The rs1058587 of growth differentiation factor 15 increased the development and mortality risk of end-stage renal disease

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Abstract

Background

Growth differentiation factor 15 (GDF-15) is a member of transforming growth factor β (TGF-β).

Recent studies have shown that the serum level of GDF-15 increased and was associated with the occurrence and progression of the many diseases. However, the effect of GDF15 variants on end-stage renal disease (ESRD) is still unclear. The study was to explore the effect of GDF15 rs 1058587 on the susceptibility to ESRD and mortality risk of ESRD.

Methods

A total of 296 ESRD patients and 300 healthy individuals were enrolled. All the patients were followed-up for 3 years. The genotype of GDF-15 rs 1058587 was determined PCR amplification and Sanger sequencing. The baseline serum GDF-15 level was measured by enzyme-linked immunosorbent assay (ELISA).

Results

The frequency of G gene in ESRD patients was higher in ESRD patients than in controls (30.14% vs. 25.17%, P =0.027). The patients with CG/GG genotype had higher mortality risk in the dominant model (CG+GG vs. CC, OR= 1.708, 95% CI: 1.288 - 2.461, P = 1.00×10^-3 ). Multivariate analysis showed that age > 60 years, total cholesterol > 6 mmol/L, and genotype GG were independent risk factors for mortality in patients with ESRD (P <0.05), and survival analysis informed us that the three-year overall survival of patients with GG genotype was significantly lower than other genotype patients.

Conclusions

The G allele or CG/GG genotype of GDF15 was associated with higher susceptible risk of ESRD and the genotype GG was an independent risk factor for overall mortality in patients with ESRD.

1. Introduction

Chronic kidney disease has become a major public health concern worldwide recently[1–4]. In the United States, disability-adjusted life years (DALYs) due to CKD increased by 52.6% from 2002 to 2016, and death increased by 58.3% [5]. A recent study analyzed the change in burden of CKD
globally from 1990 to 2016 by using the Global Burden of Disease study data, the results showed that the incidence of CKD increased by 89%, prevalence increased by 87%, death due to CKD increased by 98% [3]. According to the data of a national epidemiological survey, it was estimated that the number of CKD patients in China was more than 100 million [6], and the number of patients receiving renal replacement therapy has increased rapidly in recent years [7]. There were about 553000 people received the hemodialysis and 55000 patients underwent peritoneal dialysis in 2015 [7]. It was estimated that the prevalence of CKD and end-stage renal disease (ESRD) would increase rapidly in the future [8, 9].

The mortality of ESRD patients was very high, and cardiovascular diseases (CVD) were the main cause of death of ESRD patients [4, 7]. How to predict and prevent CVD in CKD patients and decrease the mortality of CKD patients, was still a difficult problem in clinical practice. Genetic variation might play an important risk factor in the occurrence and progression of many diseases. In recent years, a new gene, Growth Differentiation Factor–15 (GDF15) has been proved to play an important role in the development and progression of many diseases, including CKD, cancer, diabetes, as well as CVD [10, 11]. The previous studies revealed that the variations or increased serum levels of GDF15 are closely related to obesity [12], cardiovascular disease [13], inflammation [14], multiple tumors [15], renal function deterioration and the development of ESRD [16, 17]. The variants of GDF15 were showed to be related to several diseases [18–22]. The G allele of nonsynonymous single nucleotide polymorphism (SNP) rs1058587 was reported to increase the risk of hypertension [20], mortality of prostate cancer [19], tumorigenesis, metastasis and prognosis in colorectal cancer [22]. Recently, Nair demonstrated that after adjusting for potential confounders, the circulating GDF–15 levels strongly correlated with intrarenal expression of GDF15 and significantly associated with increased risk of CKD progression in two independent cohorts [17]. Circulating GDF15 might be a marker for intrarenal GDF15-related signaling pathways associated with CKD and CKD progression [17]. But the relationship between the variants of GDF15 and the increased risk of mortality for patients with ESRD is still unclear. In this study, we investigated the role of SNP rs1058587 in the susceptibility to the ESRD patients and the risk for the mortality of ESRD patients.
2. Subjects And Methods
2.1 Subjects
This was a prospective cohort study. Total 296 patients with ESRD received maintenance hemodialysis (hemodialysis for more than 3 months) were included in this study and followed-up for 3 years. Those patients recently received renal transplantation or had malignant tumor were excluded. Three hundred healthy individuals were selected as the control group. This study was approved by the institutional review board at Sichuan Provincial People’s Hospital and informed consents were obtained before study.

