**Role of Gene Polymorphisms/Haplotypes and Plasma Level of TGF-β1 in Susceptibility to In-Stent Restenosis Following Coronary Implantation of Bare Metal Stent in Chinese Han Patients**

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**Summary**

Transforming growth factor (TGF)-β1 has been implicated in the pathogenesis of restenosis. However, the role of TGF-β1 polymorphisms in development of in-stent restenosis (ISR) after coronary bare metal stent (BMS) implantation in Chinese Han population has not been reported to date. The aim of this study was to explore the association between TGF-β1 gene polymorphisms (-509C/T and 869T/C) and its plasma level in Chinese Han patients with BMS-ISR.

We investigated 419 patients after successful coronary stent placement. All patients were reexamined by angiography. Genotyping for the two TGF-β1 gene polymorphisms was performed using polymerase chain reaction-restriction fragment length polymorphism analysis. Plasma TGF-β1 levels were measured by enzyme-linked immunosorbent assay.

Ninety-two patients (21.96%) developed ISR during the follow-up period. The multivariable analysis adjusted for potential confounders and it revealed that the C allele of TGF-β1 869T/C polymorphism was linked to an increased risk of ISR in both additive (Per each C allele) and dominant (TC+CC versus TT) models with odds ratios (ORs) of 1.88 (95% confidence interval [CI]: 1.21-2.84, \( P = 0.008 \)) and 2.52 (95% CI: 1.40-4.80, \( P = 0.005 \)), respectively. In accord with this, C-dominant CC/CT genotype was linked to higher plasma TGF-β1 level compared to TT genotype. One haplotype (TC) (-509T, +869C) was associated with an increased risk for ISR (OR = 1.48, 95% CI: 1.06-2.06, \( P = 0.010 \)).

The C allele of TGF-β1 869T/C polymorphism, correlated with high plasma TGF-β1 level, represented an independent risk factor for BMS-ISR in Chinese Han patients with coronary artery disease.

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**Key words:** Transforming growth factor-β1, Genetic variation, Coronary stenting, Risk factors

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**Experimental Study**

Semercutaneous coronary intervention (PCI) has become the most common revascularization procedure for coronary artery disease (CAD). Despite the remarkable advances in the pharmacology and technology of PCI, in-stent restenosis (ISR) is still an unsolved problem of this therapeutic strategy. ISR occurs in approximately 30% of patients using bare metal stents and 10%-15% with drug eluting stents.

Transforming growth factor (TGF)-β1 plays a certain role in all stages of the restenosis process: thrombogenesis, inflammation, and neointimal hyperplasia. The TGF-β1 expression increases in restenotic vessels and serum of patients with ISR. Arteries exposed to TGF-β1 after injury lead to a stimulatory effect on platelets activation and thrombogenesis, inflammatory cell infiltration, vascular smooth muscle cell migration and proliferation, extracellular matrix protein synthesis and deposition, and transformation of vascular fibroblasts to myofibroblasts, which finally results in eventual luminal narrowing. On the contrary, attenuating TGF-β1 activity is assuredly efficacious in diminishing intimal hyperplasia and reducing ISR.

It has been confirmed that there are multiple functional genetic polymorphisms in the TGF-β1 gene which are associated with TGF-β1 expression or function. They include rs1800468 (-800G/A) and rs1800469 (-509C/T) in the promoter region, rs1800470 (869T/C, Leu10Pro) and rs1800471 (915G/C, Arg25Pro) in the signal peptide region, and rs1800472 (11929C/T, Thr263Ile) in the region encoding the precursor of TGF-β1. Regarding the serum and vascular levels of TGF-β1 in patients with ISR, it could be speculated that patients with a pathological se-
cretion of TGF-β1, which could be determined by genetic polymorphisms of the gene encoding TGF-β1, may be more susceptible to develop ISR. However, there are limited data assessing the impact of these variants on ISR.25-36 Additionally, whether the genetic variations in TGF-β1 are associated with the risk of ISR after coronary implantation of bare metal stent (BMS) among Chinese Han patients has not been studied.

