LV hypertrophy had higher levels of TGF- β_1 than hypertensive patients with no target organ damage. Furthermore, increased levels of TGF- β_1 have also been shown to induce pathologic interstitial fibrosis in several other contexts, including post-transplant reactions^{20, 21} and myocardial infarction,²² and to correlate positively with blood pressure.²³

There is growing evidence that cardiac fibrosis induced by angiotensin II may be mediated by TGF- β_1 .^{2, 5, 24} The AT₁-receptor has been identified on cultured cardiac fibroblasts,^{25, 26} and stimulation of this receptor has been shown to induce formation of TGF- β_1 . Furthermore, the release of TGF- β_1 can stimulate cardiac myocyte hypertrophy by paracrine action.²⁷

Both ACE inhibitors and AT₁-receptor antagonists can reduce the TGF- β_1 mRNA levels in hypertensive rats.²⁸⁻³¹ In a recent study, Yu *et al.*²² demonstrated that treatment with an AT₁-receptor antagonist was associated with a reduced TGF- β_1 expression in the heart after acute myocardial infarction. Suppressed expression of extracellular matrix proteins after treatment with AT₁-receptor antagonists has also been described.^{11, 22, 29}

Production of TGF- β_1 is in part under genetic control.³² The G + 915C single nucleotide polymorphism (SNP), which changes codon 25 in the signal sequence and substitutes an arginine for a proline, is functionally associated with TGF- β_1 production.^{33, 34} Awad *et al.*³⁴ showed that lymphocytes from individuals homozygous for the G allele have a higher production of TGF- β_1 in vitro than heterozygotes. There is suggestive evidence of a clinical impact of this polymorphism. Densem *et al.*³³ found that patients undergoing heart transplants, who were carriers of the C allele, that is, related to lower TGF- β_1 levels, were less likely to develop coronary vasculopathy than those homozygous for the G allele. In an additional study by Li *et al.*,²³ circulating levels of TGF- β_1 correlated with blood pressure levels, and the G allele was more common among hypertensives than normotensives.

The aim of this study was to investigate whether the TGF- β_1 G + 915C SNP was related to the change in LV mass in hypertensive patients with LV hypertrophy during antihypertensive treatment with either the AT₁-receptor antagonist irbesartan or the beta 1-adrenoceptor blocker atenolol. The TGF- β_1 G + 915C SNP was hypothesized to have an impact mainly on the irbesartan group. Beta 1-adrenoceptor blockers have been shown to lower angiotensin II levels significantly,³⁵ which could be sufficient to result in a genotype-dependent change in LV mass, but the effect, if any, would probably be less pronounced.

Methods

Study Population

The subjects participated in the Swedish Irbesartan Left Ventricular Hypertrophy Investigation versus Atenolol (SILVHIA) trial, which has been described in detail earlier.¹² Briefly, Caucasian men and women with mild to moderate essential hypertension and echocardiographically verified LV hypertrophy were enrolled, with the primary goal of evaluating the efficacy of irbesartan compared with atenolol on blood pressure reduction and regression of LV hypertrophy. Left ventricular hypertrophy was considered present if left ventricular mass index (LVMI) was > 131 g/m² for men and > 100 g/m² for women. The Penn convention was used for calculation of LV mass, which was corrected for body mass index (LVMI). Secondary hypertension was excluded by means of a physical examination and routine laboratory tests. Patients were excluded if they had an LV ejection fraction < 45%, any significant concomitant diseases, or were taking any other medications that might interfere with the efficacy assessments or that would present safety hazards. On average, patients had been diagnosed with hypertension 8 years previously. All antihypertensive agents were withdrawn before the start of a 4-6 week, single-blind, placebo lead-in period, after which the patients received either irbesartan 150 mg or atenolol 50 mg once daily as monotherapy in a double-blind fashion. The doses were doubled after 6 weeks if diastolic blood pressure (DBP)

was ≥ 90 mmHg. If DBP remained ≥ 90 mmHg at Week 12, hydrochlorothiazide (12.5–25 mg once daily) was added. At Week 24, felodipine (5–10 mg once daily) was added if required. In all, 101 patients completed the study; DNA and echocardiographic data were available in 90 of these patients.

The appropriate ethics committees approved the study, the participating patients gave their informed consent, and the study was completed in accordance with institutional guidelines and the Declaration of Helsinki.

