Correlation of TNF-α and IL-10 gene polymorphisms with primary nephrotic syndrome

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Abstract. The study explored the correlations of tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) gene polymorphisms with susceptibility and the condition of primary nephrotic syndrome. A total of 200 patients with primary nephrotic syndrome in Qilu hospital were collected as disease group, and 200 healthy people were selected as control group. Genomic deoxyribonucleic acids (DNAs) of nucleated cells in the peripheral blood were extracted to detect the gene polymorphisms of TNF-α (rs1799724 and rs1800629) and IL-10 (rs1800872 and rs141219090). Allele distributions at rs1799724 (P=0.003) and rs1800629 (P=0.011) of TNF-α gene and at rs1800872 (P=0.033) of IL-10 gene in disease group were different from those in control group. In the disease group, allele C frequency at rs1799724 and allele A frequency at rs1800629 of TNF-α gene and allele T frequency at rs1800872 of IL-10 gene were higher. There were differences between rs1799724 (P=0.007) and rs1800629 (P=0.002) of TNF-α gene. In addition, there was a difference in the frequency of the dominant model of TNF-α gene rs1800629 between disease group and control group (P=0.035), and the frequency of dominant model GG+GA was remarkably lower in the disease group. Additionally, TT genotype at rs1799724 of TNF-α gene was obviously related to the plasma TNF-α level (P<0.05), and the plasma TNF-α level was significantly increased in disease group. AA genotype at rs141219090 of IL-10 gene had a notable correlation with the plasma IL-10 level (P<0.05), and the plasma IL-10 level in disease group was markedly raised. Additionally, CT genotype at rs1799724 of TNF-α gene was related to the 24-h urine protein level (P=0.035), GG genotype at rs1800872 of IL-10 gene was associated with the plasma albumin level (P=0.031), and GG genotype at rs141219090 was related to the serum creatinine level (P=0.047). TNF-α and IL-10 gene polymorphisms are predominantly correlated with the susceptibility and the condition of primary nephrotic syndrome.

Introduction

Nephrotic syndrome is a disease with a complicated mechanism related to various causes, and it frequently occurs in women and children (1,2). The pathogenesis of nephrotic syndrome is related to immune factors (3) mainly involving glomeruli, thereby causing changes in its permeability and massive proteinuria, hypoproteinemia and other symptoms, but the specific mechanism is still unclear. Nephrotic syndrome can be divided into many pathological types according to the specific injury sites and causes. The pathogenesis of each type is relatively different, resulting in complicated and unpredictable occurrence and progression of the disease (4). Primary nephrotic syndrome is defined as nephrotic syndrome, excluding diabetes, systemic lupus erythematosus, adverse drug reactions and other secondary factors. The secretion imbalance of various cytokines such as interleukin-2 (IL-2) and IL-4 may be an important reason for the occurrence and progression of primary nephrotic syndrome (5).
Gene polymorphisms refer to the condition that alleles at the same locus of each biological individual may be different, which leads to different genotypes, thus affecting the release of some key substances or important metabolites in the body, and even changing the individual's susceptibility to certain diseases (6,7). The importance of gene polymorphisms in influencing the occurrence of diseases has been proved in various diseases. For example, rs2108622 of CYP4F2 gene is related to the susceptibility to hypertension (8), SEMA3 gene polymorphism is associated with Hirschsprung's disease (9), and PEAR1 gene polymorphism is associated with pulmonary embolism (10). Tumor necrosis factor-α (TNF-α) and IL-10 are important cytokines with a wide range of biological effects, which affect the immune environment. Their synthesis is affected by genetic factors (gene polymorphism). The gene polymorphisms of TNF-α and IL-10 may be related to the susceptibility and development of immune diseases, but their correlations with primary nephrotic syndrome have not been reported.