A high degree of linkage disequilibrium (LD) has been detected between rs1800469 (-509C/T) and rs1800470 (869T/C, Leu10Pro) in Chinese Han population.37-40 whereas minor allele frequencies (MAFs) of the rs1800468 (-800G/A), rs1800471 (915G/C, Arg25Pro), and rs1800472 (1192C/T, Thr263Ile) are extremely rare.36,38-40 We therefore selected rs1800469 and rs1800470 to investigate whether the two single nucleotide polymorphisms (SNPs) and their haplotypes are involved in genetic susceptibility to ISR in Chinese Han patients with CAD after BMS implantation via linking with TGF-β1 serum level.

Methods

Study population: The study population was comprised of 419 unrelated Chinese Han patients with CAD from the South region of China who had successfully undergone target vessel revascularization with BMS for the first time in participating hospitals (Gaozhou People’s Hospital and Guangdong Provincial People’s Hospital) between April 2010 and December 2016. Patients were eligible for inclusion if they were treated successfully for stable angina, non-ST elevation acute coronary syndromes, or silent ischemia by PCI. Exclusion criteria included complex lesions such as saphenous vein graft lesions, a target lesion in the left main trunk, bifurcated lesions, and reference vessel diameter < 2.5 mm; acute ST-segment elevation myocardial infarction within the previous 4 weeks; any contraindication to the use of aspirin, heparin, or clopidogrel; disturbances of blood coagulation; current infectious inflammatory condition; known malignant diseases; autoimmune disorders; hepatic or renal failure; tumor; and lack of consent to participate in the study. Before the procedure, patients received clopidogrel 300 mg and aspirin 100-300 mg. After the intervention, all patients received 100 mg/day aspirin indefinitely, 150 mg clopidogrel for the first 3 days followed by 75 mg/day for at least three months and other cardiac medications (including statins, β-blockers, angiotensin-converting enzyme inhibitors and Ca2+-antagonists) according to the judgment of the patient’s physician. Re-hospitalization for angiography reexamination was scheduled 6 months after stenting or earlier if there was any angina or any adverse event occurred. The restenosis was defined as stenosis diameter ≥ 50% within the stent or in 5-mm segments proximal or distal to the stent from follow-up angiography.41 Events occurring within the first month were excluded from the analysis since these events were attributable mainly to sub-acute stent thrombosis or occluding dissections, but less likely to restenosis. The study protocol conformed to the Declaration of Helsinki and was approved by the medical ethics committees of the participating institutions. Written informed consent was obtained from each participant before the PCI procedure.

Measurement of plasma TGF-β1 levels: Plasma samples were obtained from all patients during follow-up angiography. The samples were stored at -70 °C until laboratory testing. The plasma TGF-β1 concentration was detected using the Human TGF-β1 ELISA Kit (BioSource International, Camarillo, California, USA) in duplicates. Measurements for each patient were made with the same kit to avoid inter-kit variability. The detection minimum of this assay was 15.6 pg/mL, and the intra-assay and inter-assay coefficients of variation (% CVs) were 4.7% and 6.9%, respectively. The laboratory technician who performed the immunological assays was blind to the treatment groups.

Genotyping: Genomic DNA was extracted from ethylene diamine tetraacetic acid anti-coagulated peripheral blood using TIANamp Blood DNA kits (Tiangen Biotech Co. Ltd., Beijing, China) following the manufacturer’s instructions and stored at -20 °C. Genotypes for rs1800469 (-509 C/T) and rs1800470 (869T/C, Leu10Pro) polymorphisms were determined by polymerase chain reaction-restriction fragment length polymorphism, as described by Zheng, et al.42,43 respectively. Genotyping was performed in a blinded fashion so that the performers did not know the ISR/non-ISR status of the subjects. A random double-check was conducted to detect potential genotyping errors. To confirm our results, about 5% of the samples were directly sequenced using the same primers, and the results were 100% concordant.