The hospitalization history of all the patients in the group was recorded, and the age, gender as well as other baseline information of the patients were collected. The baseline serum and peripheral blood cells anticoagulated by EDTA were collected for further analysis.

h2>2.2 Determination of single nucleotide polymorphism of GDF15 gene and detection of serum GDF15 level
Whole blood DNA was extracted by using the TIANamp Blood Genomic DNA Extraction Kit (Tiangen Biotech Co., LTD, Beijing, China) and operated in accordance with the kit instructions. After DNA extraction, the genotypes of GDF–15 gene were determined by direct sequencing after polymerase chain reaction (PCR) amplification with the forward primer sequence: CTGCTGGCAGAATCTTCGTC and reverse primer sequence: GCACCATGGGATTGTAGCTG. PCR reaction system was 50 μL and Taq enzyme was purchased from Beijing TsingKe Biotech Company (Beijing, China). The PCR productions were sent to Beijing TsingKe Biotech Company for Sanger sequencing with the sequencing instrument ABI3730XL. The sequence was analyzed by Chromas software (Supplementary Fig. 1).

Total 45 serum samples from patients with different kinds of genotypes CC, CG and GG of GDF15 were selected, and the serum GDF15 concentration was determined by enzyme-linked immunosorbent assay (ELISA) (Raybio, USA) (Supplementary Fig. 2).

2.3 Statistical analyses
The genetic statistical analysis was performed using R software (version 3.5.1). Hardy-Weinberg equilibrium was conducted on the genetics package implemented in R. The patients were divided into three groups according to their genotypes. We analyzed the baseline data of each group by using SPSS 23 software version (SPSS, Inc, Chicago, IL). The baseline data were compared by chi-square
test, Fisher’s exact test, as well as one-way ANOVA test. The survival rates were analyzed by Kaplan-Meier survival analysis and COX regression analysis.

3. Results
3.1 The baseline characteristics of patients with maintenance hemodialysis and healthy controls

The characteristics of studied population (n = 596) at baseline are shown in Table 1. The mean age of controls and cases was 52.59 ± 17.19 and 52.68 ± 12.94 years, respectively. The ESRD group and the control group were comparable in age and gender. There were 73% of patients with hypertension and 10% with hyperlipidemia in the case group. The median duration of hemodialysis time was 48 months at baseline. There were significant differences in blood urea nitrogen, serum creatinine and albumin between the cases and controls (P < 0.001) (Table 1).

3.2 Distribution of alleles and genotypes of GDF15 rs1058587

The actual genotype frequencies of GDF15 rs1058587 observed in the control group was in accordance with the Hardy-Weinberg equilibrium (P = 0.068). The frequency of G allele of the ESRD group was significantly higher than that of healthy group (30.14% vs. 25.17%), it suggested that G allele was associated with higher risk of development of ESRD (Odds ratio [OR] 1.341, 95% confidence internal 1.041-1.728, P = 0.027). The comparison of GDF-15 gene polymorphism between the healthy and ESRD groups was conducted based on the assumption of a specific genetic model (recessive, dominant, or codominant). The CC, CG, and GG genotype frequencies were significantly different between the two groups in the codominant model (P = 1.04×10^{-3}); in the dominant model (CG+GG vs. CC), the OR value of the GG genotype in the ESRD group relative to the healthy group was 1.708 (95% CI: 1.288 - 2.461, P = 1.00×10^{-3}) ; in the recessive model (CC+CG vs. GG), the OR value was 0.613 (95% CI: 0.250 - 1.501, P = 0.391) (Table 2).