Polymorphism at rs1799724 and rs1800629 of the TNF-α gene promoter has been linked to increased TNF transcription, earlier study showing an increase of TNF-α synthesis and gene expression in primary nephrotic syndrome (11). IL-10 promoter variants rs1800872 and rs141219090 were associated with an increased risk of IgAN (12). Therefore, in this study, the polymorphisms and haplotypes of TNF-α gene rs1799724 and rs1800629 and IL-10 gene rs1800872 and rs141219090 in the peripheral blood nucleated cells of primary nephrotic syndrome patients before treatment and healthy people were analyzed, and the correlations of TNF-α and IL-10 gene polymorphisms with susceptibility and the condition of primary nephrotic syndrome were explored based on renal function-related clinical indexes of each research subject.

Patients and methods

General data. A total of 200 patients with primary nephrotic syndrome (disease group) and 200 healthy people (control group) from the Physical Examination Center in the hospital from 2017 to date were taken as the research subjects. The basic and clinical data of patients, including name, sex, admission ID, age, height, weight, family history and drug allergy history were collected in the disease and control group. Disease group included 53 males and 147 females with an average age of 46.12±3.41 years, while control group included 53 males and 147 females, with an average age of 43.92±4.73 years. There were no statistically significant differences in age, sex distribution and other general data between the disease and control group.

Diagnostic criteria for primary nephrotic syndrome in disease group: 24-h urine protein ≥3.5 g, hypoalbuminemia, plasma albumin ≤30 g/l, lipid metabolism disorder, hyperlipidemia, hematuria, red blood cells detected in urine sediment microscopy, excluding diabetes, systemic lupus erythematosus, adverse drug reactions and other secondary factors, and confirmed by percutaneous renal biopsy, including minimal change disease (n=69), membranous nephropathy (n=42), focal segmental glomerular sclerosis (n=39), mesangial proliferative glomerulonephritis (n=30), IgA nephropathy (n=9) and others (n=11). The study was approved by the Ethics Committee of Qilu Hospital of Shandong University (Jinan, China). Signed informed consents were obtained from all participants before the study.

Sample collection and processing. A total of ~6-8 ml of peripheral blood was collected from patients in disease group and control group by nurses on duty in our department. Then the blood was centrifuged at 4°C, 3,450 x g for 8 min within 2 h to separate the upper serum and the nucleated cells in the middle layer into new centrifuge tubes, respectively. Serum in the upper layer was stored in liquid nitrogen for detection, and the genomic deoxyribonucleic acids (DNAs) were extracted from the nucleated cells in the middle layer.

Genomic DNA extraction. Genomic DNAs in the peripheral blood in disease group and control group were extracted using Tiangen blood genomic extraction kit (Tiangen Biotech). All steps were strictly carried out according to the standard operation of the kit as follows: The centrifuge tube was added with an appropriate amount of protein K solution according to the sample volume, peripheral blood samples and buffer GE. Then mixed evenly using a vortex oscillator for 30 sec, and the samples were incubated at 65°C for 8 min. Then the samples were added with 2 ml of anhydrous ethanol, mixed well and transferred to the adsorption column. After that, 2 ml of the buffer was added to the adsorption column for centrifugation at 4°C, 3,850 x g for 1 min. Subsequently, 200 µl of elution buffer was added to the adsorption column, and the obtained solution was the genomic DNA of research subjects.

Polymerase chain reaction (PCR) amplification and analysis of TNF-α and IL-10 gene polymorphisms. The polymorphic regions at rs1799724 and rs1800629 of TNF-α gene and at rs1800872 and rs141219090 of IL-10 gene were amplified using PCR apparatus. The total PCR system was 25 µl, including primers (1 µl each), template DNAs (0.5 µl), high-fidelity thermostable Taq enzymes (12.5 µl) and dH2O (10 µl). PCR conditions: 96°C for 5 min, (96°C for 30 sec, 56°C for 45 sec and 72°C for 35 sec) x 45 cycles and 72°C for 5 min. Primers for polymorphic loci: TNF-α gene rs1799724: F: (5’-3’)‘CCCTCTCTTATACGCGCCCTCTG’ and R: (5’-3’)‘GAGGACCTGGGGAGTAGATGAG’, TNF-α gene rs1800629: F: (5’-3’)‘GAGGCCAAGCCCTGGATGATG’ and R: (5’-3’)‘CGGGCGGATTGATCTCAGC’, IL-10 gene rs1800872: F: (5’-3’)‘CAGGCCGATCTTCAGCAG’, and R: (5’-3’)‘CTGAACTTGGGTTGATCGG’ and R: (5’-3’)‘GGCTGGTCAGTCAAGTGGAG’, PCR products were sent to Zhejiang Biotechnology Co., Ltd. for sequencing via Sanger technology, and the genotype and allele frequencies were obtained by comparing the sequence with reference sequence using Seqman software in DNAStar software. TNF-α and IL-10 gene polymorphisms in disease group and control group were obtained.