Statistical analysis: All statistical analyses were performed using the SPSS version 13.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Continuous variables were expressed as mean ± standard deviation (SD) and were compared by means of Student’s t-test or Mann-Whitney U test or one-way analysis of variance. Categorical data are presented as frequencies and percentage. Differences between categorical variables, genotype/allele frequencies, and Hardy-Weinberg equilibrium were tested by χ² analysis or Fisher’s exact test. Haploview software 4.2 (Daly Lab, Cambridge, MA, USA) was used to analyze the linkage disequilibrium (LD) block and haplotypes between two TGF-β1 SNPs.42 The standardized disequilibrium coefficient (D’) > 0.7 and squared correlation coefficient (r²) > 1/3 were defined as statistical significance of the LD.44-46 We also utilized expectation-maximization-based haplotype frequency estimation with a permutation test to confirm whether a specific haplotype was associated with ISR.45,47 Logistic regression was performed to assess the association between the presence of a particular genotype and the angiographic outcome. Additive, dominant, and recessive genetic models of the minor allele were assumed in association analyses, and analyses were performed with or without adjustment for confounding risk factors. All variables that resulted with a P value < 0.30 in group comparison were entered into a multivariate model for ISR to test for independent effects.48-50 All statistical analyses were performed using the SPSS version 13.0 for Windows (SPSS Inc.). A two-tailed P value of less than 0.05 was considered statistically significant.
Results

Characteristics in the ISR and non-ISR groups: Angiographic follow-up was performed in all patients. Of the 419 patients with angiographic follow-up, 92 (22.0%) developed ISR. Table I shows the baseline characteristics of the ISR and non-ISR groups. Compared with the non-ISR group, patients with ISR had higher BMI (26.56 ± 3.22 versus 25.80 ± 3.18 kg/m², P = 0.044) and incidence of smoking (32.6% versus 19.0%, P = 0.022). There were no significant differences between the ISR and non-ISR groups in terms of other clinical variables. Table II illustrates the angiographic and procedural features of the CAD patients included in the study. The group of patients with ISR revealed a longer length of lesions (15.52 ± 7.01 versus 13.88 ± 6.75 mm, P = 0.042) and stent per lesion (19.45 ± 5.28 versus 17.98 ± 5.45 mm, P = 0.022) compared to the non-ISR group.

Association of TGF-β1 polymorphisms with ISR after BMS implantation: The genotype distributions and allele frequencies of rs1800469 (-509C/T) and rs1800470 (869T/C, Leu10Pro) polymorphisms in TGF-β1 gene are shown in Table III. The genotype distributions were in Hardy-Weinberg equilibrium for both patients with and without ISR after BMS implantation (all P > 0.05), minimizing the possibility of selection bias. The frequency of C allele of rs1800470 (869T/C, Leu10Pro) polymorphism was significantly higher in the ISR group than in the non-ISR group (P = 0.008). ISR was more frequent in patients with CC genotype (P = 0.011), CT genotype (P = 0.007), and C-dominant CC/CT genotype (P = 0.003) of 869T/C polymorphism versus patients with TT genotypes. No significant difference in the genotype and allele frequency distribution was observed in TGF-β1 rs1800469 (-509C/T) polymorphism between the subjects in the ISR and non-ISR groups (P > 0.05).

Of the two SNPs, only rs1800470 (869T/C, Leu10Pro) polymorphism was associated with an increased risk of ISR in additive and dominant models with ORs of 1.56 (95% CI: 1.13-2.17, P = 0.010) and 2.29 (95% CI: 1.31-4.02, P = 0.005), respectively. When additionally adjusted for age, male sex, body mass index (BMI), hypertension, diabetes mellitus, smoking, TC, HDL-C, LDL-C, lesion complexity according to ACC/AHA angiographic classification, reference diameter after stenting, minimal lumen diameter immediately after stenting and balloon-to-vessel ratio, the significance remained in the additive and dominant models with ORs of 1.88 (95% CI: 1.21-2.84, P = 0.008) and 2.52 (95% CI: 1.40-4.80, P = 0.005), respectively (Table IV).

LD and haplotype analysis: We used the LD test with D' and r² to assess the pair-wise linkage between two polymorphic sites of TGF-β1 gene. The overall pairwise LD constructed by the two TGF-β1 SNPs is strong (D': 0.813, r² = 0.457).