3.3 The baseline characteristics among the three groups with different genotypes of GDF15 rs1058587

We divided the ESRD patients into three groups based on their genotypes of GDF15 rs1058587. The results showed that there were no significant differences in age, gender, iPTH, ferritin, serum creatinine, urea nitrogen, serum calcium and serum phosphorus among these ESRD patients (P > 0.05). The differences in WBC and LDL-C were statistically significant (P < 0.05), as shown in Table
3.4 The genotypes of GDF15 rs 1058587 on the mortality of patients with MHD Kaplan-Meier survival analysis showed that the three-year overall survival rate of patients in the GG group was significantly lower than that in the CC group and the CG group (log-rank test $P = 0.027$) (Fig. 1). The difference of the total survival rate in the recessive model (CC+CG vs. GG) was statistically significant (log-rank test $P = 0.009$) (Supplementary Fig. 3A). There was no significant difference in the overall survival rate between the three groups in the dominant model (CG+GG vs. CC) (log-rank test $P = 0.72$) (Supplementary Fig. 3B).

Univariate analysis of the cox regression model showed that age $> 60$ years (HR = 3.77 [95%CI: 1.94 - 7.33] $P < 0.001$), total cholesterol $> 6$ mmol/L (HR = 9.48 [95%CI: 3.01 - 29.88] $P < 0.001$), GG genotype of GDF15 (HR = 3.08 [95%CI: 1.91 - 10.72] $P = 0.001$), and dominant models (HR = 1.57 [95%CI: 1.13 - 2.19] $P = 0.007$) were significantly associated with the overall survival in ESRD patients. There was no statistically significant association among gender, BMI, low-density lipoprotein, high-density lipoprotein, CRP, Ca, P, Hb, PTH, BUN, hypertension, and recessive models with prognosis ($P > 0.05$). Multivariate analysis showed that age $> 60$ years old (HR = 4.73 [95%CI: 2.18 - 10.26] $P < 0.001$), total cholesterol $> 6$ mmol/L (HR = 13.97 [95%CI: 3.87 - 50.47] $P < 0.001$), and GG genotype (HR = 5.89 [95%CI: 1.33 - 26.05] $P = 0.019$) were independent risk factors for mortality, but the dominant model was not an independent risk factor for prognosis in patients with ESRD ($P = 0.192$) (Fig. 2).

3.5 The serum GDF15 concentration among patients with different genotypes Total 45 patients with different genotypes were selected for the detection of serum GDF15 concentration by ELISA. The Kruskal-Wallis H-test showed that there was no significant difference in the concentration of GDF15 among the three subgroups of CC, CG and GG (Fig. 3). Pearson correlation analysis showed that the correlation coefficients between serum GDF-15 concentration and Cr, BMI and BUN were 0.18, 0.12 and 0.13 ($P > 0.05$), respectively. It was suggested that there was no significant correlation between serum GDF15 concentration and Cr, BMI and BUN in patients with ESRD (Supplementary Fig. 4).

4. Discussion
The GDF15, a member of the transforming growth factor β (TGF-β) superfamily, is located at 19p12.1-13.1[23], and also named as “placenta BMP”(PLAB), “macrophage inhibitory cytokine–10 (MIC–1), etc. [11]. GDF15 is expressed broadly in multiple tissues, and is increased in circulation and highly expressed in placenta during pregnancy [11, 23]. After tissue injury or in diverse disease states, including cancer, cardiovascular, and kidney diseases, GDF15 is robustly increased in circulation, and the increased serum GDF15 often correlates with poor prognosis [11]. The variants of GDF15 were demonstrated to be related to the prostate cancer, colorectal cancer, hypertension, but the relationship between these variants and the susceptibility to ESRD and mortality of ESRD patients was still unclear. In our study, we found that the frequency of G allele of the ESRD group was significantly higher than that of healthy group and the patients with CG or GG genotype had higher risk of ESRD in the dominant model. It suggested that the patients who had G allele or CG/GG genotype would be susceptible to ESRD.

