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Possible role of IL-6 and TIE2 gene polymorphisms in predicting the initial high transport status in peritoneal dialysis patients: an observational study

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Running title: Role of IL-6 and TIE2 Gene Polymorphisms

Abstract

Objectives: The aim of this study was to investigate the effect of IL-6 and TIE2 gene polymorphisms on baseline peritoneal transport property.

Design: An observational study

Setting: A hospital.

Participants: This study included 220 continuous ambulatory peritoneal dialysis (PD) patients.

Outcome measures: Patients were divided into two groups based on the results of an initial peritoneal equilibration test performed within 3 months of starting PD therapy: group 1 consisted of low/low average transporters (n = 123), and group 2 consisted of high/high average transporters (n = 97). We genotyped TIE2 and IL-6 polymorphisms and analysed their effects on baseline transport status.

Results: The genotype AT in IL-6 Rs13306435 and the genotype CC in TIE2 Rs639225 were both negatively associated with a higher initial peritoneal transport status (IL-6 Rs13306435: OR = 0.408, 95% CI, 0.227~0.736; TIE2 Rs639225: OR = 0.188, 95% CI, 0.044~0.806).

Conclusions: IL-6 and TIE2 polymorphisms are associated with baseline peritoneal transport property.

Keywords: peritoneal dialysis, high transport status, interleukin-6, TIE2, gene polymorphism

Article summary

Strengths of this study

This study was the first study exploring the possible association between TIE2 gene polymorphisms and the characteristics of peritoneal transport.

This study confirmed the association between IL-6 polymorphism and baseline peritoneal transport among the Chinese Han population.

This study may provide a convenient and non-invasive method to determine transport status before starting dialysis.

This study may be helpful in improving the prognosis of ESRD patients.

This study may help us elucidate the mechanisms underlying high transport status.

Limitations of this study

The TA genotype of rs13306435 presents in only 7% of the total population; therefore, it is not the main determinant of peritoneal transport in most patients.

This study was a single center research.

This study needs to be improved by increasing the sample size.

We did not examine dialysate IL-6/TIE2 concentration to investigate its relationship with SNPs.

We found no statistically significant difference in the data of D/P creatinine and D/D0 glucose among different genotypes.

Introduction

Peritoneal dialysis (PD) is an effective renal replacement therapy for end stage renal disease (ESRD) patients[1]. Patients undergoing peritoneal dialysis have significantly different small solute transport rates. The standard peritoneal equilibration test (PET) proposed by Twardowski in 1987 is the most widely used method to assess the peritoneal small solute transport rate[2]. Patients can be divided into four types: high (H), high-average (HA), low-average (LA), and low (L) based on PET results. Studies have shown an association between high transport status and poor outcome[3-6]. Results of meta-analysis[7] have indicated that for every 0.1 increase in the dialysate over plasma ratio for creatinine (D/P Cr), the relative risks for mortality and technique failure increase by 1.15 and 1.18, respectively. Compared with that of the low transport group, the mortality of the low-average, high-average, and high transport groups increased by 21.9%, 45.7%, and 77.3%, respectively. As technology advances, new peritoneal dialysate (icodextrin)[8-11] and automated peritoneal dialysis (APD)[12-14] have been shown to improve the prognosis of high transporters. However, in developing countries, icodextrin and APD cannot be widely used for PD patients. Initial high transport is still an important factor that influences the outcome of these patients without icodextrin or APD. Therefore, it is important to know the baseline peritoneal transport property before starting PD therapy. We can advise probable high transporter patients to choose haemodialysis (HD) or renal transplantation for renal replacement therapy. Researchers have attempted to find non-invasive biomarkers to predict the baseline peritoneal membrane function before starting dialysis. Previous studies found that age, gender, and complications such as hypertension, diabetes, and malnutrition might influence transport characteristics[1]. However, they are not sufficient to predict high transport status.