Detection of plasma TNF-α and IL-10. Plasma TNF-α and IL-10 levels in the peripheral blood of patients in disease group and control group were detected by enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Inc.). Plasma TNF-α and IL-10 levels were detected using commercial ELISA kit (R&D Systems, Inc.). The experimental procedures
were strictly carried out in accordance with the kit instructions, and standard wells were set, with 3 replicates for each patient. After the experiment, the absorbance value in the 96-well plate was read using a microplate reader (Bio-Rad), and the plasma TNF-α and IL-10 concentrations of each research subject were converted according to the standard curve.

Detection of clinical indicators. Clinical indicators, 24-h urine protein, plasma albumin and creatinine of patients in disease group and control group were detected in the Clinical Laboratory of the hospital. 24-h urine protein was detected in the clinical examination room within 24 h using the urine of subjects, while plasma albumin and creatinine were detected in the biochemical room using plasma.

Statistical analysis. Statistical Product and Service Solutions (SPSS) 23.0 (IBM Corp.) was adopted for statistical analysis. Comparisons of count data were conducted by the χ² test, and Hardy-Weinberg equilibrium analysis was carried out. The haplotype analysis was conducted on line through SHEsis website. Comparisons of quantitative data among multiple groups was performed using one-way analysis of variance test followed by Bonferroni as the post hoc test, and P<0.05 suggested that the difference was statistically significant.

Results

Allele distributions at rs1799724 and rs1800629 of TNF-α gene and at rs1800872 and rs141219090 of IL-10 gene. Allele distributions at rs1799724 and rs1800629 of TNF-α gene and at rs1800872 and rs141219090 of IL-10 gene were shown in Table I. It was found that allele distributions at rs1799724 (P=0.003) and rs1800629 (P=0.011) of TNF-α gene and at rs1800872 (P=0.033) of IL-10 gene in disease group were different from those in the control group. Among them, in disease group, allele C frequency at rs1799724 and allele A frequency at rs1800629 of TNF-α gene and allele T frequency at rs1800872 of IL-10 gene were higher.

Genotype distributions at rs1799724 and rs1800629 of TNF-α gene and at rs1800872 and rs141219090 of IL-10 gene. The genotype distributions at rs1799724 and rs1800629 of TNF-α gene and at rs1800872 and rs141219090 of IL-10 gene are shown in Table II. The genotype distributions at rs1800872

| Gene Locus | Allele | Control group | Disease group | OR   | 95% CI   | χ² value | P-value |
|------------|--------|---------------|---------------|------|----------|----------|---------|
| TNF-α      | rs1799724 | C 184 (0.460) | 226 (0.565)  | 1.52 | 1.15-2.01 | 8.82     | 0.003   |
|            |         | T 216 (0.540) | 174 (0.435)  | 1.43 | 1.08-1.89 | 6.51     | 0.011   |
|            | rs1800629 | G 206 (0.515) | 170 (0.425)  | 1.43 | 1.08-1.89 | 6.51     | 0.011   |
|            |         | A 194 (0.485) | 230 (0.575)  | 1.43 | 1.08-1.89 | 6.51     | 0.011   |
| IL-10      | rs1800872 | G 203 (0.507) | 173 (0.432)  | 0.73 | 0.55-0.97 | 4.51     | 0.033   |
|            |         | T 197 (0.492) | 227 (0.568)  | 0.73 | 0.55-0.97 | 4.51     | 0.033   |
|            | rs141219090 | G 201 (0.502) | 212 (0.530)  | 0.89 | 0.67-1.18 | 0.61     | 0.431   |
|            |         | A 199 (0.497) | 188 (0.470)  | 0.89 | 0.67-1.18 | 0.61     | 0.431   |