GDF15 can inhibit the activation of macrophages, promote cell maturation, protect vascular endothelial cells and anti-apoptosis [24]. GDF–15 can be secreted rapidly under stress, which is closely related to the occurrence and development of various diseases. GDF15 levels were shown to increase significantly in the early stages of kidney injury in the mouse model [25]. Elevated plasma GDF15 levels in patients with type 2 diabetes are independent risk factors for impaired renal function [26]. Nair also demonstrated that the circulating GDF–15 levels strongly correlated with intrarenal expression of GDF15 and significantly associated with increased risk of CKD progression in two independent cohorts [17]. Lukaszyk found a significant negative correlation between GDF15 and eGFR ($P < 0.05$), and GDF–15 concentrations were significantly higher in elderly and anemia CKD patients [16]. Moreover, the higher the plasma GDF15 level, the higher the mortality rate in CKD patients [27]. In this study, we revealed the association between GDF15 rs1058587 and increased mortality risk in ESRD patients, but we didn’t find there was a linear correlation between the genotypes of rs1058587 and serum GDF15 level. This result was consistent with previous studies [28]. Although the previous genome-wide association study demonstrated that 9 SNPs (top SNP, rs888663) were significantly associated with blood GDF15 concentration, and explained 21.47% of its variance[29]. But the 9 SNPs
didn’t include the rs1058587. It suggested that rs1058587 could also affect the mortality risk in ESRD patients through other pathophysiological mechanisms.

Lots of studies demonstrated that the GDF15 can activate various signal pathways such as MAPK-ERK, PI3K-Akt and NF-κB [30, 31] after binding to its receptor GFRAL [12, 32, 33]. Increased circulating GDF15 level might be an essential role in obesity and metabolic disorders [33]. It could also explain why it was associated with an increased risk of cardiovascular disease in general population, and might be one of the reasons for a significant increased risk of cardiovascular mortality in ESRD patients.

In conclusion, our results demonstrated that the G allele or CG/GG genotype of GDF15 rs1058587 was associated with higher susceptible risk of ESRD and the genotype GG was an independent risk factor for overall mortality in patients with ESRD. It might be one of the reasons for the increased risk of cardiovascular mortality in ESRD patients. This relationship does not depend on the increased circulating GDF15 concentration. Further study is needed to explore its possible mechanism.

Declarations

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Disclosure statement
No potential conflict of interest was reported by the authors.

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Ethics statement
This study was approved by Institutional Review Boards of the Sichuan Academy of Medical Sciences and Sichuan Provincial People’s Hospital, and informed consent was obtained before study. Written informed consent for the study was obtained from all patients.

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Tables

Table 1. Baseline and demographic characteristics of the patients with maintenance hemodialysis and controls

| Variables                        | Controls (n=300) | Cases (n=296) | P value |
|----------------------------------|-----------------|---------------|---------|
| Age (years)                      | 52.59 ± 17.19   | 52.68 ± 12.94 | 0.943   |
| Age ≥ 60 (%)                     | 120(40)         | 96(33)        | 0.07    |
| Male (%)                         | 167(56)         | 153(52)       | 0.372   |
| Hypertension (%)                 | 0               | 217(73)       |         |
| Hyperlipidemia (%)               | 0               | 30(10)        |         |
| Duration of hemodialysis (months)| 0               | 48(16)        |         |
| Albumin (g/L)                    | 45.43 ± 2.55    | 40.35 ± 4.44  | 2e-16   |
| Hemoglobin (g/L)                 | NA              | 101.17 ± 20.59|         |
| Blood urea nitrogen (mmol/L)     | 4.82 ± 1.11     | 27.18 ± 8.28  | 2e-16   |
| Serum creatinine (μmol/L)        | 66.97 ± 13.6    | 1056.97 ± 337.97 | 2e-16 |