In recent years, many studies have shown that genetic variants may play an important role in mechanisms contributing to the baseline variability in peritoneal transport[15-20]. It has been

suggested that chronic inflammation mediated by various inflammatory cytokines may have an effect on peritoneal transport[21]. Studies have shown that the IL-6 level in peritoneal dialysates is associated with the peritoneal solute transport rate in dialysis patients[22-24]. Polymorphism of IL-6 is reported to correlate with baseline peritoneal transport status in Caucasian and Korean patients[15, 19]. An increase in the effective solute exchange area caused by peritoneal vascular proliferation is also an important factor for high peritoneal transport status[25]. TIE2 is the receptor of angiogenin (Ang) 1 and 2. Ang/Tie2 has been confirmed to play an important role in angiogenesis in the peritoneum[26]. The angiogenesis of the peritoneum induced by PD can be inhibited using sTie2/Fc in a uremic rat model[27]. Therefore, it is possible that the genetic polymorphisms of Il-6 and TIE2 might be involved in the mechanism of high peritoneal transport status. This study aimed to determine whether TIE2 and IL-6 gene polymorphisms have an effect on the baseline peritoneal transport property and explore its possible role in predicting initial high transport status.

Materials and Methods

Patient selection

All PD patients having an initial PET performed within 3 months of starting PD therapy were included. Those who switched from failed renal allograft or maintenance haemodialysis were excluded. Two hundred and twenty continuous ambulatory PD patients in the Peritoneal Dialysis Center, School of Medicine, in Shanghai Jiaotong University were enrolled in the study. The study was approved by the ethical committee of Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200127, China. Written informed consent was obtained from each patient.

Study of peritoneal transport

A standard PET was performed for each of the enrolled patients. Dialysate as well as plasma

creatinine and glucose levels were measured at 4 h using 2 L of 2.5% glucose dialysis fluid. Creatinine dialysate to plasma ratios at 4 h (D/P Cr) were calculated. Patients were classified into four types based on the D/P Cr value: high (D/P Cr > 0.8), high average (D/P Cr 0.66 to 0.8), low average (D/P Cr 0.5 to 0.65), and low transporters (D/P Cr < 0.5). Then, they were divided into two groups: group 1 consisted of low/low average transporters, and group 2 consisted of high/high average transporters. The residual urine volume was assessed after 24 h of urine collection. Weekly peritoneal Kt/V (peritoneal Kt/V) and residual urine Kt/V (urine Kt/V) were calculated and presented as total weekly Kt/V (total Kt/V).

DNA extraction and genotyping

DNA was extracted from whole blood using a DNA purification kit (Promega, USA). The SNPs of IL-6 and TIE2 were genotyped by a single base primer extension assay. The genomic DNA flanking the SNP was amplified by polymerase chain reaction (PCR) using forward and reverse primer pairs (Tables 1 and 2), and standard PCR reagents in a 10-μL reaction volume containing 20 ng DNA sample, 0.4 μmol of each primer, 10X PCR buffer, 0.4 μmol dNTPs (Generay Biotech, China), 10 mmol $MgCl_2$, and 0.25 units HotStarTaq DNA Polymerase (QIAGEN, Germany). After 40 cycles of PCR (MJ Research PT-100), the products were purified by 2 U SAP and 2 U Exonuclease I (Epicentre, USA). The purified amplification products (2 μL) were added into a SNaPShot Multiplex Ready reaction mixture (Applied Biosystems, USA) containing 1 μL of genotyping primer for the primer extension reaction (Table 1, Table 2). The primer extension reaction was carried out with 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 30 s. The reaction products were purified by 0.5 U SAP. The final reaction samples (0.5 μL) were added into 9.25 μL Hi-Di formamide (Applied Biosystems) and 0.25 μL GS-120 LIZ (Applied Biosystems). The mixture was incubated at 95°C for 5 min and then analysed by electrophoresis using the ABI Prism 3730xl DNA analyser (Applied Biosystems). Results were analysed using GeneScan analysis software

(Applied Biosystems).

Statistical analysis

Statistical analysis was conducted using SPSS version 17.0. All categorical data were presented as absolute counts or percentages, mean and standard deviation (SD) were provided for continuous data. To compare the differences between two baseline transport groups, categorical data were analysed by Fisher's exact test and continuous variables were analysed by an unpaired t-test. Logistic regression analysis was applied to determine whether polymorphism of IL-6 and TIE2 affected the baseline peritoneal transport status. P-values of < 0.05 were considered statistically significant.

Results

Clinical parameters between different transport groups

In total, 220 patients were enrolled in this study. The average age of the patients was 52.54 ± 14.56 years; the male to female ratio was 118:102 and the average body mass index was 21.83 ± 3.47 kg/m^2. Residual renal function was 3.98 ± 3.47 mL/min. The causes of ESRD were as follows: chronic glomerulonephritis (n = 71; 32.3%), diabetic nephropathy (n = 32; 14.5%), hypertensive nephropathy (n = 9; 4.1%), and other/unknown (n = 107; 48.6%). Based on the first PET results, there were 97 patients (44.1%) in the H/HA group, and 123 patients (55.9%) in the L/LA group. Comparisons of clinical characteristics between the two groups are shown in Table 3.

