Original Article

Transforming growth factor-β receptor 2 gene polymorphisms are associated with end-stage renal disease

Hye-Jin Ki1, Se Yun Kim1, Sang Ho Lee1, Ju-Young Moon1, Kyung Hwan Jeong1, Tae Won Lee1, Chun Gyoo Ihm1, Su Kang Kim2, Joo-Ho Chung2, Sun Woo Kang3, Tae Hee Kim3, Yeong-Hoon Kim3, Yang Gyun Kim1, *n

1 Division of Nephrology, Department of Internal Medicine, Kyung Hee University School of Medicine, Seoul, Korea
2 Kohwang Medical Research Institute, School of Medicine, Kyung Hee University, Seoul, Korea
3 Division of Nephrology, School of Medicine, Inje University Paik Hospital, Seoul, Korea

Article history:
Received 22 January 2015
Received in revised form 14 May 2015
Accepted 15 May 2015
Available online 29 May 2015

Keywords:
End-stage renal disease
Fibrosis
Transforming growth factor-beta

ABSTRACT

Background: Transforming growth factor-beta (TGF-β) is a multifunctional cytokine involved in immune disorders, cancer, asthma, lung fibrosis, and chronic kidney disease, and its signal pathways are considered crucial mediators of a variety of cellular processes. In addition, several recent studies have reported that TGF-β receptor (TGF-βR) gene polymorphism is associated with chronic kidney disease. However, the association between end-stage renal disease (ESRD) and the TGF-β gene polymorphism has not been sufficiently investigated. In this study, we hypothesized that polymorphisms of the TGF-β ligands or their receptors may be related to ESRD.

Methods: We assessed the relationship between four single-nucleotide polymorphisms (SNPs) in the TGF-βR2 and TGF-β2 genes and ESRD, in 312 patients with ESRD and 258 controls.

Results: Compared with the control participants, the frequencies of the TGF-βR2 (rs764522 C) and TGF-βR2 (rs3087465 G) alleles were significantly higher in the patients with ESRD. Genotyping analysis demonstrated that two SNPs in TGF-βR2 of the four SNPs included in the study were significantly associated with ESRD in the codominant 1 (rs764522, odds ratio (OR) = 1.65; rs3087465, OR = 1.63), dominant (rs764522, OR = 1.63; rs3087465, OR = 1.57), and log-additive (rs764522, OR = 1.54; rs3087465, OR = 1.39) models after adjusting for age and sex.

Conclusion: We suggest that TGF-βR2 polymorphisms (rs764522 and rs3087465) increase the risk of development of ESRD.

Copyright © 2015. The Korean Society of Nephrology. Published by Elsevier. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Introduction

Recent economic prosperity and medical developments since 2010 have stabilized the rate of patients with end-stage renal disease (ESRD) [1]. However, the absolute numbers continue to increase, leading to growing morbidity and mortality rates directly related to ESRD. Diabetes, hypertension, and glomerular renal disease are documented as common
causes of ESRD worldwide. However, environmental and genetic factors that lead to progressing renal failure in patients with this disease are not well understood [2].

Many researchers have suggested that cytokines associated with immune response and fibrosis are related to the etiology and progression of renal disease. Tumor necrosis factor-alpha, interleukin-10 (IL-10), and the transforming growth factor-beta (TGF-β) complex regulate various inflammation reactions and activate progressive fibrogenesis in chronic renal injury [3]. TGF-β is a multifunctional protein known to be involved in various processes associated with the progression of chronic kidney disease (CKD), including tubular apoptosis and interstitial fibrosis. The three isoforms of TGF-β (TGF-β1, -β2, and -β3) regulate downstream signals and the combination of TGF-β receptor 1 (TGF-βR1) and TGF-β receptor 2 (TGF-βR2) [4,5]. There are few reports that TGF-β2 and TGF-βR2 of the three TGF-β isoforms are associated with CKD and renal fibrosis.

The hypothesis that common genetic variations predispose to common diseases has increased the interest in single-nucleotide polymorphisms (SNPs). A number of recent studies have suggested that SNPs in TGF-β and its receptor, and epigenetic factors are associated with the progression of CKD [4,6–8]. Additionally, several SNPs in TGF-βR2 have been reported to be associated with various diseases [9–13]. As mentioned in the previous context, genetic variants of inflammatory markers (IL-10, TGF-α, IL-17) have been studied in relation to the progression of CKD. However, the link between genetic polymorphism of TGF-β2 and TGF-βR2 and the development of ESRD has not been studied. Therefore, we assessed the association between ESRD and four SNPs located within the genes of TGF-β2 and TGF-βR2.

