Transforming growth factor-β1 869T/C, but not interleukin-6 –174G/C, polymorphism associates with hypertension in rheumatoid arthritis

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OBJECTIVES. Part of the deleterious effects of systemic inflammation on the cardiovascular system of patients with RA may be exerted via increased propensity to hypertension. IL-6 and TGF-β1 are important regulators of the inflammatory response. In some, but not all, studies, IL6 –174G/C and TGFB1 869T/C gene polymorphisms have been associated with hypertension in the general population. The present study addressed their potential association with hypertension in RA patients.

METHODS. TGFB1 869T/C and IL6 –174G/C were identified in 400 RA patients and 422 local, non-RA controls using real-time PCR and melting curve analysis. Binary logistic and linear regression models were used to identify the independence of the effects of the polymorphisms on hypertension.

RESULTS. Genotypic and allelic frequencies of the two polymorphisms were similar in RA and controls. Within the RA group, there was no significant association between IL6 –174G/C and hypertension, but TGFB1 869T-allele carriers had significantly increased prevalence of hypertension compared with CC homozygotes (70.2 vs 55.2%; P = 0.023). This association remained significant after adjustment for other hypertension risk factors and medication (odds ratio = 1.96; 95% CI 1.02, 3.77; P = 0.044), and was more pronounced in patients with increased systemic inflammation.

CONCLUSIONS. This study suggests an association of TGFB1 869T/C, but not of IL6 –174G/C, with hypertension in RA patients. If this finding is confirmed in prospective studies, this polymorphism could be used as a screening tool for RA patients with higher risk of developing hypertension and lead to increased surveillance and earlier treatment.

KEY WORDS: Rheumatoid arthritis, Hypertension, Genetics, Gene, Transforming growth factor, Interleukin.

Introduction

Mounting evidence demonstrates an excess morbidity and mortality due to cardiovascular disease (CVD) in RA patients [1] that can be partially attributed to ongoing systemic inflammation [2]. Part of the deleterious effects of inflammation on the CVD profile of RA patients can be exerted via hypertension, a highly prevalent comorbidity in RA [3–5]. Even though the link between hypertension and inflammation is well established in prospective studies [6] the exact underlying mechanisms are yet to be elucidated.

IL-6 is one of the major cytokines driving inflammation in RA [7]. It is the main driver of the synthesis of acute-phase reactants by the liver and regulates inflammatory/immune pathways, as well as bone metabolism and endocrine functions [8]. Fishman et al. [9] first described a polymorphism (chromosome 7, position 22 539 885-hapmap [10], rs1800795) in the 5’ flanking region of the IL-6 gene promoter (IL6 –174G / C) that alters in vitro the transcriptional response to stimuli, such as lipopolysaccharide and IL-1 in HeLa cells; the IL6 –174C allele has been associated in vivo with increased levels of IL-6 [11, 12] and CRP [13] in the general population. A couple of studies [14, 15] indicate that high IL-6 levels correlate with increased blood pressure (BP) and may be an independent risk factor for hypertension.

TGF-β, in contrast, is an anti-inflammatory cytokine [16] which is considered to be an important (down)regulator of inflammation in RA [17]. The T-allele of a single nucleotide polymorphism (SNP) in the TGFB1 869T/C polymorphism (chromosome 19, position 46 550 761-hapmap [10], rs1982073) has been associated with decreased levels of TGFB1 and worse disease outcome in a study of 208 RA patients [18]. Polymorphisms in the TGFB1 gene have also been associated with increased prevalence of hypertension [19] and myocardial infarction [20] in the general population, thus rendering TGF-β1 a particularly interesting candidate gene for hypertension in RA patients. Of note, two recent genome-wide association studies (GWAS) [21, 22] did not detect a statistically significant association between either IL6 –174G/C or TGFB1 869T/C polymorphisms and hypertension. However, given the increased systemic inflammatory background of RA patients, such an association may be enhanced in these patients and thus easier to detect.

In the present study, we aimed to examine: (i) whether IL6 –174G/C or TGFB1 869T/C polymorphisms have different genotypic or allelic frequencies in RA patients compared with non-RA controls; (ii) whether, within the RA population, hypertension indices differ across the genotypes of the above SNPs and if so, whether this is independent of other established risk factors of hypertension in patients with RA [3].