Four haplotypes in the TGF-β1 gene were identified to examine the relationship of these variations with ISR (Table V). The TT haplotype (-509T, +869T) occurred at a remarkably lower frequency in the ISR group compared with that in the non-ISR group (haplotype frequency: 0.0652 vs. 0.1391; OR = 0.43, 95% CI: 0.23-0.81, P = 0.004). The TC haplotype (-509T, +869C) showed an ex-
Table II. Details of Coronary Interventional in Patients with or without ISR

| Variable                                      | ISR (n = 92) | non-ISR (n = 327) | P    |
|-----------------------------------------------|--------------|-------------------|------|
| Artery treated                                |              |                   |      |
| LAD [n, (%)]                                  | 51 (55.4)    | 169 (51.7)        | 0.741|
| LCx [n, (%)]                                  | 14 (15.2)    | 60 (18.3)         |      |
| RCA [n, (%)]                                  | 27 (29.4)    | 98 (30.0)         |      |
| Number of diseased vessels                    |              |                   |      |
| Single vessel disease [n, (%)]                | 31 (33.7)    | 125 (38.2)        | 0.679|
| Double vessel disease [n, (%)]                | 34 (37.0)    | 118 (36.1)        |      |
| Triple vessel disease [n, (%)]                | 27 (29.3)    | 84 (25.7)         |      |
| ACC/AHA angiographic classification A [n, (%)] | 12 (13.0)    | 58 (17.7)         | 0.124|
| B1 [n, (%)]                                   | 38 (41.3)    | 157 (48.0)        |      |
| B2+C [n, (%)]                                 | 42 (45.7)    | 112 (34.3)        |      |
| Number of diseased vessels                    |              |                   |      |
| Lesion length (mm)                            | 15.52 ± 7.01 | 13.88 ± 6.75      | 0.042|
| Total length of stent per lesion (mm)         | 19.45 ± 5.28 | 17.98 ± 5.45      | 0.022|
| Diameter stenosis                             |              |                   |      |
| Before stenting (%)                           | 83.04 ± 10.68| 82.37 ± 11.01     | 0.604|
| After stenting (%)                            | 12.97 ± 4.53 | 13.26 ± 4.51      | 0.587|
| Reference diameter                            |              |                   |      |
| Before stenting (mm)                          | 2.66 ± 0.72  | 2.73 ± 0.71       | 0.405|
| After stenting (mm)                           | 3.34 ± 0.45  | 3.27 ± 0.49       | 0.219|
| Minimal lumen diameter                        |              |                   |      |
| Before stenting (mm)                          | 0.54 ± 0.35  | 0.57 ± 0.37       | 0.487|
| After stenting (mm)                           | 2.98 ± 0.54  | 2.91 ± 0.52       | 0.259|
| Acute gain (mm)                               | 2.38 ± 0.61  | 2.35 ± 0.57       | 0.661|
| Maximal balloon pressure (atm)                | 14.37 ± 1.89 | 14.27 ± 1.85      | 0.049|
| Balloon-to-vessel ratio                       | 1.06 ± 0.12  | 1.04 ± 0.11       | 0.132|

Values refer to the number of subjects (%) or the means (± SEM). ISR indicates in-stent restenosis; LAD, left anterior descending coronary artery; LCx, left circumflex coronary artery; RCA, right coronary artery; and AHA/ACC, American Heart Association/American College of Cardiology.

Table III. Genotype and Allele Frequency of the TGF-β1 Polymorphisms in Patients with and without ISR

| SNP              | Genotypes and Alleles | ISR (n = 92) | non-ISR (n = 327) | P    |
|------------------|-----------------------|--------------|-------------------|------|
| TGF-β1 C-509T    | CC                    | 20 (21.7)    | 78 (23.9)         |      |
|                  | CT                    | 49 (53.3)    | 173 (52.9)        |      |
|                  | TT                    | 23 (25.0)    | 76 (23.2)         | 0.890|
|                  | C/T                   | 89 (48.4)/95 (51.6) | 329 (50.3)/325 (49.7) | 0.643|
| TGF-β1 T + 869C  | CC                    | 20 (21.7)    | 52 (15.9)         |      |
|                  | TC                    | 54 (58.7)    | 158 (48.3)        |      |
|                  | TT                    | 18 (19.6)    | 117 (35.8)        | 0.012|
|                  | CC/TT                 | 20 (21.7)/18 (19.6) | 52 (15.9)/117 (35.8) | 0.011|
|                  | TC/TT                 | 54 (58.7)/18 (19.6) | 158 (48.3)/117 (35.8) | 0.007|
|                  | CC + TC/TT            | 74 (80.4)/18 (19.6) | 210 (64.2)/117 (35.8) | 0.003|
|                  | C, T                  | 94 (51.1)/90 (48.9) | 262 (40.1)/392 (59.9) | 0.008|

ISR indicates in-stent restenosis; and TGF-β1, transforming growth factor beta gene.

tremely higher frequency in the ISR group than in the non-ISR group (haplotype frequency: 0.45 versus 0.36; OR = 1.48, 95% CI: 1.06-2.06, P = 0.010).