Methods

Participants

This study included 312 patients with ESRD (age > 18 years) and 258 control individuals enrolled at three hospitals in Korea (Kyung Hee University Medical Center, Kyung Hee University Hospital at Gangdong, and Inje University Busan Paik Hospital) from 2000 to 2009. All participants provided informed consent. All patients with ESRD caused by hypertension, glomerulonephritis, or other diseases (polycystic kidney, renal tuberculosis, hemolytic uremic syndrome, etc.) or with ESRD of unknown origin were recruited. Some patients were on hemodialysis, peritoneal dialysis, or both. The control participants were enrolled during regular checkups at the study hospitals from 2002 to 2005. All of them were healthy and had no past medical history of renal disease. The age and sex of the control participants were matched to those of the patients with ESRD (Table 1).

Blood collection, genotyping, and SNP selection

Peripheral blood samples were collected in EDTA tubes and genomic DNA was extracted from peripheral blood lymphocytes using a commercially available Qiagen DNA extraction kit (Qiagen, Tokyo, Japan). All participants were genotyped using direct sequencing, and genomic DNA was amplified using specific primers. The polymerase chain reaction products were then sequenced with an ABI PRISM 3730XL analyzer (PE Applied Biosystems, Foster City, CA, USA), and sequencing results were evaluated with the SeManII software (DNASTAR Inc., Madison, WI, USA). Gene SNPs were identified using the National Center for Biotechnology Information dbSNP database, version 131 (National Center for Biotechnology Information, Bethesda, MD, USA; http://www.ncbi.nlm.nih.gov/SNP) and the International HapMap Project database (http://www.Hapmap.org/index.html). SNPs with unidentified heterozygosity or low allele frequency (<5%) were expected for the Asian population. Finally, four SNPs located in two genes (rs764522, rs3087465, and rs2228048 in TGF-βR2 and rs7550232 in TGF-β2) were selected.

Table 1. Clinical characteristics of ESRD patients versus controls

| Variable                  | ESRD (n = 312) | Normal (n = 258) | P     |
|---------------------------|---------------|-----------------|-------|
| Age                       | 38.47 ± 11.64 | 37.91 ± 12.96   | 0.617 |
| Male:female               | 186:126       | 151:107         | 0.694 |
| Serum creatinine (mg/dL)  | 8.30 ± 2.69   |                 |       |
| Duration of dialysis (mo) | 25.53 ± 38.75 |                 |       |
| Causes of ESRD, n (%)     |               |                 |       |
| Glomerulonephritis        | 141 (45.19)   |                 |       |
| Hypertension              | 95 (30.45)    |                 |       |
| Others                    | 24 (7.69)     |                 |       |
| Unknown origin            | 52 (16.67)    |                 |       |
| Dialysis method, n (%)    |               |                 |       |
| Hemodialysis              | 181 (58.01)   |                 |       |
| Peritoneal dialysis       | 75 (24.04)    |                 |       |
| Both (HD + PD)            | 5 (1.60)      |                 |       |
| No dialysis               | 51 (16.35)    |                 |       |

Data are presented as mean ± SD. ESRD, end-stage renal disease; HD, hemodialysis; PD, peritoneal dialysis.

Statistical analysis

Compliance with the Hardy–Weinberg equilibrium was evaluated using the SNPstats software (http://bioinfo.iconcologia.net/index.php module = SNPStats) for all the SNPs. All continuous variables were expressed as mean ± standard deviation of the allelic frequencies and were assessed using the Chi-square test. For association tests, we calculated the odds ratios (ORs), 95% confidence intervals (CIs), and P values with SNPstats, Hap Analyzer version 1.0, and SNPAnalyzer (ISTECH, Inc., Goyang, Korea). The differences between the ESRD group and the control group in terms of genotype distribution were analyzed using a multiple logistic regression test to adjust for age and sex. We used multiple inheritance models, including codominant 1 (major allele homozygotes vs. heterozygotes), codominant 2 (major allele homozygotes vs. minor allele homozygotes), dominant (major allele homozygotes vs. minor allele homozygotes + heterozygotes), recessive (major allele homozygotes + heterozygotes vs. minor allele homozygotes), overdominant (heterozygotes vs. major allele homozygotes + minor allele homozygotes), and log-additive (major allele homozygotes vs. heterozygotes vs. minor allele homozygotes). The presence of a linkage disequilibrium block of polymorphisms was assessed using Haploview, version 4.1 (Broad Institute of MIT and Harvard, Cambridge, MA, USA; http://www.broadinstitute.org/haploview/haploview). We also used the online program AlibiBaba2.1 (Labmom.com; http://www.gene-regulation.com/pub/programs/alibaba2). Clinical characteristics were compared using the Chi-square test and Student unpaired t test. A value of P < 0.05 was considered to represent statistical significance.