Distribution of IL-6 and TIE2 polymorphisms in different transport groups

The distribution of IL-6 and TIE2 genotypes in the peritoneal transport groups are summarized in Tables 4 and 5. Distributions of the 24 alleles (12 polymorphisms) were within the Hardy–Weinberg equilibrium. For the IL-6 polymorphism, there was a statistically

significant correlation between AT genotype of rs13306435 and peritoneal transport group (p = 0.023). For the TIE2 polymorphism, the distribution of rs10967789 and rs639225 genotypes differed significantly between the two groups (p = 0.039 and 0.047, respectively).

Parameters for peritoneal solute transport rate for different genotypes

We further compared the peritoneal solute transport rate among groups with the three SNPs (rs13306435, rs10967789, and rs639225). We found no statistically significant difference in the data of D/P creatinine and D/D0 glucose among different genotypes (Table 6).

Roles of IL-6 and TIE2 gene polymorphisms in predicting initial peritoneal high transport status

With possible clinical factors controlled in a multiple logistic regression model, the genotype AT in IL-6 Rs13306435 and CC in TIE2 Rs639225 were both negatively associated with a higher initial peritoneal transport status (IL-6 Rs13306435: OR=0.408, 95% CI, 0.227~0.736; TIE2 Rs639225: OR = 0.188, 95% CI, 0.044~0.806) (Table 7).

Discussion

The relationship between gene polymorphisms and disease had been gaining greater attention by researchers. It has been shown that SNPs of IL6 may influence the development of cardiovascular disease[28], cancer[29,30], fractures[31], and autoimmune diseases[32-34]. In contrast, there is less available research regarding TIE2 gene polymorphisms, except for a study on the relationship between rs638203/ rs639225 and vascular malformations[35].

In this study, we investigated the effect of genetic polymorphisms of IL-6 and TIE2 on the baseline peritoneal transport property. Results showed that IL-6 and TIE2 gene

polymorphisms were both negatively associated with initial high transport status. The genotypes of rs13306435 and rs639225 were shown to be independent predictors of initial high transport status in PD patients.

Initial transport status can determine the patients' dialysis prescription, which may influence the outcome for PD patients. Previous studies have shown an association between initial high transport status and poor outcome. Although icodextrin and APD have been shown to improve the prognosis of high transporters, most of the PD patients in developing countries are unable to use them. In China, for instance, icodextrin has not been approved for sale yet, and few patients can afford the cost of APD therapy. Therefore, predicting the baseline transport status is important to select a better treatment strategy. Our study provided a potential solution to predict initial high transport status before beginning PD.

There have been several genetic studies of peritoneal solute transport rate in PD patients. Polymorphisms of endothelial nitric oxide synthase[16], receptor of advanced glycation end products[17], and transforming growth factor –βl[18] were reported to be involved in baseline transport status. In 2005, Gillerot et al. showed that the SNP of IL-6 (rs1800795) influenced baseline peritoneal permeability in Caucasian PD patients[15]. Additionally, Hwang et al. reported that the rs1800795 polymorphism was associated with dialysate IL-6 concentration and baseline peritoneal transport status in Korean PD patients[19]. However, for the Chinese Han population, the minor allele frequency (MAF) of rs1800795 was reported to be very low (MAF = 0.02). Thus, it might not be appropriate to directly apply these results to this population. Our finding similar to those of the aforementioned studies. This supports the role of the IL-6 polymorphism in predicting baseline peritoneal transport.

Angiogenesis in the peritoneum could directly increase the effective solute exchange area and influence the transport characteristics. Previous studies have found that polymorphisms of vascular endothelial growth factor were not associated with initial peritoneal transport type[15,

[17]. Ang/Tie2 has recently been confirmed to play an important role in angiogenesis in the peritoneum[26]. Whether the polymorphisms of TIE2 are involved in initial peritoneal transport status remains unknown. In this study, we carefully selected nine SNPs with MAF > 0.05 in the Chinese Han population to investigate their possible role in predicting high transport status. Results showed that TIE2 could be a new factor in predicting the baseline transport type. This study is the first to explore the possible association between TIE2 gene polymorphisms and characteristics of peritoneal transport. Rs639225 is located in exon 13 of TIE2. A study reported its association with venous malformation and presumed that this polymorphism might cause abnormal splicing of *TIE2* into a defective protein[28]. Functional validation of this polymorphism is warranted in the future.

