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Transforming growth factor-β1 gene polymorphism rs1800471 and end-stage renal disease

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The mortality rate in the ultimate form of chronic kidney disease (CKD), that is, end-stage renal disease (ESRD), is high, the main cause being cardiovascular disease (CVD). While typical risk factors such as hypertension, diabetes mellitus, dyslipidemia, age, and smoking are prevalent in ESRD patients on dialysis, the high prevalence of CVD in these patients can only be partly explained. In the development of CKD and its complications, as in other multifactorial disorders, genetic factors interact with environmental factors [1]. Several studies have linked chronic inflammation and morbidity and mortality in maintenance-haemodialysis patients with ESRD. Pro-inflammatory cytokines play an important role, providing a link between accelerated atherogenesis, and excessive morbidity and mortality in ESRD [2].

Specific single-nucleotide polymorphisms (SNPs) in genes may directly or indirectly lead to variations in their activity and have significant impacts in different diseases [3–8]. There are several SNPs in the transforming growth factor-β1 (*TGF-β1*) gene located at 19q13, some of which affect TGF-β1 protein levels. At position 74 of the *TGF-β1* signal chain, the G/C transition causes the amino acid sequence to shift in codon 25 from arginine (CCG) to proline (CCG). TGF-β1 overexpression reduces the accumulation of macrophages and T cells and decreases the release of inflammatory mediators in renal disease. A typical pathological phenomenon characteristic of ESRD is progressive fibrosis of kidney tissue and subsequent sclerosis. TGF-β1 exerts its pro-fibrotic activity by inducing fibroblast proliferation, extracellular matrix synthesis and epithelial-to-mesenchymal transformation. Although studies have investigated the effects of the *TGF-β1* SNPs in the pathogenesis of different diseases few have investigated the influence of *TGF-β1* SNPs on ESRD, and the results are contradictory. This study tested the hypothesis of a link between *TGF-β1* and its product with SNPs in rs1800471 with ESRD.

The hypothesis was tested in 150 patients with ESRD (>1 year dialysis) and 150 healthy controls free of any renal disease. Common co-morbidities in the patients were high blood pressure, loss of appetite, and fatigue. Written informed consent was obtained from all participants. The study was approved by the high graduate committees of Hawler Medical University, Erbil, Kurdistan Region-Iraq.

Seven millilitres of blood samples were taken from all participants and put in two different tubes, some to obtain serum for the determination of serum TGF-β1 by ELISA (R&D Systems, Minneapolis, USA) and for routine renal indices and some (into EDTA) for DNA analysis by amplification refractory mutation system PCR. The genomic DNA was isolated and extracted from the venous blood of the studied samples according to standard salting out procedures. Primer sequences were a *TGF-β1* generic primer, 5′-GG CGAGCCGCAGCTTGGACA-3′, *TGF-β1* (G) allele primer 5′-TGGTGCTGACGCCTGGCCG-3′ and *TGF-β1* (C) allele primer 5′-TGGTGCTGACGCCTGGCCC-3′. The PCR reaction was carried out in the thermal cycler (PX2) with the following program. The samples were placed in a 20 µL reaction volume containing 40 ng genomic DNA, 1.5 mM dNTPs, 25 mM $MgCl_2$, 1 µL of 10 pmol of each primer and 0.4 units of Taq polymerase (Fermentas, Maryland, USA) in 1X reaction buffer. Cycling conditions were a primary 4-min denaturation at 95 °C, followed by 35 30-s cycles at 95°C, 60°C and 74°C. The final extension step was at 74°C for 6 min. The amplified products resulted 125 bp were analysed on a 2% agarose gel. To evaluate the biological function of rs1800471 polymorphism, *in silico* analysis was also conducted. Different bioinformatics tools were used to assess the effects of this polymorphism on serum TGF-β1.

All statistical analyses were done using both SNPAlyze software (ver.8.1, Dynacom, Japan) and SPSS (ver.22). Allele and genotype frequencies among control and case groups were compared and checked using Pearson χ^2 statistic. Analyses were also performed assuming recessive, codominant and dominant models of inheritance and crude odds ratio (OR)

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and their 95% confidence interval ranges. The significance level was p<0.05.

Cases and controls were matched for body mass index (23.9 ± 0.7 kg/m^2 vs. 24.2 ± 0.5, P = 0.23), age (47.3 ± 1.9 years vs. 44.2 ± 2.3, P = 0.11) and sex (89 males/61 females, 75 males/75 females, P = 0.1). Urea and electrolytes (U&Es) were Na^+ 133 ± 3.4 mmol/L vs. 138 + 2.5, K^+ 4.7 ± 0.3 mmol/L vs. 4.2 ± 0.5, Cl^- 108 ± 4 mmol/L vs. 100 ± 4) (all P > 0.05), creatinine 293 ± 53 µmol/L vs. 60 ± 10 and urea 14.4 ± 2.1 mmol/L vs. 3.3 ± 0.7 (both P < 0.001). Serum levels of TGF-β1 were increased in cases (410 ± 154 pg/mL) versus controls (280 ± 73, P = 0.01).

Allele and genotype distribution frequencies of rs1800471 in the case and control groups are shown in Table 1. The difference in genotype frequency was marked, with an eightfold increase in the CC genotype in cases. This translates into significant ORs for the disease in both co-dominant and recessive genotype models. However, there was no difference in G or C allele distribution between the groups. Genotype was a strong influence on serum TGF-β1 levels: GG 386 ± 136 vs. 285 ± 79 (P = 0.028), GC 398 ± 141 vs. 277 ± 65 (P = 0.001) and CC 354 ± 156 vs. 267 ± 81 (P = 0.031). Different *in silico* analysis predicted rs1800471 G > C variation to be damaging. PredictProtein and SNAP servers indicated that Arg25Pro substitution could have detrimental effects on the protein structure (Scores: 20; Expected accuracy: 63%) (Figure 1). The effects of rs1800471 G > C polymorphisms on *TGF-β1* mRNA secondary structure are indicated in Figure 1(b). Our results revealed that R25P polymorphism results in a severe and destructive change in the secondary structure of the mRNA (Figure 1).