Results

Baseline characteristics

A total of 312 patients with ESRD and 258 healthy controls were studied. The characteristics of the participants are shown in
The whole TGF-βR2 and TGF-β2 genes were genotyped to identify the four SNPs. The genotype distributions of all SNPs were in agreement with the Hardy–Weinberg equilibrium ($P < 0.05$). The allele frequencies of the genetic polymorphisms in the patients with ESRD and control individuals are shown in Table 2. Two SNPs out of four were associated with ESRD in an allele-specific manner. The major alleles were TGF-βR2 rs764522C (OR = 1.39, 95% CI = 1.06–2.20, $P = 0.023$) and TGF-βR2 rs3087465C (OR = 1.37, 95% CI = 1.03–1.89, $P = 0.033$). Logistic regression analysis showed significant differences in the allele frequencies of the TGF-βR2 genes between the patients with ESRD and controls. After adjustment for age and sex, the following two SNPs were still significantly associated with ESRD: TGF-βR2 rs764522 and TGF-βR2 rs3087465 (Table 3). Tests for association with individual SNPs showed significance for two SNPs in the codominant 1 (rs764522; OR = 1.65, 95% CI = 1.10–2.44, $P = 0.016$) and recessive (rs764522; OR = 2.37, 95% CI = 1.21–4.63, $P = 0.013$) models. In addition, we used Haploview (version 4.1) to determine whether a specific TGF-βR2 haplotype is associated with ESRD. All four SNPs were explored to estimate pair-wise linkage disequilibrium. The resulting single linkage disequilibrium block in TGF-βR2 rs764522 and rs3087465 was evaluated based on the
standard of Gabriel et al [14]. Three haplotypes, including CG, GA, and CA, in TGF-βR2 (rs764522 and rs3087465) were identified. Among these three haplotypes, the frequency of CG and GA was significantly increased in patients with ESRD compared with the control participants (Table 4).

Discussion

CKDs are irreversible degenerative disorders in which excessive fibrosis in the glomeruli and tubular epithelium eventually leads to ESRD. In simple terms, the renal inflammatory reaction and fibrogenesis are because of the failure of tissue wound healing and subsequent persistent damage. Therefore, TGF-β has been gradually recognized as an important mediator of these cellular processes [7,15,16].

TGF-β signal pathways, which are associated with cell proliferation, differentiation, and apoptosis; are considered important for the immune system [5]. TGF-β stimulates specific receptors on the cell surface membrane and causes both pathological and physiological events. The three isoforms of TGF-β (TGF-β1, -β2, and -β3) are part of the TGF-β superfamily, and they are expressed and activated in most of cell types. TGF-β1 and TGF-β3 are primarily responsible for early morphogenesis, and TGF-β2 is responsible for epithelial cell differentiation [7,17]. The TGF-β ligand binds to a dimeric receptor complex that consists of Type I and II serine/threonine kinase receptors. TGF-β2 ligand binding leads to the formation of TGF-β2R dimer and phosphorylation of threonine and serine residues then activates the TGF-β1R. The activated receptor recruits the downstream signaling regulator, SMAD (mothers against decapentaplegic homolog; R-SMAD), which initiates a signaling cascade that ultimately alters gene expression, leading to renal fibrosis [17]. Therefore, as described earlier, the TGF-β ligands contribute to all the processes that involve proliferation, apoptosis, hypertrophy, mesangial cell fibrosis, and tubulointerstitial fibrosis; resulting in glomerulosclerosis, tubular atrophy, and renal scarring that lead to ESRD [16,18,19]. An experimental study showed that inhibition of the TGF-β functions efficiently prevented chronic renal damage, whereas overexpression of TGF-β2 induced fibrotic changes of the renal matrix [20].

Although the significance of the TGF-β1 ligand in CKD pathology has been established by several studies [21,22], few investigators have examined the role of TGF-β2 and TGF-βR2 in ESRD. According to several studies, SNPs in TGF-β2 and TGF-βR2 have been reported to be associated with a number of other diseases. The following results were obtained for the four types of SNPs in different studies. One study showed that the TGF-β2 rs7550232 polymorphism played a key role in protecting against the development of high myopia [23]. The rs2228048 SNP in TGF-βR2 was shown to be associated with cerebral hemorrhage [24], whereas the rs764522 TGF-βR2 SNP was related to high susceptibility of essential hypertension [25].