DNA Extraction and Genotyping

Genomic DNA was extracted from ethylene diamine tetraacetic acid (EDTA) blood using spin columns (QIAamp[®] DNA Blood Mini Kit, Qiagen, Hilden, Germany). Polymerase chain reaction (PCR) was conducted using AmpliTaq[™] Gold DNA polymerase (Applied Biosystems, Foster City, Calif., USA) with the forward primer (5'-CCT ACC TTT TGC CGG GAG AC-3') and the 5'-biotinylated reverse primer (5'-CCG CAG CTT GGA CAG GAT CT-3') (The Virtual Laboratory Interactiva, Ulm, Germany), giving a 278 bp product. Reaction volumes (50 μ l) containing 1xPCR buffer II, 2.25 mmol/l MgCl₂, 0.25 mmol/l of each dNTP, 0.2 μ mol/l of each primer, 1 U of taq polymerase, and approximately 100 ng of genomic DNA were used. Polymerase chain reaction conditions consisted of an initial activation step at 95°C for 10 min, followed by 40 cycles of 96°C for 30 s, 61°C for 30 s, and 72°C for 60 s, after which a final elongation step at 72°C for 10 min concluded the reaction (GeneAmp PCR system 9700, Applied Biosystems). Genotyping for the TGF- β_1 polymorphism was conducted with solid-phase minisequencing.³⁶ Polymerase chain reaction products were captured in streptavidin-coated microtiter plate wells and rendered single stranded. The polymorphic nucleotide was detected by single nucleotide extension with a radioactively labeled nucleotide (G or C) of a detection primer (5'-ACT GGT GCT GAC GCC TGG CC-3'), which was designed to anneal immediately adjacent to the polymorphic site. The genotype of the samples was defined by the ratio between the incorporated ³(H)-labeled nucleotides.

Statistical Analysis

Data are presented as mean values \pm standard error. The estimated adjusted mean difference in LVMI change at 48 weeks between G/G and carriers of the C allele (two categories) was calculated with the general linear models (GLM) procedure of the SAS software (SAS Institute, Cary, N.C., USA) for each treatment group. Two different models were used: one univariate and one multivariate model, including the potential covariates age, DBP and systolic blood pressure (SBP), LVMI at study entry, dose of atenolol or irbesartan, change in SBP and DBP (all continuous), gender, as well as the addition of hydrochlorothiazide or felodipine (yes/no). A p value of < 0.05 was considered significant.

Results

Genotype distribution (78 G/G [87%], 11 G/C [12%], and 1 C/C [1%]) was consistent with Hardy-Weinberg equilibrium. There was no significant correlation between LVMI and age (data not shown). Carriers of the C allele were considered as one group. Forty-seven patients (40 G/G and 7 carriers of the C allele) had been given atenolol and 43 (38 G/G and 5 carriers of the C allele) irbesartan. Among patients given atenolol, 48% received 50 mg, 39% 100 mg, and the remainder either 75 or 25 mg. Among patients given irbesartan, 66% received 300 mg, 29% 150 mg, and the remainder either 75 or 225 mg. A total of 57% of the patients required additional treatment with felodipine and/or hydrochlorothiazide. Baseline characteristics of the patients stratified by treatment and genotype are shown in Table I. There were no significant differences between the genotypes in each treatment group.

As was previously shown in the SILVHIA trial,¹² both irbesartan and atenolol progressively reduced LVMI; by 26 ($p < 0.001$) and 14 g/m² ($p < 0.001$) (16 and 9%), respectively, at Week 48, with a greater reduction in the irbesartan group ($p = 0.024$). Change in blood pressure and LVMI at 48 weeks, stratified by treatment and genotype, is shown in Table II. The

TABLE I Baseline patient characteristics stratified by treatment and genotype

	Irbesartan			Atenolol		
	G/G (n = 38)	G/C and C/C (n = 5)	p Value	G/G (n = 40)	G/C and C/C (n = 7)	p Value
Age (years)	53.6 (1.3)	53.2 (3.1)	0.92	54.0 (1.3)	52.0 (3.0)	0.57
Sex (male/female)	22/16	3/2	0.93	25/15	6/1	0.24
BMI (kg/m ²)	27.3 (0.4)	25.5 (1.9)	0.21	27.2 (0.6)	27.9 (1.1)	0.69
SBP (mmHg)	162.7 (3.1)	163.8 (9.0)	0.90	159.3 (3.3)	153.5 (5.1)	0.49
DBP (mmHg)	103.9 (1.1)	107.4 (2.6)	0.28	102.2 (1.3)	102.8 (2.9)	0.87
LVMI (g/m ²)	150.7 (5.6)	147.9 (9.4)	0.85	142.5 (3.9)	154.2 (9.9)	0.27
Dose (mg)	245.8 (12.5)	270.0 (30.0)	0.50	67.3 (4.2)	75.0 (12.2)	0.50
Additional treatment (%)	61	60	0.98	58	29	0.16

Mean values \pm standard error.