Characteristics of the rs1800470 (869T/C, Leu10Pro) genotypes: Comparisons of the clinical and angiographic baseline characteristics among the 869T/C genotypes are listed in Table VI, VII. The observed distribution of the 869T/C genotypes among the 419 patients after coronary stenting was 72 (17.2%) for CC, 212 (50.6%) for TC, and 135 (32.2%) for TT, respectively. The genotype distribution was in Hardy-Weinberg equilibrium (χ² = 0.523, P = 0.469). There was no significant difference among the three different 869T/C genotypic groups in terms of baseline clinical characteristics, lesion variables before coronary intervention, procedural parameters, and types of stents.

The results of angiographic follow-up according to the genotypes of the TGF-β1 869T/C polymorphism...
Table IV. Association of TGF-β1 Polymorphisms with BMS-ISR after BMS Implantation

| SNP               | Additive model OR (95% CI) | Dominant model OR (95% CI) | Recessive model OR (95% CI) |
|-------------------|---------------------------|---------------------------|-----------------------------|
| TGF-β1 C-509T     |                           |                           |                             |
| Non-adjusted model| 1.08 (0.78-1.50)          | 1.13 (0.65-1.97)          | 1.10 (0.64-1.88)            |
| Adjusted model    | 1.01 (0.70-1.45)          | 1.19 (0.72-2.21)          | 1.14 (0.69-1.94)            |
| TGF-β1 T + 869C   |                           |                           |                             |
| Non-adjusted model| 1.56 (1.13-2.17)          | 2.29 (1.31-4.02)          | 1.47 (0.83-2.67)            |
| Adjusted model    | 1.88 (1.21-2.84)          | 2.52 (1.40-4.80)          | 1.28 (0.73-2.45)            |

TGF-β1 indicates transforming growth factor beta gene; ISR, in-stent restenosis; BMS, bare metal Stent; OR, odds ratio; and CI, confidence interval.

Table V. Haplotype Frequency of the TGF-β1 Gene Polymorphisms in ISR and Non-ISR Groups

| Haplotype | Overall n (%) | ISR n (%) | Non-ISR n (%) | OR 95% CI | P* |
|-----------|---------------|-----------|---------------|-----------|----|
| C-509     |               |           |               |           |    |
| C         | 379 (45.227)  | 78 (42.391) | 301 (46.024)  | 0.86      | 0.62-1.20 | 0.237 |
| T         | 317 (37.828)  | 83 (45.109) | 234 (35.780)  | 1.48      | 1.06-2.06 | 0.010 |
| T + C     | 103 (12.291)  | 12 (6.522)  | 91 (13.914)   | 0.43      | 0.23-0.81 | 0.004 |
| C + C     | 39 (4.653)    | 11 (5.978)  | 28 (4.281)    | 1.42      | 0.69-2.91 | 0.215 |

*There results were confirmed through permutation test. TGF-β1 indicates transforming growth factor beta gene; ISR, in-stent restenosis; OR, odds ratio; and CI, confidence interval.

Table VI. Baseline Characteristics According to the Genotypes of the TGF-β1 869T/C Polymorphism