Patients and methods

Study populations

The study was approved by the local research ethics committee and Research and Development Directorate and all participants gave written informed consent. Four hundred consecutive patients fulfilling 1987 ACR criteria for RA [23] were recruited from routine rheumatology outpatient clinics of the Dudley Group of Hospitals NHS Trust, UK, between 1 August 2004 and 31 July 2006. A previously collected anonymized DNA biobank from...
422 local general population controls without RA was used for comparison.

RA patients underwent a thorough clinical and contemporary laboratory evaluation including complete medical history and physical examination. Blood tests included full blood and differential blood count, ESR, full biochemistry, CRP, fasting lipids, glucose and insulin. The following information was systematically recorded: demographic characteristics, RA disease duration, seropositivity for RF, anti-cyclical citrullinated peptide (anti-CCP) antibody; current disease activity score (DAS-28) [24] and current physical function using the HAQ [25]. Hypertension risk factors (including BMI [26], smoking status and total cholesterol) were also investigated and recorded. RA patients were categorized into ‘current smokers’, ‘ex-smokers’ and ‘non-smokers’. BMI was calculated as the ratio of weight (kilograms) divided by height (centimetres) squared.

BP was the mean of three measurements taken at 5 min intervals on the right arm with the patient in a seated position after at least 5 min rest, using an appropriately sized cuff of the CRITICARE 506DXX machine (CRITicare Systems, Waukesha, USA). Hypertension was defined as having a BP of \( \geq 140 \) mmHg systolic (SBP) and/or \( \geq 90 \) mmHg diastolic (DBP); measured as per British Hypertension Society guidelines [27] and/or receiving any anti-hypertensive therapy (with indication hypertension). All medications and their exact indication were recorded, including anti-hypertensives, non-selective NSAIDs, specific cyclo-oxygenase-II inhibitors (coxibs) and DMARDs. Oral prednisolone dose was defined as low if \( < 7.5 \) mg/day, medium if \( 7.5 \leq \) dose \( < 30 \) mg/day and high if \( \geq 30 \) mg/day [28]. Renal function was assessed by glomerular filtration rate estimation using the six-variable Modification of Diet in Renal Disease (MDRD) equation [29].

IL-6 was measured using the Evidence® analyser (Randox Laboratories, Crumlin, UK), which is a fully automated protein biochip array system, in serum specimens stored at \(-70^\circ\)C, which were available only in 135 of the RA patients; post hoc analysis did not demonstrate any significant differences between this subpopulation and the total RA population assessed in this study (data not shown). The Evidence analyser was calibrated weekly and Randox tri-level cytokine controls were included in each run. Randomly selected samples were sent to Randox Laboratories for quality assurance.

DNA extraction

DNA was extracted from whole blood using the QuickGene-810 system [30]. Blood was collected in EDTA-containing tubes, placed in an isolation vessel and the red cells were lysed. The white blood cells were then captured in a filter matrix and lysed, so that the DNA was physically entrapped around the fibres. Isolated DNA was released from the matrix and eluted into a collection vessel in the enclosed environment of the QuickGene 810 system. Resulting DNA samples were stored at \(4^\circ\)C until analysis. Quality control with each batch of extractions was performed by running a blank tube through the whole process, omitting only the addition of blood. If any DNA was found in the blank, the whole DNA batch was rejected.

Genotyping

The TGFB1 869T/C and IL6 \(-174\)/G/C SNPs were identified using the Roche LightCycler® 2.0 System (Roche Diagnostics, Sussex, UK).

**IL6** \(-174\)/G/C. PCR was performed with a reaction volume of \(10\mu l\) containing \(1\mu l\) of DNA, \(0.5\mu M\) of each of the primers (forward) 5′-TTACCTTTGGCAAGACATGGCA-3′, reverse primer; 5′-ATGAGGCTCAGACATTCACAG-3′, \(2\mu l\) of reaction buffer (LightCycler Faststart DNA master plus hybridization probes 10× buffer; Roche Diagnostics), \(0.2\mu M\) of the sensor probe (specific for the G-allele), 5′-CTAAGCTGACATT TCCCCCTAGT-3′, labelled at the 3′ end with fluorescent, and \(0.3\mu M\) of anchor probe LC Red 640-5′-GTGCTTCG CAGTGCTAAAGGA-3′-ph, labelled at the 5′ end with Light Cycler Red 640 fluorophore.