| Gene Locus | Genotype | Control group | Disease group | OR   | 95% CI   | χ² value | P-value |
|------------|----------|---------------|---------------|------|----------|----------|---------|
| TNF-α      | rs1799724 | CC 41 (0.205) | 68 (0.340)  | 1.24 | 1.09-1.56 | 9.71     | 0.007   |
|            | CT 102 (0.510) | 90 (0.450)  | 0.96 | 0.81-1.13 | 1.68     | 0.194   |
|            | TT 57 (0.285) | 42 (0.210)  | 0.96 | 0.81-1.13 | 1.68     | 0.194   |
|            | rs1800629 | GG 51 (0.255) | 46 (0.230)  | 1.51 | 1.25-1.89 | 11.91    | 0.002   |
|            | GA 104 (0.520) | 78 (0.390)  | 0.96 | 0.81-1.13 | 1.68     | 0.194   |
|            | AA 45 (0.225) | 76 (0.380)  | 0.96 | 0.81-1.13 | 1.68     | 0.194   |
| IL-10      | rs1800872 | GG 52 (0.260) | 38 (0.190)  | 0.82 | 0.65-0.97 | 4.44     | 0.108   |
|            | GT 99 (0.495) | 97 (0.485)  | 0.82 | 0.65-0.97 | 4.44     | 0.108   |
|            | TT 49 (0.245) | 65 (0.325)  | 0.82 | 0.65-0.97 | 4.44     | 0.108   |
|            | rs141219090 | GG 55 (0.275) | 54 (0.270)  | 0.67 | 0.45-0.77 | 2.37     | 0.304   |
|            | GA 91 (0.455) | 104 (0.520) | 0.67 | 0.45-0.77 | 2.37     | 0.304   |
|            | AA 54 (0.270) | 42 (0.210)  | 0.67 | 0.45-0.77 | 2.37     | 0.304   |

TNF-α, tumor necrosis factor-α; IL-10, interleukin-10; OR, odds ratio; CI, confidence interval.

Table I. Allele distributions at rs1799724 and rs1800629 of TNF-α gene and at rs1800872 and rs141219090 of IL-10 gene.

Table II. Genotype distributions at rs1799724 and rs1800629 of TNF-α gene and at rs1800872 and rs141219090 of IL-10 gene.
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(P=0.108) and rs141219090 (P=0.304) of IL-10 gene in disease group were not different from those in control group, but there was a difference between rs1799724 (P=0.007) and rs1800629 (P=0.002) of TNF-α gene. Besides, the frequencies of CC genotype at rs1799724 and AA genotype at rs1800629 of TNF-α gene were higher in the disease group.

Analysis of TNF-α gene polymorphisms (rs1799724 and rs1800629) and IL-10 gene polymorphisms (rs1800872 and rs141219090). Results of TNF-α gene polymorphisms (rs1799724 and rs1800629) and IL-10 gene polymorphisms (rs1800872 and rs141219090) are shown in Table III. The frequency of the recessive model of TNF-α gene rs1799724 in disease group was different from that in control group (P=0.043), and the frequency of recessive model CT+TT was obviously lower. In addition, there was a difference in the frequency of dominant model of TNF-α gene rs1800629 between disease group and control group (P=0.035), and the frequency of dominant model GG+GA was remarkably lower in the disease group.