As mentioned earlier, only a small number of researchers have directly examined the role of TGF-β2 and TGF-βR2 in renal progressive diseases. The rs2228048 SNP in TGF-βR2 was shown to be associated with acute rejection in kidney transplantation recipients [26]. It was also significantly associated with the prevalence of CKD [27]. However, the same SNP was found to have no significant relationships with ESRD in the present study; instead, rs764522 and rs3087465, two other SNPs in TGF-βR2, were shown to be associated with the progression of ESRD.

This article deals solely with TGF-βR2. However, the TGF-β pathway, as we have explained previously, begins with the binding of the TGF-β ligand to the TGF-βR2 to induce its activation, consequently causing the phosphorylation of TGF-β1R, and consequently leading to upregulation, which seems to be the culprit of renal disease progression. We suggest that the pathophysiology of ESRD is partly related to the TGF-βR2 signaling-dependent fibrosis pathway.

Our study has several limitations. The reliability of the relationship between SNP and ESRD may be affected by the small sample size. In addition, we only analyzed the relationship between the genetic polymorphism of TGF-β and the development of ESRD, whereas the biological role of TGF-β in ESRD was not investigated and the comparative concentration of relevant cytokines was not assessed. It is possible that one or more of the gene polymorphisms related with ESRD in the present study are in other adjacent genes that are actually responsible for the progression of this state. In future studies, we need to measure the quantitative cytokines and determine the degree of functional genetic effects.

In conclusion, this study suggests that genomic variations in TGF-βR2 are associated with the occurrence of ESRD. This result supports the notion that inflammation and renal fibrosis might play an essential role in increasing the risk of ESRD. Additional studies assessing the biological roles of TGF-βR2 genes will be required to confirm our conclusions.

Conflict of interest

The authors have no conflicts of interest to declare.

References

[1] Collins AJ, Foley RN, Chavers B, Gilbertson D, Herzog C, Ishani A, Johansen K, Kasiske BL, Kutner N, Liu J, St Peter W, Guo H, Hu Y, Kats A, Li S, Li S, Maloney J, Roberts T, Skeans M, Snyder J, Solid C, Thompson B, Weinhandl E, Xiong H, Yusuf A, Zaun D, Arko C, Chen SC, Daniels F, Ebben J, Frazier E, Johnson R, Sheets D, Wang X, Forrest B, Berrini D, Constantini E, Everson S, Eggers P, Agodoa L: US Renal Data System 2013 annual data report. Am J Kidney Dis 63 (Suppl 1):A7, 2014

[2] Ortiz A, Covic A, Fiser D, Fouque D, Goldsmith D, Kanbay M, Mallamaci F, Massy ZA, Rossignol P, Vanholder R, Wieck A, Zoccali C, London GM: Epidemiology, contributors to, and clinical trials of mortality risk in chronic kidney failure. Lancet 383:1831–1843, 2014

[3] Shlipak MG, Fried LF, Crump C, Bleyer AJ, Manolio TA, Tracy RP, Furberg CD, Psaty BM: Elevations of inflammatory and procoagulant biomarkers in elderly persons with renal insufficiency. Circulation 107:87–92, 2003

[4] Tampe B, Zeisberg M: Contribution of genetics and epigenetics to progression of kidney fibrosis. Nephrol Dial Transplant 29 (Suppl 4):S72–S79, 2014

[5] Garcia-Sanchez O, Lopez-Hernandez FJ, Lopez-Noova JM: An integrative view on the role of TGF-beta in the progressive tubular deletion associated with chronic kidney disease. Kidney Int 77:950–955, 2010

[6] Bottinger EP: Bitzer M: TGF-beta signaling in renal disease. J Am Soc Nephrol 13:2600–2610, 2002

[7] He J, Xu Y, Koya D, Kanasaki K: Role of the endothelial-to-mesenchymal transition in renal fibrosis of chronic kidney disease. Clin Exp Nephrol 17:488–497, 2013
Kim YG, Kim EY, Ihm CG, Lee TW, Lee SH, Jeong KH, Moon JY, Chung JH, Kim YH: Gene polymorphisms of interleukin-17 and interleukin-17 receptor are associated with end-stage kidney disease. *Am J Nephrol* 36:472–477, 2012

Border WA, Noble NA: Transforming growth factor beta in tissue fibrosis. *N Engl J Med* 318:1286–1292, 1994

Alansari A, Hajeer A, Teh LS, Bayat A, Mysercough A, Gul A, Inanc M, Ordi-Ros J, Ollier W: Transforming growth factor-12 polymorphism and systemic lupus erythematosus. *J Rheumatol* 29:1189–1191, 2002