Abbreviations: BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, LVMI = left ventricular mass index.

TABLE II Blood pressure and left ventricular mass index change after 48 weeks stratified by treatment and genotype

	Irbesartan			Atenolol		
	G/G (n = 38)	G/C and C/C (n = 5)	p Value	G/G (n = 40)	G/C and C/C (n = 7)	p Value
SBP change (mmHg)	-27.9 (3.5)	-29.2 (8.5)	0.89	-21.5 (2.5)	-19.3 (5.5)	0.74
DBP change (mmHg)	-18.5 (1.4)	-19.9 (1.5)	0.73	-15.8 (1.3)	-16.3 (1.9)	0.88
LVMI change (g/m ²)						
- univariate	-22.4 (4.4)	-46.1 (12.0)	0.071	-13.5 (3.4)	-17.5 (8.1)	0.65
LVMI change (g/m ²)						
- multivariate	-22.2 (2.6)	-44.7 (7.2)	0.007	-14.1 (3.3)	-13.9 (8.3)	0.99

Mean values \pm standard error.

In the multivariate model, adjustment was made for age, gender, dose, baseline systolic and diastolic blood pressure (SBP and DBP) and LVMI, changes in SBP and DBP, as well as additional antihypertensive medication.

Abbreviations as in Table I.

blood pressure response was similar between the different genotypes in each treatment group. Left ventricular mass index change, however, depended markedly on genotype in the irbesartan group. According to the multivariate model, regression of LVMI in this group was about two-fold in patients carrying the C allele compared with patients with the G/G genotype at 48 weeks (-44.7 g/m² [7.2] vs. -22.2 g/m² [2.6], $p = 0.007$). In the atenolol group, on the other hand, LVMI change did not differ between the genotypes.

Discussion

To our knowledge, this is the first study to investigate the impact of the TGF- β_1 G + 915C polymorphism on response to antihypertensive treatment. The results suggest that the TGF- β_1 G + 915C polymorphism is related to the degree of reduction of LV mass during antihypertensive treatment with an AT₁-receptor antagonist such as irbesartan. Hypertensive patients who were carriers of the C-allele, which is associated with low expression of TGF- β_1 , showed a two-fold greater decrease in LVMI than subjects with the G/G genotype. This effect was independent of the reduction in blood pressure.

The involvement of TGF- β_1 in cardiac complications of hypertension has been previously demonstrated. Our observations further suggest that the G + 915C TGF- β_1 polymorphism, presumably by influencing TGF- β_1 levels, may determine the effect of irbesartan on LV mass.

In addition to a chronic increase in pressure and/or volume overload, elevations in ACE activity and angiotensin II concentrations are believed to play a major role in the development of LV hypertrophy.^{25, 37} There is evidence that stimulation of TGF- β_1 production, via the AT₁-receptor, is important in its development. Blockade of the AT₁-receptor by AT₁-receptor antagonists has been proposed to be responsible for the beneficial effects of this drug class beyond the blood pressure-lowering activity, and decreased TGF- β_1 production may be an important mechanism.

An increased level of TGF- β_1 has been shown to be related to essential hypertension and LV hypertrophy.¹⁹ In this study, hypertensive patients with the + 915 GG genotype, which has been associated with higher TGF- β_1 levels, showed a less pronounced reduction of LVMI in response to irbesartan treatment compared with those with the C allele at this locus. A possible explanation might be a heavier load of TGF- β_1 in the former. Even though beta₁-adrenoceptor blockers have been shown to decrease angiotensin II levels, we found no influence of the TGF- β_1 genotype in the atenolol group. The effect on the angiotensin II levels may be of less importance than a direct blockade of AT₁-receptors, thus making a theoretical influence of the TGF- β_1 genotype impossible to discern on this level.

The major limitation of the present study is the small number of subjects. The strength of the study, on the other hand, is that the subjects represent a clinically well-characterized group, randomized to treatment in a prospective, double-blind trial. It would be of great interest to study the impact of this SNP on the incidence of cardiovascular endpoints during AT₁-receptor antagonist treatment in larger trials.

Conclusions

Our findings suggest an impact of the G + 915C SNP in the TGF- β_1 gene on LV mass reduction during antihypertensive treatment with the AT₁-receptor antagonist irbesartan, and suggest that this polymorphism could be used to predict the response to this drug.

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