|          | 869TT (n = 135) | 869TC (n = 212) | 869CC (n = 72) | P |
|----------|----------------|-----------------|----------------|---|
| Age (years) | 64.55 ± 8.96  | 64.37 ± 8.74  | 64.98 ± 8.90  | 0.880 |
| Male [n, %] | 105 (77.8)    | 154 (72.7)     | 54 (75.0)     | 0.561 |
| BMI (kg/m²) | 25.96 ± 3.15  | 26.04 ± 3.24  | 25.77 ± 3.09  | 0.824 |
| Hypertension [n, %] | 58 (43.0) | 101 (47.6) | 30 (41.7) | 0.564 |
| Diabetes mellitus [n, %] | 47 (34.8) | 85 (40.1) | 26 (36.1) | 0.585 |
| Current smoking [n, %] | 34 (25.2) | 44 (20.8) | 21 (29.2) | 0.305 |
| Family history of CAD [n, %] | 24 (17.8) | 46 (21.7) | 11 (15.2) | 0.421 |
| Left ventricular ejection fraction (%) | 59.48 ± 10.65 | 59.60 ± 10.37 | 59.43 ± 10.78 | 0.991 |
| Clinical presentation | Unstable disease [n, %] | 74 (54.8) | 104 (49.1) | 42 (58.3) | 0.320 |
| Stable disease [n, %] | 61 (45.2) | 108 (50.9) | 30 (41.7) | 0.320 |
| Biochemistry | Plasma glucose level (mmol/L) | 5.72 ± 1.98 | 5.89 ± 1.93 | 6.04 ± 2.15 | 0.520 |
| TG (mmol/L) | 2.09 ± 1.53 | 1.99 ± 1.49 | 2.03 ± 1.55 | 0.291 |
| TC (mmol/L) | 5.78 ± 1.31 | 5.65 ± 1.29 | 5.93 ± 1.35 | 0.266 |
| HDL-C (mmol/L) | 0.98 ± 0.33 | 1.06 ± 0.31 | 1.03 ± 0.29 | 0.110 |
| LDL-C (mmol/L) | 4.11 ± 1.01 | 4.02 ± 1.03 | 4.14 ± 0.98 | 0.587 |
| Baseline medication | Beta-blockers [n, %] | 98 (72.6) | 165 (77.8) | 53 (73.6) | 0.503 |
| Statins [n, %] | 118 (87.4) | 182 (85.8) | 59 (81.9) | 0.562 |

Values refer to the number of subjects (%) or the means ± (SEM). TGF-β1 indicates transforming growth factor beta gene; BMI, body mass index; CAD, coronary artery disease; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; and ACE, angiotensin-converting enzyme.

among the whole population are summarized in Table VIII. TGF-β1 869CC genotype is associated with a decrease in the mean minimal lumen diameter compared to the 869TC and 869TT genotypes at follow-up (2.08 ± 0.52 versus 1.91 ± 0.68 versus 1.75 ± 0.65 mm, P = 0.001); furthermore, the mean late luminal loss, diameter stenosis, and loss index were markedly higher in the 869 CC homozygotes (P = 0.008, P = 0.046, and P = 0.035, respectively). As expected, 869CC genotype was associated with higher rates of restenosis compared to 869TC and 869TT genotypes (27.8% versus 25.5% and 13.3%, respectively, P = 0.012).

Plasma TGF-β1 levels in the two TGF-β1 polymorphisms: As shown in Table IX, the plasma TGF-β1 level
was dramatically higher in the ISR group than in non-ISR group (23.34 ± 5.24 versus 22.07 ± 4.72 ng/mL, \( P = 0.027 \)). rs1800470 (869T/C, Leu10Pro) polymorphism was associated with plasma TGF-\( \beta \)1 level. The CC genotype
corresponded to the highest plasma TGF-β1 level while the TT genotype corresponded to the lowest in both ISR (CC versus TC versus TT = 26.52 ± 5.61 versus 23.18 ± 5.08 versus 20.31 ± 5.32 ng/mL, P = 0.002) and non-ISR groups (CC versus TC versus TT = 24.58 ± 4.98 versus 22.97 ± 4.65 versus 19.74 ± 4.71 ng/mL, P < 0.001). For rs1800469 (-509C/T) polymorphism, no significant difference was observed in plasma TGF-β1 levels between genotypes in both ISR and non-ISR groups.