The cycle conditions were: 1 cycle of 95°C for 10 min, 40 cycles of 95°C for 5 s, 57°C for 10 s and 72°C for 15 s each with a temperature transition rate of 20°C/s; melting curve analysis, 1 cycle of 95°C for 8 s and 50°C for 30 s, ramping to 72°C at 0.1°C/s continuous. The temperatures for the melting peaks were 56°C for the CC genotype and 63°C for the GG genotype.

**TGFB1 869T/C.** Reaction volumes were the same as for IL6 \(-174\)/G/C. Primers were (forward) 5′-AGACACCCCCG GTCCAAG-3′ and (reverse) 5′-CTGTGTGACAGCCGACAA C-3′. Anchor probe was LC Red 640-5′-CGTCTGAGCTGGCTA CTGTGCTGACCGCCT-3′-ph, and sensor probe (specific for the C-allele) 5′-CTGCTGCCTGCCTGTCG-3′-FL.

The cycle conditions were as follows: 1 cycle of 95°C for 10 min, programmed transition rate of 20°C/s; 40 cycles of 95°C for 8 s, 61°C for 20 s and 72°C for 15 s each with a temperature transition rate of 20°C/s; then melting curve analysis for 1 cycle of 95°C for 8 s and 40°C for 20 s, each with a transition temperature rate of 20°C/s, and then ramping to 75°C at 0.2°C/s continuous. The temperatures for the melting peaks were 53°C for the TT genotype and 62°C for the CC genotype.

Statistical analysis

Statistical analysis was performed using SPSS software, version 14 (SPSS, Chicago, IL, USA). Variables were tested for normality by the Kolmogorov–Smirnov test. Using chi-square (df = 1) test no differences of genotype frequencies from Hardy–Weinberg equilibrium were observed for the alleles tested individually in each group.

Means and standard deviations (s.d.s) or medians and 25th–75th percentile values (interquartile range) were calculated for continuous variables (normally and not normally distributed, respectively) and proportions for categorical variables. Group differences were tested using one-way analysis of variance (ANOVA) and Kruskal–Wallis test for parameters with normal and non-normal distributions, respectively. Chi-square test or Fisher’s exact test was used as appropriate, to test group differences of proportions. Hypertension prevalence was compared across the three genotypic groups of the two SNPs in all RA patients and hypertension indices (i.e. SBP, DBP) in those not on any anti-hypertensive treatment (to exclude the potential confounding effect of anti-hypertensive drugs).

The association between hypertension and TGFB1 T869C genotype was analysed with a web-based module [31] which uses likelihood ratio tests to compare nested models in an attempt to determine which of the six models (general, multiplicative, additive, dominant, recessive, no association) is most appropriate for any given data set. The likelihood ratio tests showed that the multiplicative, additive and recessive models were not significantly better than a model assuming no association (\(P = 0.08, P = 0.07\) and \(P = 0.32\), respectively). The dominant model (T dominant allele) was significantly better than the null model (\(P = 0.03\)) and was not significantly worse than the more general model (\(P = 0.82\)). For this reason a dominant model was assumed in subsequent analysis. Regarding IL6 \(-174\)/G/C, none of the above models was better than the null one (results not shown). However, since previous studies demonstrated an association of IL6 \(-174\)-allele carriers with hypertension [13] and higher IL-6 levels [9], we also performed comparisons for \(-174\)-allele carriers vs GG.

Post hoc power analysis based on a web-based module [32], revealed that our study had a 99% power at 0.05 significance level to detect an association of TGFB1 genotypes and hypertension in...
patients for RA [genotypic (OR) for hypertension: OR869TT/CC = 1.97 and an OR869CT/CC = 1.87].

Logistic regression analysis was used to investigate the effect of the IL6 –174G/C allele and TGFB1 869T/C allele on the presence of hypertension crudely and after adjustment for age, sex, BMI and medium dose prednisolone use, i.e. the previously described independent hypertension associations in this RA population [3]. To test for interaction of severe disease patterns (seropositivity for RF, anti-CCP) or moderate severe disease activity (DAS > 3.2) with TGFB1 869T/T allele on hypertension prevalence, three additional models were created by adding each of the following variables at a time, at the above logistic regression model: TGFB1 869C/T*RF-positivity (no/yes), TGFB1 869T/C*anti-CCP positivity (no/yes), TGFB1 869T/C* disease activity [no–mild (DAS < 3.2)/moderate–severe (DAS > 3.2)]. Linear regression was used to evaluate the independence of the effect of TGFB1 869T/T allele on SBP. Significance was defined as P < 0.05 throughout.