Haplotype analysis of TNF-α gene rs1799724 and rs1800629 and IL-10 gene rs1800872 and rs141219090. The haplotype analysis results of TNF-α gene rs1799724 and rs1800629 and IL-10 gene rs1800872 and rs141219090 are displayed in Table IV. The frequencies of CA haplotype at rs1799724 and rs1800629 of TNF-α gene in disease group were markedly higher than those in the control group (P<0.001, OR=1.972). Furthermore, disease group had notably lower frequencies of GG haplotype (P=0.041, OR=0.719) and markedly

### Table III. Polymorphism analysis and model construction.

| Model          | Gene   | Type          | Genotype | Control group | Disease group | χ² value | P-value |
|----------------|--------|---------------|----------|---------------|---------------|----------|---------|
| Dominant model | TNF-α  | rs1799724     | CC+CT    | 143 (0.715)   | 158 (0.790)   | 0.96     | 0.845   |
|                |        |               | TT       | 57 (0.285)    | 42 (0.210)    |          |         |
|                |        |               | GG+GA    | 155 (0.775)   | 124 (0.620)   | 6.63     | 0.035   |
|                |        |               | AA       | 45 (0.225)    | 76 (0.380)    |          |         |
|                | IL-10  | rs1800872     | GG+GT    | 151 (0.755)   | 135 (0.675)   | 2.65     | 0.531   |
|                |        |               | TT       | 49 (0.245)    | 65 (0.325)    |          |         |
|                |        |               | GG+GA    | 146 (0.730)   | 158 (0.790)   | 3.15     | 0.241   |
|                |        |               | AA       | 54 (0.270)    | 42 (0.210)    |          |         |
| Recessive model| TNF-α  | rs1799724     | CC       | 41 (0.205)    | 68 (0.340)    | 5.35     | 0.043   |
|                |        |               | CT+TT    | 159 (0.795)   | 132 (0.660)   |          |         |
|                |        |               | GG       | 51 (0.255)    | 46 (0.230)    | 3.86     | 0.135   |
|                |        |               | GA+AA    | 149 (0.745)   | 154 (0.770)   |          |         |
|                | IL-10  | rs1800872     | GG       | 52 (0.260)    | 38 (0.190)    | 1.39     | 0.736   |
|                |        |               | GT+TT    | 148 (0.740)   | 162 (0.810)   |          |         |
|                |        |               | GG       | 55 (0.275)    | 54 (0.270)    | 2.84     | 0.463   |
|                |        |               | GA+AA    | 145 (0.725)   | 146 (0.730)   |          |         |
| Heterozygous model| TNF-α  | rs1799724     | CC       | 41 (0.205)    | 68 (0.340)    | 3.98     | 0.093   |
|                |        |               | CT       | 102 (0.510)   | 90 (0.450)    |          |         |
|                |        |               | GG       | 51 (0.255)    | 46 (0.230)    | 1.83     | 0.673   |
|                |        |               | GA       | 104 (0.520)   | 78 (0.390)    |          |         |
|                | IL-10  | rs1800872     | GG       | 52 (0.260)    | 38 (0.190)    | 3.64     | 0.103   |
|                |        |               | GT       | 99 (0.495)    | 97 (0.485)    |          |         |
|                |        |               | GG       | 55 (0.275)    | 54 (0.270)    | 2.71     | 0.323   |
|                |        |               | GA       | 91 (0.455)    | 104 (0.520)   |          |         |
| Homozygous model| TNF-α  | rs1799724     | CC       | 41 (0.205)    | 68 (0.340)    | 1.45     | 0.548   |
|                |        |               | TT       | 57 (0.285)    | 42 (0.210)    |          |         |
|                |        |               | GG       | 51 (0.255)    | 46 (0.230)    | 3.42     | 0.231   |
|                |        |               | AA       | 45 (0.225)    | 76 (0.380)    |          |         |
|                | IL-10  | rs1800872     | GG       | 52 (0.260)    | 38 (0.190)    | 2.04     | 0.575   |
|                |        |               | TT       | 49 (0.245)    | 65 (0.325)    |          |         |
|                |        |               | GG       | 55 (0.275)    | 54 (0.270)    | 2.21     | 0.513   |
|                |        |               | AA       | 54 (0.270)    | 42 (0.210)    |          |         |

When a dominant effect was assumed, genotype wild (W)/W was coded as 0 and W/variant (V) and V/V combined were coded as 1. Scores of 0 for W/W and W/V combined and of 1 for V/V were used in a model that assumed a recessive effect. When a heterozygous effect was assumed, VV was codes as 1. While W/V was coded as 1 in a model that assumed a homozygous effect. TNF-α, tumor necrosis factor-α; IL-10, interleukin-10.
higher frequencies of TG haplotype (P=0.003, OR=1.624) at rs1800872 and rs141219090 of IL-10 gene than the control group.