There are some limitations to our study. The TA genotype of rs13306435 presents in only 7% of the total population; therefore, it is not the main determinant of peritoneal transport in most patients. Additionally, this study was a single centre research and the number of cases was limited; increasing the sample size would improve this study. Research has shown that the IL-6/TIE2 concentration was associated with baseline transport status. We hypothesized that the SNPs may participate in the formation of high transport status by influencing the dialysate IL-6/TIE2 concentration. We did not examine the dialysate IL-6/TIE2 concentration in this study. As shown in Table 6, we did not find any statistically significant difference in the data of D/P creatinine and D/D0 glucose between the different genotypes. We believe this may be due to the limited sample size.

In conclusion, IL-6 and TIE2 polymorphisms are associated with baseline peritoneal transport property. Functional study of the polymorphisms is required in the future.

Author contributions statement

Li Ding, Xinghua Shao and Zhaohui Ni contributed to conception and design, Li Ding, Xinghua Shao, Liou Cao1, Wei Fang, Hao Yan, Jiaying Huang, Aiping Gu, Zanzhe Yu, Chaojun Qi, Xinbei Chang and Zhaohui Ni contributed to acquisition of data, or analysis and interpretation of data; Li Ding, Xinghua Shao, Liou Cao1, Wei Fang, Hao Yan, Jiaying Huang, Aiping Gu, Zanzhe Yu, Chaojun Qi, Xinbei Chang and Zhaohui Ni contributed to drafting the manuscript or revising it critically for important intellectual content. All authors reviewed the manuscript.

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Competing interests

The authors declare that they have no competing interests.

Data sharing statement

Technical appendix, statistical code and dataset are available from the corresponding author.

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Tables

Table 1 Primer Sequences of IL6 for Genotyping Using SNapShot Assay

SNP	PCR-L	PCR-R	Target
rs1800795	AACCTCCTCTAAGTGGGCTG	GGTGGGGCTGATTGGAAAC	TCCCCCTAGTTGTGTCTTGC
rs1800796			CCAGGCAGTTCTACAACAGCC
rs13306435	GAAGGGTCCTACTCAGAGCA	GTTGGGTCAGGGGTGGTTAT	TTCCTTCAGGCAAAGAATCTAGA

Table 2 Primer Sequences of TIE2 for Genotyping Using SNapShot Assay

SNP	PCR-L	PCR-R	Target
rs10967717	TTTCCTCTGGTGGGTAGGAA	GGGCTACTGGGATCTCTGAC	GAGGAGTATAATGATTTCCTCAGGC
rs667867			CCACATGGTTTGAATTGGGA
rs639225	TTCTTCCTCCTCAACCAGAAA	TCACATCAACGTGCTGGTCT	CAATATTGTCCAAGAAATCACAGC
rs542913	ACGGGTGGGTCTGTTTCTC	GAGGCTTGCCTAAGGGAAAT	CATTCTCCTTTGCACATTTGC
rs3737188	GATTGTCCCGAGGTCAAGAG	TTTCCCAGGGCACACAGTAT	TTGTCCCGAGGTCAAGAGGTGTA
rs2273719	TGGCACTGTTTGTCTTCCAG	ACCGGCTGACTTTGCTAGAG	AGGCACACCCTACTGCGG
rs2273718			AGTCTGTAGCCCTGGGGCA
rs10967789	ATGGGCTGAAATCAGAATGC	AACCTGTACTATCAGGGTCATTG	AATGCTATTAAATGTTTTCCTGTGT
rs9987817	CTGGGTGACATTTGGGAGAC	CACTCCTGGATGAGACGTGA	GATGAGACGTGAGTAGGCAAGA

Table 3 Comparison of Clinical Characteristics and Peritoneal Parameters between the PeritonealTransport Groups of 220 Peritoneal Dialysis Patients