Table 2. The distribution of genotypes between control and case group

| rs1058587 Allele or genotypes | Controls (%) (n=300) | Cases (%) (n=296) | P Value | Odds ratio [95% CI] |
|------------------------------|---------------------|-------------------|---------|--------------------|
| Allele                       | C allele            | 449(74.83)        | 408(69.86) | 0.027   | 1.341 (1.041-1.728) |
|                             | G allele            | 151(25.17)        | 176(30.14) |         |                   | |
| Codominant                   | C/C                 | 162(54.0)         | 120(40.54) | 1.04×10⁻³|                     | |
|                             | C/G                 | 125(41.67)        | 168(56.76) |                     |                   | |
|                             | G/G                 | 13(4.33)          | 8(2.70)   |                     |                   | |
| Dominant                     | C/C                 | 162(54.0)         | 120(40.54) | 1.00×10⁻³| 1.780 (1.288-2.461) |
|                             | C/G                 | 138(46.0)         | 176(59.46) |                     |                   | |
|                             | G/G                 | 13(4.33)          | 8(2.70)   |                     |                   | |
| Recessive                    | C/C-C/G             | 287(95.67)        | 288(97.30) | 0.391   | 0.613 (0.250-1.501) |
|                             | G/G                 | 13(4.33)          | 8(2.70)   |                     |                   | |

Table 3. Comparisons of clinical characteristics among patients with different genotypes of
| Characteristics                  | GDF15 | C/G  | G/G  | P-value |
|---------------------------------|-------|------|------|---------|
| Male/female                     | 53/42 | 66/59| 7/2  | 0.363   |
| Age (year)                      | 52.77±12.49 | 52.21±12.6 | 52.78±13.21 | 0.827   |
| Hypertension (%)                | 77.9  | 71.2 | 55.6 | 0.147   |
| Serum creatinine (μmol/L)       | 1040.14±331.27 | 1054.05±351.76 | 1037.49±294.65 | 0.416   |
| Blood urea nitrogen (mmol/L)    | 26.11±7.93  | 27.08±8.04  | 30.17±4.81  | 0.201   |
| Serum calcium (mmol/L)          | 2.23±0.3    | 2.17±0.32   | 2.28±0.43   | 0.525   |
| Serum phosphate (mmol/L)        | 1.95±0.51   | 2.06±0.69   | 2.12±0.65   | 0.338   |
| Intact parathyroid hormone (pg/ml) | 445.41±407.61 | 563.33±594.94 | 531.56±750.46 | 0.362   |
| Ferritin (μg/L)                 | 238.5±191.36 | 303.8±254.24 | 250.35±229.4 | 0.360   |
| C reaction protein (mg/L)       | 6.69±13.5   | 5.51±7.86   | 9.45±14.03  | 0.455   |
| Albumin (g/L)                   | 40.64±4.62  | 40.75±4.48  | 38.8±5.19   | 0.135   |
| Alanine aminotransferase (U/L)  | 19.61±16.51 | 17.89±19.05 | 15±9.97    | 0.795   |
| Aspartate aminotransferase (U/L)| 19.31±14.34 | 17.23±8.74  | 18.66±10.28 | 0.448   |
| total cholesterol (mmol/L)      | 3.91±0.96   | 3.86±1.02   | 3.74±0.72   | 0.960   |
| Low Density Lipoprotein         | 2±0.64      | 2.11±0.81   | 3.31±4.79   | 0.001   |
| Cholesterol (mmol/L)            | 1.23±0.39   | 1.19±0.36   | 1.1±0.33    | 0.994   |
| High density lipoprotein        | 104.11±21.09 | 99.73±20.35 | 102±23.01   | 0.759   |
| Hemoglobin (g/L)                | 6.3±2.23    | 5.73±1.84   | 5.57±1.5    | 0.029   |
| white blood cell (10⁹/L)        | 1.27±0.47   | 1.21±0.47   | 0.92±0.32   | 0.170   |

**Figures**

![Kaplan-Meier curves](image)

**Figure 1**

Kaplan-Meier curves show the distinct outcome between GG, CC and CG groups in testing set (log-rank test P-value=0.027).
Figure 2

Forest plot of hazard ratios for OS assessed by the GDF-15 genotype and clinical characteristics in the case group. Error bars represent 95% confidence intervals.
The Kruskal-Wallis H-test showed that there was no significant difference in the concentration of GDF15 among the three subgroups of CC, CG and GG.

Supplementary Files
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