Correlation analysis of genotypes at rs1799724 and rs1800629 of TNF-α gene and at rs1800872 and rs141219090 of IL-10 gene with plasma TNF-α and IL-10. The results of the correlation analysis of genotypes at rs1799724 and rs1800629 of TNF-α gene and at rs1800872 and rs141219090 of IL-10 gene with plasma TNF-α and IL-10 are shown in Figs. 1-4. It was discovered that TT genotype at rs1799724 of TNF-α gene was obviously related to the plasma TNF-α level (P<0.05), and the plasma TNF-α level was evidently increased in the disease group. AA genotype at rs141219090 of IL-10 gene had a notable correlation with the plasma IL-10 level (P<0.05), and the plasma IL-10 level in disease group was markedly raised.

Correlation of genotypes at rs1799724 and rs1800629 of TNF-α gene and at rs1800872 and rs141219090 of IL-10 gene with renal function indexes. Correlation of genotypes at rs1799724 and rs1800629 of TNF-α gene and at rs1800872 and rs141219090 of IL-10 gene with renal function indexes (24-h urine protein, plasma albumin and creatinine) were analyzed (Table V), which revealed that CT genotype at rs1799724 of TNF-α gene was related to the 24-h urine protein level (P=0.035), GG genotype at rs1800872 of IL-10 gene was associated with the plasma albumin level (P=0.031), and GG genotype at rs141219090 of IL-10 gene was related to the serum creatinine level (P=0.047).

Discussion

Nephrotic syndrome is a disease with a complicated pathogenesis caused by heredity, infection and other factors, in which the immune microenvironment is often out of balance (13,14). Primary nephrotic syndrome is nephrotic syndrome without definite cause. Disorders of various cytokines and inflammatory factors such as IL-17A cause glomerulus to be damaged and its permeability to be increased, triggering various symptoms (15).

TNF-α, primarily secreted by mononuclear macrophages, has an outstanding function of killing tumor cells, thus protecting the body from the infringement of tumor cells. However, TNF-α also exerts lethal effects on normal tissues and cells. High-level TNF-α may lead to autoimmune diseases and damage different tissues. Gene polymorphisms may influence TNF-α secretion and disease susceptibility. In addition, TNF-α has been proved to be related to various diseases, such as schistosomiasis, periportal fibrosis (16) and diabetic nephropathy (17). IL-10 is also a cytokine exerting pro-inflammatory effects, and it originates from numerous cells, with a complex mechanism of action. IL-10 gene polymorphism can influence the occurrence of diseases, such as viral infection (18) and ischemic stroke (19).

Electron microscopy with podocyte detachment plays an increasingly important role in the diagnosis and classification of kidney diseases. Unfortunately, the role of TNF-α and IL-10 in nephrotic syndrome has not been confirmed by electron microscopy as far as we know. It was found in this study that allele distributions at rs1799724 (P=0.003) and rs1800629 (P=0.011) of TNF-α gene and at rs1800872 (P=0.033) of IL-10 gene in disease group were different from those in control group. Among them, in disease group, allele C frequency at rs1799724
and allele A frequency at rs1800629 of TNF-α gene and allele T frequency at rs1800872 of IL-10 gene were higher. It can be concluded that allele C at rs1799724 and allele A at rs1800629 of TNF-α gene and allele T at rs1800629 and rs1800872 of IL-10 gene are susceptible factors for primary nephrotic syndrome. However, there were differences between rs1799724 (P=0.007) and rs1800629 (P=0.002) of TNF-α gene, and the frequency of CC genotype at rs1799724 and AA genotype at rs1800629 of TNF-α gene were higher in the disease group. The above results prove that the gene polymorphisms (rs1799724 and rs1800629) of TNF-α gene may have strong correlation with the occurrence of primary nephrotic syndrome.