Results

IL6 –174G/C polymorphism was successfully genotyped in 383 RA patients [mean age ± s.d.: 61.13 ± 12.1; 280 (73.1%) female; 372 (97.1%) Caucasian] and all 422 controls [mean age ± s.d.: 50.13 ± 15.73; 255 (60.4%) female; 409 (97%) Caucasian]. TGFB1 869T/C SNP was successfully genotyped in 395 RA patients [mean age ± s.d.: 61.49 ± 12.03; 289 (73.2%) female; 383 (96.9%) Caucasian] and 401 of the controls [mean age ± s.d.: 50.41 ± 15.72; 242 (60.3%) female; 388 (96.8%) Caucasian].

There were no significant differences observed between RA patients and controls, before and after adjustment for age and sex, either for IL6 or for TGFB1 genotypes and alleles studied (Table 1).

No significant differences were observed in age or gender between the genotypic groups of the two SNPs. There were also no significant differences for most RA characteristics, including RF, disease duration, CRP, ESR, DAS-28 or HAQ, as well as usage of any DMARD, MTX, NSAID or Cox-2 inhibitor. Anti-CCP antibodies were present more frequently in IL6 –174GG and GC compared with CC patients (70.5% vs 58.5%; P = 0.049), and in TGFB1 869TT vs CT and CC genotypic groups (75.2% vs 64.5% and 64.7%, respectively; P = 0.024). There was a significant trend for increased usage of medium-dose oral prednisolone moving from TGFB1 869TT patients to CT and CC (22.4% vs 15.6% vs 12.1%, respectively; P = 0.04). IL6 levels were significantly elevated amongst IL6 –174C-allele carriers when compared with GG homozygotes [14.02 (3.21, 38.82) pg/ml vs 4.48 (2.25, 16.50) pg/ml; P = 0.028]. Furthermore, TGFB1 869T/T-allele carriers presented with raised IL-6 levels compared with CC homozygotes [10.35 (3.04, 34.72) pg/ml vs 3.8 (2.17, 7.60) pg/ml; P = 0.020]. Established risk factors for hypertension, including smoking, obesity (BMI), dyslipidaemia, insulin resistance (IR) and renal function (calculated as estimated glomerular filtration rate) were not significantly different among IL6 –174G/C or TGFB1 869T/C genotypic groups (Table 2).

No significant differences were observed in the prevalence of hypertension or levels of SBP or DBP across the three IL6 –174G/C genotypic groups (Table 3). When adjusting for hypertension risk factors in RA, no significant associations or interactions were identified.

However, for the TGFB1 869T/C SNP there was a significantly increased prevalence of hypertension among T-allele carriers compared with CC homozygotes (70.2 vs 55.2%; P = 0.023), which remains significant after Bonferroni correction for multiple testing (Padj = 0.023*2 = 0.046 < 0.05) (Table 3). When adjusting for hypertension risk factors in RA [age, gender, BMI and medium-dose prednisolone use (Model 1)], there was a 2-fold increase in the odds for hypertension amongst TGFB1 869T-allele carriers compared with CC homozygotes (OR = 1.95; 95% CI 1.02, 3.56; P = 0.045).

In order to assess possible gene–environment interactions we added individually the terms TGFB1 869T/C*RF positivity, TGFB1 869T/C* anti-CCP and TGFB1 869T/C* disease activity in Model 1. RF positivity enhanced the hypertensive effect of TGFB1 869T-allele (OR = 4.88; 95% CI 1.02, 23.39; P = 0.047), whereas a borderline non-significant interaction was observed with moderate/high disease activity (OR = 4.47; 95% CI 0.852, 23.5; P = 0.077). No interaction was observed with anti-CCP positivity (OR = 0.89; 95%CI 0.22, 3.6; P = 0.874).