	L/LA (n=123)	H/HA (n=97)	*P*
Age (y)	52.85±14.94	52.27±14.42	0.761
Male (%)	68(55.3)	60(61.8)	0.291
BMI (kg/㎡)	21.82±3.58	21.85±3.03	0.939
DM (%)	22(17.8)	23(23.7)	0.266
Hypertension (%)	94(68.1)	75(70.1)	0.740
Periods between operation and initial PET(d)	48(39,69)	47(35,68)	0.924
Hemoglobin (g/L)	104.60±22.67	99.13±21.29	0.056
Serum albumin (g/L)	36.37±4.7	34.8±5.33	0.017
hs-CRP (mg/L)	2.65(0.71,3.83)	2.58(1,3.86)	0.657
ACEI/ARB(%)	64(52)	53(54.6)	0.624
D4/D0 (glu)	0.44±0.08	0.34±0.08	＜0.01
D/P Cr	0.55±0.08	0.74±0.07	＜0.01
RRF(ml/min)	4±2.92	3.95±2.63	0.901
nPCR[g/(kg.d)]	1.05±0.6	0.96±0.26	
UF(ml)	160(-200,520)	80(-45,500)	0.004
Urine volume (ml)	1000(560,1400)	1000(500,1500)	0.217
Kt/V	2.23±0.58	2.20±0.56	0.647

Table 4 IL-6 Gene Polymorphisms in Two Groups

Rs13306435	L/LA (n=123)	H/HA (n=97)	P
AT	14	3	0.023
TT	109	94	
Rs1800796			
CC	77	61	0.986
CG	37	28	
GG	9	8	
Rs1800795			
GG	121	96	0.506
GC	2	1	

Table 5 TIE2 Gene Polymorphisms in Two Groups

	L/LA (n=123)	H/HA (n=97)	P
Rs10967717			
AA	15	16	0.652
AG	56	47	
GG	52	34	
Rs10967789			
CC	90	83	0.039
CG	33	14	
Rs2273718			
AG	14	4	0.081
GG	109	93	
Rs2273719			
AA	4	2	0.868
AG	36	27	
GG	83	68	
Rs3737188			
CC	6	1	0.373
CT	44	31	
TT	73	65	
Rs542913			
CC	100	80	0.403
CT	22	17	
TT	1	0	
Rs639225			
CC	40	18	0.047
CT	53	46	
TT	30	33	
Rs657867			
AA	112	87	0.732
AG	11	10	
Rs9987817			
CC	97	74	0.730
CT	22	22	
TT	4	1	

Table 6 The parameters for peritoneal solute transport rate according to different genotypes

IL-6 Rs13306435	AT	TT	P
D/P creatinine	0.61±0.99	0.63±0.12	0.357
D/D0 glucose	0.41±0.092	0.39±0.085	0.424
TIE2 Rs639225	CC	CT/TT	P
D/P creatinine	0.61±0.11	0.64±0.11	0.056
D/D0 glucose	0.40±0.09	0.39±0.08	0.518
TIE2 Rs10967789	CC	CG	P
D/P creatinine	0.64±0.12	0.61±0.09	0.187
D/D0 glucose	0.39±0.09	0.40±0.06	0.839

Table 7 Multivariate Logistic Regression Model to Identify Factors Associated with High/High-Average Transport Status

	OR	95%CI	P
TIE2 Rs639225 (CC vs. CT/TT)	0.188	0.044~0.806	0.024
IL-6 Rs13306435 (AT vs. TT)	0.408	0.227~0.736	0.043
Age	0.966	0.930~1.004	0.081
Male	1.401	0.519~3.788	0.506
DM	3.28	0.952~11.360	0.060
Periods between operation and initial PET(d)	0.996	0.987~1.005	0.401
hs-CRP(mg/L)	1.081	0.964~1.212	0.182
Serum albumin (g/L)	0.898	0.796~1.014	0.083
TIE2 Rs10967789 (CC vs. CG)	1.061	0.371~1.632	0.197
Hemoglobin (g/L)	0.984	0.796~1.014	0.192

Section & Topic	No	Item	Reported on page #
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ABSTRACT			
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Possible role of IL-6 and TIE2 gene polymorphisms in predicting the initial high transport status in peritoneal dialysis patients: an observational study

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Possible role of IL-6 and TIE2 gene polymorphisms in predicting the initial high transport status in peritoneal dialysis patients: an observational study

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Running title: Role of IL-6 and TIE2 Gene Polymorphisms

Abstract

Objectives: The aim of this study was to investigate the effect of IL-6 and TIE2 gene polymorphisms on baseline peritoneal transport property.

Design: An observational study

Setting: Renji Hospital in Shanghai, China

Participants: This study included 220 continuous ambulatory peritoneal dialysis (PD) patients.