Through polymorphism analysis and recessive or the dominant model construction, it was discovered that the frequency of recessive model of TNF-α gene rs1799724 in disease group was different from that in control group (P=0.035), and the frequency of dominant model GG+GA was remarkably lower. The above results imply that TNF-α gene may affect the occurrence of primary nephrotic syndrome through the combination of two genotypes at a single locus. Besides, the frequencies of CA haplotype at rs1799724 and rs1800629 of TNF-α gene may have strong correlation with the occurrence of primary nephrotic syndrome. However, there were differences between rs1799724 (P=0.007) and rs1800629 (P=0.002) of TNF-α gene, and the frequency of CC genotype at rs1799724 and AA genotype at rs1800629 of TNF-α gene were higher in the disease group. The above results prove that the gene polymorphisms (rs1799724 and rs1800629) of TNF-α gene may have strong correlation with the occurrence of primary nephrotic syndrome.

Through polymorphism analysis and recessive or the dominant model construction, it was discovered that the frequency of recessive model of TNF-α gene rs1799724 in disease group was different from that in control group (P=0.043), and the frequency of the recessive model (CT+TT) was significantly lower. In addition, there was a difference in the frequency of dominant model of TNF-α gene rs1800629 between disease group and control group (P=0.035), and the frequency of dominant model GG+GA was remarkably lower. The above results imply that TNF-α gene may affect the occurrence of primary nephrotic syndrome through the combination of two genotypes at a single locus. Besides, the frequencies of CA haplotype at rs1799724 and rs1800629 of TNF-α gene in disease group were markedly higher than those in control group (P<0.001, OR=1.972). Furthermore, disease group had evidently lower frequencies of GG haplotype (P=0.041, OR=0.719) and markedly higher frequencies of TG haplotype (P=0.003, OR=1.624) at rs1800872 and rs141219090 of IL-10 gene than control group, indicating that TNF-α gene and IL-10 gene may affect the susceptibility of primary nephrotic syndrome through two loci. It can be concluded that gene polymorphisms may exert a holistic effect on primary nephrotic syndrome, rather than a unilateral effect of a single locus or genotype of gene, which contributes to the understanding of the disease.

Table IV . Haplotype analysis of TNF-α gene rs1799724 and rs1800629 and IL-10 gene rs1800872 and rs141219090.

| Gene       | Haplotype | Control group | Disease group | OR    | 95% CI      | χ² value | P-value |
|------------|-----------|---------------|---------------|-------|-------------|----------|---------|
| TNF-α      | CA        | 92.50 (0.231) | 148.93 (0.372)| 1.972 | 1.449-2.684 | 18.889   | <0.001  |
|            | CG        | 91.50 (0.229) | 77.07 (0.193) | 0.805 | 0.572-1.131 | 1.565    | 0.211   |
|            | TA        | 101.50 (0.254)| 81.07 (0.203) | 0.748 | 0.536-1.042 | 2.962    | 0.085   |
|            | TG        | 114.50 (0.286)| 92.93 (0.232) | 0.755 | 0.549-1.037 | 3.029    | 0.082   |
| IL-10      | GA        | 85.31 (0.213) | 80.74 (0.202) | 0.933 | 0.663-1.313 | 0.159    | 0.690   |
|            | GG        | 117.69 (0.294)| 92.26 (0.231) | 0.719 | 0.524-0.987 | 4.175    | 0.041   |
|            | TA        | 113.69 (0.284)| 107.26 (0.268)| 0.923 | 0.677-1.258 | 2.588    | 0.111   |
|            | TG        | 83.31 (0.208) | 119.74 (0.299)| 1.624 | 1.176-2.242 | 8.758    | 0.003   |

TNF-α, tumor necrosis factor-α; IL-10, interleukin-10; OR, odds ratio; CI, confidence interval.