The sub-analysis of patients not on anti-hypertensive drugs revealed that the increased hypertension prevalence among TGFB1 869T-allele carriers may be attributed to increased SBP (T-allele vs CC; 137.79 vs 130 mmHg; P = 0.032) (Table 3). After adjustment for age, gender, BMI and medium-dose prednisolone use, T-allele carriers had still a significantly increased SBP value (β = 6.97; P = 0.041).

Similar results were obtained for all of the above analyses when excluding the small number of non-Caucasians (data not shown).

Discussion

The present study shows that genotypic and allelic frequencies of IL6 –174G/C and TGFB1 869T/C are not significantly different between RA patients and local controls without RA. There was no obvious association between IL6 –174G/C SNP and hypertension within RA patients. However, TGFB1 869T/T-allele carriers had 2-fold increased odds for hypertension compared with CC homozygotes after adjustment for hypertension risk factors specific to the RA population (age, sex, BMI, prednisolone use) [3]. The BP-raising effect of this allele seemed to be enhanced in patients who were seropositive for RF or those with increased disease activity (DAS > 3.2), but not in those who had anti-CCP antibodies.

IL6 and TGFB-β play a vital role in the pathogenesis and course of RA [33, 34]. Therefore, genes regulating IL-6 or TGFB-β production, such as IL6 –174G/C and TGFB1 869T/C, may exhibit a different genotypic pattern in RA patients compared with normal controls. This did not appear to be the case in the present study. Our findings concur with those of another recent study [35] that found no association between TGFB1 869T/C polymorphism and RA in Caucasians, but not with a Japanese study [36], which reported significantly increased T-allele carrier status amongst RA patients. These discrepancies may be due to the different ethnicities or due to the limited power of the Japanese study. Our results concur with the findings of recent genome-wide analyses [21, 37].

The primary aim of the present study was to examine the potential association of hypertension indices with the above SNPs.
IL-6 and SNPs

**T869C**

- CC (n=178)
- TT (n=154)
- CT (n=180)

**P**

- **Age, yrs** 59 (47–66.2) 58 (45.1–66.78) 57.7 (43.5–67.9) 0.89 0.787 63.1 (53.8–69.6) 62.7 (55.7–69.5) 63.3 (55.6–69.8) 1 1
- **RA characteristics**
  - **n (%)** 118 (75.6) 131 (76.2) 44 (75.9) 0.994 0.822 95 (73.1) 135 (76.7) 51 (75) 0.768 0.317
- **Anti-CCP positive, (%)** 111 (78.2) 100 (64.5) 33 (64.7) 0.024 0.361 86 (70.5) 112 (75.2) 38 (58.5) 0.049 0.939
- **Disease duration, yrs** 12 (6–19) 8.5 (3.75–18) 10.5 (3.75–17.25) 0.049 0.654 10 (4–17) 11 (5–18.25) 9.5 (3.25–19) 0.444 0.444
- **CRP, mg/l** 9.5 (5–20) 8 (5–18) 8.5 (4–19.5) 0.43 0.823 8 (4–16) 10.5 (6–21) 6 (4–18) 0.071 0.071
- **HAQ** 1.63 (0.63–2.25) 1.38 (0.5–2) 1.63 (1.06–2.28) 0.113 0.136 1.5 (0.38–2.13) 1.63 (0.63–2.25) 1.5 (0.63–2.25) 0.306 0.306
- **RA treatment**
  - **DMARDs, (%)** 143 (88.8) 152 (86.4) 51 (87.9) 0.789 0.705 119 (88.1) 151 (85.8) 65 (90.3) 0.599 0.799
  - **NSAIDs or coxibs, (%)** 37 (23) 59 (33.5) 15 (25.9) 0.091 0.75 33 (24.4) 54 (30.7) 20 (27.8) 0.478 0.160

**HT risk factors**

- **Smoking, %:** never/ex/current 45.6/38.6/15.8 42.7/38/19.3 50/39.7/10.3 0.601 0.355 44.7/39.4/15.9 44.4/38/17.5 45.8/37.5/16.7 0.995 0.767
- **BMI, kg/m²** 27.59 28.03 28.03 4.79 28.03 5.62 0.849 0.649 27.89 4.57 28.02 5.17 0.434 0.38 5.49 1.17 5.36 1.06 0.434 0.38 5.49 1.17 5.36
- **TC, mmol/l** 5.35 1.08 5.48 1.16 5.57 1.06 0.434 0.38 5.49 1.17 5.36
- **HDL, mmol/l** 1.6 (1.3–1.85) 1.6 (1.2–1.9) 1.7 (1.3–1.9) 0.771 0.567 1.6 (1.3–1.9) 1.6 (1.3–1.9) 1.6 (1.3–2) 0.660 0.905
- **IR** 63 (41.7) 59 (33.9) 23 (41.1) 0.309 0.227 45 (35.4) 66 (38.6) 25 (35.7) 0.831 0.66
- **Full MDRD** 83.11 19.58 0.102 0.142 81.78 23.21 82.51 21.32 0.933 0.842