Discussion

TGF-β1 is a pleiotropic cytokine that plays a certain role in all stages of the restenosis process: thrombogenesis, inflammation, and neointimal hyperplasia. Current studies have examined the relationship between TGF-β1 polymorphisms and ISR. However, none of the TGF-β1 genetic variations has ever been studied in relation to the risk of ISR after BMS implantation in the Chinese Han population. In the present study, we observed that C-domain CC/CT genotype frequency of TGF-β1 869T/C polymorphism T869C (rs1800470; Leu10/Pro10; T29C, codon10) is significantly higher in the ISR group after coronary implantation of BMS among Chinese Han patients. The plasma TGF-β1 level was higher in the ISR group than in non-ISR group. Furthermore, the plasma TGF-β1 level was remarkably higher in the CC/CT genotypes than in the TT genotypes in both ISR and non-ISR groups. These data suggested that the CC/CT genotypes of TGF-β1 869T/C polymorphism with higher plasma TGF-β1 might be associated with disease susceptibility to ISR after BMS implantation in the Chinese Han population. We did not find an association of TGF-β1 -509C/T polymorphism with ISR. However, the haplotype analysis indicated that the haplotype of TC (-509T, +869C) was associated with an increased risk for ISR, whereas the haplotype of TT (-509T, +869T) was associated with a decreased risk for ISR.

Our findings partially agreed with Osadnik, et al., who had reported that the C allele of the TGF-β1 869T/C polymorphism was associated with an increased risk of ISR only in dominant models (TC+CC versus TT) in the Polish population with stable CAD treated by PCI with BMS implantation. However, in our study, we found that the C allele of the TGF-β1 869T/C polymorphism was linked to an increased risk of ISR after coronary implantation of BMS in both additive (Per each C allele) and dominant (TC+CC versus TT) models in Chinese Han patients with CAD. In contrast to these results, Fragoso, et al. demonstrated that the T allele of TGF-β1 869T/C polymorphism (rs1800470, T29C) was associated with a higher risk of ISR after coronary stent placement in the Mestizo population. We propose that the discrepancy of these results could be due to the different ethnic backgrounds, different types of implanted stent, different inclusion and/or exclusion criteria for CAD patients, and different sample sizes in different studies. However, these results did not affect the main conclusion that TGF-β1 869 T/C polymorphism might be associated with the development of ISR after coronary stent placement.

As for TGF-β1 -509C/T polymorphism, our study could not demonstrate a significant relationship between the polymorphism and ISR after coronary BMS implantation in the Chinese Han population. In accordance with our result, Zeng et al. failed to confirm that the genetic variation in TGF-β1 is a risk factor for developing ISR after coronary DES implantation in the Chinese Han population. These negative results might be owing to a small sample size, which might not have sufficient statistical power to detect a slight effect of this variation. Furthermore, the studied population was limited to the Chinese Han population, which might not preclude the possibility that TGF-β1 -509C/T polymorphism exerts effects in ISR risk in a population-specific manner. Thus, we and Zeng et al. cannot generalize our findings to other populations; these findings still need to be verified in a larger sample and/or other populations.

Several lesion-, procedure- and patient-related factors have been identified to be associated with ISR. In our study, the ISR and the non-ISR groups did not differ significantly in the main demographic, clinical, angiographic and biochemical parameters, whereas BMI, incidence of smoking, length of lesions, and stent per lesion were remarkably higher or longer in the ISR group. However, we did not consider the importance of these differences in the context of the study as no difference was detected in TGF-β1 869T/C polymorphism among genotypes. Gene polymorphisms have been proved to be linked to cytokine production or function, which may potentially contribute to genetic predisposition to diseases. In our study, the ISR and the non-ISR groups did not differ significantly in the main demographic, clinical, angiographic and biochemical parameters, whereas BMI, incidence of smoking, length of lesions, and stent per lesion were remarkably higher or longer in the ISR group. However, we did not consider the importance of these differences in the context of the study as no difference was detected in TGF-β1 869T/C polymorphism among genotypes. Gene polymorphisms have been proved to be linked to cytokine production or function, which may potentially contribute to genetic predisposition to diseases. Finally, owing to the absence of functional proof, the present results have to be considered as hypothesis generating and further studies will be required to address this important issue.
Conclusion

In conclusion, this is the first study to demonstrate a phenotype/genotype interaction between a specific TGF-β1 gene genotype and susceptibility to coronary BMS-ISR in the Chinese Han population. This finding is in agreement with the functional consequences of the TGF-β1 C-509T polymorphism, and support the pivotal role of TGF-β1 chemokine in the development of ISR. Our data are preliminary, and additional studies in a larger number of samples and in other populations could help to define the true role of this marker as an independent risk factor for developing ISR after coronary implantation of BMS.

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Disclosures

Conflicts of interest: The authors do not have any potential conflicts of interest associated with this paper.

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