Table V . Correlation of genotypes at rs1799724 and rs1800629 of TNF-α gene and at rs1800872 and rs141219090 of IL-10 gene with renal function indexes.

| Gene | Locus | Genotype | 24-h urine protein (g) Normal range (≤0.15 g) | Plasma albumin (g/l) Normal range (35-55 g/l) | Creatinine (µmol/l) Normal range (44-133 µmol/l) |
|------|-------|----------|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|
|      |       |          | Control group | Disease group | P-value | Control group | Disease group | P-value | Control group | Disease group | P-value |
| TNF-α | rs1799724 | CC       | 0.1       | 3.6      | 0.035  | 42.31  | 24.42  | 0.351  | 52.34  | 145.35  | 0.175   |
|       |         | CT       | 0.3       | 5.2      |        | 45.51  | 29.52  |        | 64.14  | 148.54  |        |
|       |         | TT       | 0.1       | 3.7      |        | 39.16  | 25.42  |        | 63.42  | 132.46  |        |
|       | rs1800629 | GG       | 0.1       | 3.8      | 0.381  | 47.72  | 24.15  | 0.311  | 58.36  | 145.42  | 0.113   |
|       |         | GA       | 0.2       | 3.6      |        | 44.11  | 24.32  |        | 68.47  | 137.45  |        |
|       |         | AA       | 0.1       | 4.1      |        | 42.95  | 22.16  |        | 53.25  | 142.36  |        |
| IL-10 | rs1800872 | GG       | 0.1       | 3.9      | 0.473  | 36.17  | 18.52  | 0.031  | 69.57  | 137.13  | 0.234   |
|       |         | GT       | 0.2       | 4.2      |        | 47.13  | 26.42  |        | 56.35  | 156.43  |        |
|       |         | TT       | 0.1       | 3.7      |        | 45.53  | 25.46  |        | 58.42  | 144.31  |        |
|       | rs141219090 | GG      | 0.1       | 3.5      | 0.274  | 38.36  | 21.35  | 0.212  | 68.41  | 184.98  | 0.047   |
|       |         | GA       | 0.1       | 4.6      |        | 43.44  | 23.71  |        | 50.32  | 132.25  |        |
|       |         | AA       | 0.2       | 4.3      |        | 42.72  | 22.86  |        | 54.28  | 141.54  |        |

TNF-α, tumor necrosis factor-α; IL-10, interleukin-10.
Analysis of gene polymorphisms and the levels of plasma TNF-α and IL-10 demonstrated that TT genotype at rs1799724 of TNF-α gene was significantly related to the plasma TNF-α level (P<0.05), and the plasma TNF-α level was significantly increased in disease group. AA genotype at rs141219090 of IL-10 gene had a notable correlation with the plasma IL-10 level (P<0.05), and the plasma IL-10 level in disease group was markedly raised. The above results indicate that TNF-α and IL-10 gene polymorphisms not only affect the susceptibility of primary nephrotic syndrome, but also may promote the development of the disease by affecting plasma cytokine levels. Further research confirmed that CT genotype at rs1799724 of TNF-α gene was related to the 24-h urine protein level (P=0.035), GG genotype at rs1800872 of IL-10 gene was associated with the plasma albumin level (P=0.031), and GG genotype at rs141219090 of IL-10 gene was related to the serum creatinine level (P=0.047), implying that TNF-α and IL-10 gene polymorphisms can indeed affect the development of primary nephrotic syndrome, and the disease condition can be evaluated and predicted by detecting the gene polymorphism of patients. However, there are still some limitations in the present study. This was single center retrospective study and the sample size was small. In addition, the differences among different pathological types were not included in the present study.

In conclusion, TNF-α and IL-10 gene polymorphisms are predominantly correlated with the susceptibility and the condition of primary nephrotic syndrome. TT genotype at rs1799724 of TNF-α gene and AA genotype at rs141219090 of IL-10 gene are risk factors of primary nephrotic syndrome.

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Availability of data and materials
All data generated or analyzed during this study are included in this published article.

Authors’ contributions
MX, SB and ZH designed the study and performed the experiments. JC, LX, YaL, LQ and YoL were also involved in the conception of the study. SZ, YWu, GT, WW and YWa analyzed the data. All authors read and approved the final manuscript.

Ethics approval and consent to participate
The study was approved by the Ethics Committee of the Qilu Hospital of Shandong University (Jinan, China) (no. QL.0220161204). Signed informed consents were obtained from the patients and/or guardians.

Patients consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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