Table 1. Allelic and genotypic frequency of R25P polymorphism in ESRD patients and controls.

Genotype /Allele	Cases (*N*, %)	Controls (*N*, %)	*P*-value
GG	51, 34%	42, 28%	<0.001
GC	75, 50%	105, 70%	
CC	24, 16%	3, 2%	
G	177, 59%	189, 63%	0.31
C	123, 41%	111, 37%	
	OR (95%CI_		
Dominant (GG v GC+CC)	0.75 (0.32-1.76)		0.51
Co-dominant (GC v GG+CC)	1.66 (1.24-2.23)		<0.001
Recessive (CC v GG+GC)	9.33 (1.12 -77.7)		<0.001
Allelic level C v G	1.18 (0.67-2.09)		0.31

Number of subjects with each genotype and number of alleles (frequency in %). OR: odds ratio; CI: confidence interval.

ESRD, with an inverse relationship between inflammation and kidney function, is an irreversible and progressive disorder. One potential reason for inter-individual differences in susceptibility to CKD and ESRD may be genetic variations of the anti-inflammatory cytokines involved in inflammation. TGF-β1 is a multifunctional cytokine that controls the growth, differentiation and development of cells and induces fibrosis in a variety of tissues, such as the kidney, heart and blood vessels. TGF-β1 was a well-studied profibrogenic cytokine expected to play a crucial role in the advancement of CKD, and suppressing this cytokine prohibits CKD progression. The induction of TGF-β1 in glomeruli has been shown to induce extracellular matrix accumulation, leading to progressive kidney disease.

The *TGF-β1* gene's rs1800471 polymorphism has been shown to be associated with higher rates of CKD. A study of 109 patients with non-diabetic CKD and 218 healthy controls showed that rs1800471 polymorphism in *TGF-β1* does not have an impact on the development and progression of non-diabetic CKD caused by primary glomerulopathy and chronic interstitial nephritis. They also observed significantly higher rates of serum TGF-β1 in patients compared with controls [9]. Likewise, another study indicated no association of CKD progression with rs1800471. Their study revealed that the C allele was significantly associated

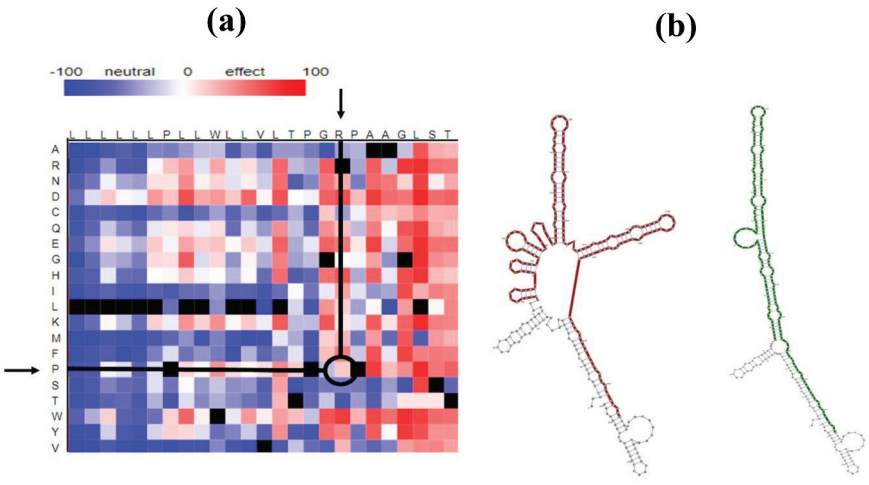

Figure 1. The results of the effect of Arg25Pro substitution on protein functions evaluated by SNAP and PredictProtein. SNAP predicted *TGF-β1* Arg25Pro mutation to be effect (a). The optimal secondary structure of global wild-type sequence depicted in green. The optimal secondary structure of global mutant sequence in red (b).

with higher risk of the disease occurrence in the dominant model of inheritance and women, opposite to male gender, was associated with higher risk of CKD development [10]. Ekrikpo and co-workers showed minor allele frequencies for *TGF-β1* polymorphisms (rs1800469, rs1800470 and rs1800471) and reported that the *TGF-β1* (rs1800470) polymorphism was associated with reduced risk of CKD [11].

Accumulating evidence indicates that elevated TGF-β1 levels have been related to renal disease progression. The rs1800471 has been involved in the development and progression of many other fibrotic processes, including pulmonary and hepatic fibrosis, in addition to renal fibrosis [12].

The *TGF-β1* rs1800471 has been investigated in relation to different diseases. Increased rates of oesophageal squamous cell carcinoma were observed among individuals with the GC and CC genotypes for rs1800471 [13]. In addition, the rs1800471 was correlated with tumour histological grades, TNM (tumour, node and metastasis) stage, and modified the serum levels of TGF-β1 among them. Significant differences in rs1800471 genotype distribution were also observed in a case–control study including 430 women with breast cancer and 498 cancer-free control [14]. Similar to this study, a significant association between rs1800471 and recurrent pregnancy loss was demonstrated [15].

The results of our study demonstrated that rs1800471 is linked to ESRD under recessive and co-dominant models. Additionally, R25P changes the secondary structure of *TGF-β1* mRNA and results in the removal of one mRNA arm and creation of two new arms. This work represents an advance in biomedical science because it shows a link between TGF-β1 rs1800471 variants and ESRD.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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