Beisner J, Buck MB, Fritz P, Dippon J, Schwab M, Brauch H, Zugenmaier K, Knabbe C: A novel functional polymorphism in the transforming growth factor-beta2 gene promoter and tumor progression in breast cancer. *Cancer Res* 66:7554–7561, 2006

Jin G, Wang L, Chen W, Hu Z, Zhou Y, Tan Y, Wang J, Hua Z, Ding W, Shen J, Zhang Z, Wang X, Xu Y, Shen H: Variant alleles of TGFbeta and TGFbeta2 are associated with a decreased risk of gastric cancer in a Chinese population. *Int J Cancer* 69:213–217, 2006

Hatsushika K, Hirota T, Harada M, Sakashita M, Kanzaki M, Takano S, Doi S, Fujita K, Enomoto T, Ebisawa M, Yoshihara S, Sagara H, Fukuda T, Masuyama K, Katoh R, Matsumoto K, Saito H, Ogawa H, Tamari M, Nakao A: Transforming growth factor-beta2 polymorphisms are associated with childhood atopic asthma. *Clin Exp Allergy* 37:1165–1174, 2007

Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D: The structure of haplotype blocks in the human genome. *Science* 296:2225–2229, 2002

Liu Y: Renal fibrosis: new insights into the pathogenesis and therapeutics. *Kidney Int* 69:213–217, 2006

Kim SI, Choi ME: TGF-β-activated kinase-1: New insights into the mechanism of TGF-β signaling and kidney disease. *Kidney Res Clin Pract* 31:94–105, 2012

Liu Y: Epithelial to mesenchymal transition in renal fibrogenesis: pathologic significance, molecular mechanism, and therapeutic intervention. *J Am Soc Nephrol* 15:1–12, 2004

Lopez-Hernandez FJ, Lopez-Novoa JM: Role of TGF-beta in chronic kidney disease: an integration of tubular, glomerular and vascular effects. *Cell Tissue Res* 347:141–154, 2012

Reeves WB, Andreoli TE: Transforming growth factor beta contributes to progressive diabetic nephropathy. *Proc Natl Acad Sci USA* 97:7667–7669, 2000

Schnaper HW, Jandeska S, Runyan CE, Hubchak SC, Basu RK, Curley JF, Smith RD, Hayashida T: TGF-beta signal transduction in chronic kidney disease. *Front Biosci (Landmark Ed)* 14:2448–2465, 2009

Border WA, Okuda S, Languino LR, Sporn MB, Ruoslahti E: Suppression of experimental glomerulonephritis by antiserum against transforming growth factor beta 1. *Nature* 346:371–374, 1990

Kitamura M, Burton S, English J, Kawachi H, Fine LG: Transfer of a mutated gene encoding active transforming growth factor-beta 1 suppresses mitogenesis and IL-1 response in the glomerulus. *Kidney Int* 48:1747–1757, 1995

Lin HJ, Wan L, Tsai Y, Liu SC, Chen WC, Tsai SW, Tsai FJ: Sclera-related gene polymorphisms in high myopia. *Mol Vis* 15:1655–1663, 2009

Lim YH, Jeong YS, Kim SK, Kim DH, Yun DH, Yoo SD, Kim HS, Baik HH: Association between TGFBR2 gene polymorphism (rs2228048, Asn389Asn) and intracerebral hemorrhage in Korean population. *Immunol Invest* 40:569–580, 2011

Chen JF, Wang HR, Yang S, Zhao YP, Zhao XH, Chen YC, Du QL, Liu SJ, Chen C, Xu YC: An association study between transforming growth factor-beta1 receptor 2 gene polymorphisms and essential hypertension. *Zhonghua Yu Fang Yi Xue Za Zhi* 46:825–830, 2012

Kim YH, Kim TH, Kang SW, Kim HJ, Park SJ, Lee KH, Kim SK, Lee SH, Ihm CG, Lee TW, Moon JY, Yoon YC, Chung JH: Association between a TGFBR2 gene polymorphism (rs2228048, Asn389Asn) and acute rejection in Korean kidney transplantation recipients. *Immunol Invest* 42:285–295, 2013

Yoshida T, Kato K, Yokoi K, Oguri M, Watanabe S, Metoki N, Yoshida H, Satoh K, Aoyagi Y, Nishigaki Y, Nozawa Y, Yamada Y: Association of gene polymorphisms with chronic kidney disease in high- or low-risk subjects defined by conventional risk factors. *Int J Mol Med* 23:785–792, 2009