**Patients not on statin. *ANOVA, t-test (T-allele vs CC, GG vs C-allele carriers), trend. Results expressed as percentages, median (25th–75th percentile values) or mean ± s.d. as appropriate. HT: hypertension; TC: total cholesterol; TG: triglycerides.**

In RA, several studies have shown that high IL-6 levels correlate with increased BP and may be an independent risk factor for hypertension. In a recent analysis [39] from the Women's Health Study, IL-6 was weakly and CRP was strongly associated with an increased risk for hypertension, after adjustment for several other variables, particularly BMI. IL-6 has been reported to induce angiotensinogen expression [40] thus leading to higher concentrations of angiotensin II [41], which is a potent vasoconstrictor. A study in dialysis patients has associated IL-6 with increased 174C-allele (which associates with increased IL-6 levels) with DBP and left ventricular hypertrophy [42]. In another study of 2751 middle-aged men, C-allele was associated with significantly higher levels of SBP [13] but no associations between IL-6 and hypertension were identified in a sample of elderly Italians [43]. Recent GWAS aiming to identify candidate SNPs for hypertension in the general population failed to demonstrate a significant association of the above SNP with hypertension in the general population [21]. Similarly, in the present study, no associations were found with IL-6 and hypertension in RA patients. Given that the effect of IL-6 levels on future development of hypertension is fairly weak [39], a much larger sample of RA patients may be needed for an association of the above SNP with hypertension to be detected.

In contrast, the prevalence of hypertension was significantly higher among TGFBI 869T-allele carriers with RA. Furthermore, the association of 869T-allele carrier status with a significant increase in SBP may identify patients at increased risk for CVD [44]; this association of the above SNP with hypertension in RA patients may be a consequence of the increased inflammatory load in such patients, since no significant association between TGFBI and hypertension was identified in recent GWAS in the general population [21]. The background inflammation in RA patients enhances the genetic influence of SNPs regulating inflammatory pathways, such as TGFBI. The presence of raised serum IL-6 levels amongst RA patients who are TGFBI 869T-allele carriers compared with CC homozygotes lends further credence to this hypothesis. TGF-β1 is synthesized in a latent form composed of 390 amino acids, with the active protein consisting of two identical disulfide-linked polypeptide chains corresponding to the 112 carboxy-terminal residues of the precursor [45]. The TGFBI polymorphism is located in the signal peptide sequence of TGF-β1, which is thought to target the newly synthesized protein to the endoplasmic reticulum [46]. Yamada et al. [47, 48] have previously shown that the serum concentration of TGF-β1 increases according to the rank order of 869T/C genotypes TT < TC < CC. This association suggests that the 869T/C substitution may affect the function of the signalling peptide, most likely influencing intracellular trafficking or export efficiency of the protein. In the general population, two genetic association studies [49, 50] have shown an increased prevalence of hypertension in TGFBI (C) carriers (which associates with increased TGF-β1 levels) in Japanese and Chinese females, a finding that contradicts our results. In a recent report, TGF-β1 excess has been associated with hypertension and TGF-β1 deficiency with atherosclerosis and inflammation [51]. However, the link between inflammation and hypertension has been well established from prospective studies [6], and therefore it appears that there are two pathways through which TGF-β1 levels may affect the future development of hypertension. One is via increased levels in (CC homozygotes), which may induce the release of endothelin-1 from the vascular endothelial cells [52] and renin from juxtaglomerular cells [53]. The other is via reduced levels (in T-allele carriers), which may lead to increased systemic inflammation (as demonstrated in our data with increased serum IL-6 levels), arterial wall stiffness [54] and hypertension [55]; this appears to be the most likely possibility in RA patients. It is well established that TGF-β1 has multiple immunosuppressive properties, including inhibition of neutrophil...
and T-cell adhesion and reduction of neutrophil transmigration, probably due to inhibition of adhesion molecule expression by endothelial cells [56–58]. Furthermore, it inhibits macrophage activation [59] and T-cell differentiation and proliferation, either directly or through suppression of antigen-presenting cell function [60, 61]. A recent study has demonstrated low TGFβ1 levels as well as increased inflammatory activity and poor functional outcome in TGFβ1 869T-allele carriers, with increasing T-allele dose being associated with reduced survival in patients with RA [62]. All of these are in line with the observation from the present study that T-allele carriers appear to have more severe RA disease patterns, as suggested by the increased serum IL-6 levels, the increased prevalence of anti-CCP positivity and usage of corticosteroid therapy. It is also possible that the candidate alleles have an enhanced BP-raising effect in a specific environmental context, for example, when they associate with states of heightened systemic inflammation, such as RF seropositivity, which has been associated with more severe RA [63] and DAS > 3.2, which defines moderate to high disease activity [64]. In the present study, this appeared to be the case for RF seropositivity, with similar trends also observed for high disease activity, suggesting a potential interaction effect of systemic inflammation and TGFβ1 869T-allele on hypertension.

Limitations of the present study include its cross-sectional character that cannot prove causality or directionality of the associations found, and the lack of TGFβ-β plasma measurements in the RA population and BP measurements among the controls, which would have provided a better understanding of the link between these particular SNPs and hypertension in the RA population. Furthermore, dichotomizing BP with a cut-off of 140/90 mmHg may have led to misclassification of normotensive patients as hypertensive, thus weakening the association of TGFβ-β with hypertension. Future studies should address this point by comparing RA patients with longstanding established hypertension to age- and sex-matched controls with several BP measurements <140/90 mmHg. However, it should be pointed out that the association of TGFβ1 869T/C SNP with SBP as a continuous variable suggests a finding that is not liable to categorization bias (cut-off points for hypertension). Other strengths of the present study include the detailed and consistent clinical and laboratory characterization of all RA patients and the use of a local non-RA control group. Also, raised serum IL-6 levels are suggestive of an intermediate phenotype characterized by increased systemic inflammation, through which the TGFβ1 869T-allele may exert its deleterious, hypertensive effect.

In summary, this is the first study to show a strong association of an SNP implicated in the inflammatory cascade with hypertension in patients with RA. If these results are confirmed in future prospective studies, TGFβ1 869T/C polymorphism could be included in a DNA microchip designed to identify RA patients at an increased risk for future development of hypertension.

Table 3. Hypertension indices across the genotypic groups of the IL6 and TGFβ1 SNPs in all RA patients and in selected populations

<table>
<thead>
<tr>
<th></th>
<th>TT (n = 161)</th>
<th>CT (n = 175)</th>
<th>CC (n = 58)</th>
<th>P*</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension, n (%)</td>
<td>114 (70.6)</td>
<td>122 (69.7)</td>
<td>32 (55.2)</td>
<td>0.074</td>
<td>0.023</td>
</tr>
<tr>
<td>Hx of hypertension, n (%)</td>
<td>69 (42.9)</td>
<td>66 (37.5)</td>
<td>19 (32.8)</td>
<td>0.346</td>
<td>0.292</td>
</tr>
<tr>
<td>All RA patients (n = 400)</td>
<td>136.59 ± 19.5</td>
<td>138.71 ± 18.96</td>
<td>130 ± 14.89</td>
<td>0.075</td>
<td>0.032</td>
</tr>
<tr>
<td>SBP</td>
<td>78.18 ± 11.09</td>
<td>78.99 ± 9.76</td>
<td>75.97 ± 8.87</td>
<td>0.35</td>
<td>0.178</td>
</tr>
<tr>
<td>DBP</td>
<td>78.18 ± 11.09</td>
<td>78.99 ± 9.76</td>
<td>75.97 ± 8.87</td>
<td>0.35</td>
<td>0.178</td>
</tr>
</tbody>
</table>


Rheumatology key messages

- This study suggests an association of TGFβ1 869T/C, but not of IL6 –174C/G, with hypertension in RA patients.
- In RA, this polymorphism may be used in the future as part of a tool for screening patients at risk of developing hypertension.

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