Outcome measures: Patients were divided into two groups based on the results of an initial peritoneal equilibration test performed within 3 months of starting PD therapy: group 1 consisted of low/low average transporters (n = 123), and group 2 consisted of high/high average transporters (n = 97). We genotyped TIE2 and IL-6 polymorphisms and analysed their effects on baseline transport status.

Results: The genotype AT in IL-6 Rs13306435 and the genotype CC in TIE2 Rs639225 were both negatively associated with a higher initial peritoneal transport status (IL-6 Rs13306435: OR = 0.408, 95% CI, 0.227~0.736; TIE2 Rs639225: OR = 0.188, 95% CI, 0.044~0.806).

Conclusions: IL-6 and TIE2 polymorphisms are associated with baseline peritoneal transport property.

Keywords: peritoneal dialysis, high transport status, interleukin-6, TIE2, gene polymorphism

Article summary

Strengths and limitations of this study

This study was the first study exploring the possible association between TIE2 gene polymorphisms and the characteristics of peritoneal transport.

This study was also the first study confirming the association between IL-6 polymorphism and baseline peritoneal transport among the Chinese Han population.

We used a convenient and non-invasive method to study the initial high transport status in PD patients.

The TA genotype of rs13306435 presents in only 7% of the total population, therefore, it is not the main determinant of peritoneal transport in most patients.

We did not examine dialysate IL-6/TIE2 concentration to investigate its relationship with SNPs.

Introduction

Peritoneal dialysis (PD) is an effective renal replacement therapy for end stage renal disease (ESRD) patients[1]. Patients undergoing peritoneal dialysis have significantly different small solute transport rates. The standard peritoneal equilibration test (PET) proposed by Twardowski in 1987 is the most widely used method to assess the peritoneal small solute transport rate[2]. Patients can be divided into four types: high (H), high-average (HA), low-average (LA), and low (L) based on PET results. Studies have shown an association between high transport status and poor outcome[3-6]. The results of a meta-analysis[7] have indicated that for every 0.1 increase in the dialysate over plasma ratio for creatinine (D/P Cr), the relative risks for mortality and technique failure increase by 1.15 and 1.18, respectively. Compared with that of the low transport group, the mortality of the low-average, high-average, and high transport groups increased by 21.9%, 45.7%, and 77.3%, respectively. As technology advances, new peritoneal dialysate (icodextrin)[8-11] and automated peritoneal dialysis (APD)[12-14] have been shown to improve the prognosis of high transporters. However, in developing countries, icodextrin and APD cannot be widely used for PD patients. Initial high transport is still an important factor that influences the outcome of these patients without icodextrin or APD. Therefore, it is important to know the baseline peritoneal transport property before starting PD therapy. We can advise probable high transporter patients to choose haemodialysis (HD) or renal transplantation for renal replacement therapy. Researchers have attempted to find non-invasive biomarkers to predict the baseline peritoneal membrane function before starting dialysis. Previous studies found that age, gender, and complications such as hypertension, diabetes, and malnutrition might influence transport characteristics[1]. However, they are not sufficient to predict high transport status.

In recent years, many studies have shown that genetic variants may play an important role in mechanisms contributing to the baseline variability in peritoneal transport[15-20]. It has been

suggested that chronic inflammation mediated by various inflammatory cytokines may have an effect on peritoneal transport[21]. Studies have shown that the IL-6 level in peritoneal dialysates is associated with the peritoneal solute transport rate in dialysis patients[22-24]. A polymorphism of IL-6 (Rs13306435) is reported to correlate with baseline peritoneal transport status in Caucasian and Korean patients[15, 19]. An increase in the effective solute exchange area caused by peritoneal vascular proliferation is also an important factor for high peritoneal transport status[25]. TIE2 is the receptor of angiogenin (Ang) 1 and 2. Ang/Tie2 has been confirmed to play an important role in angiogenesis in the peritoneum[26]. The angiogenesis of the peritoneum induced by PD can be inhibited using sTie2/Fc in a uremic rat model[27]. Therefore, it is possible that the genetic polymorphisms of Il-6 and TIE2 might be involved in the mechanism of high peritoneal transport status. This study aimed to determine whether TIE2 and IL-6 gene polymorphisms have an effect on the baseline peritoneal transport property and explore its possible role in predicting initial high transport status.

Materials and Methods

Patient selection

All PD patients having an initial PET performed within 3 months of starting PD therapy were included. Those who switched from failed renal allograft or maintenance haemodialysis were excluded. Two hundred and twenty continuous ambulatory PD patients in the Peritoneal Dialysis Center, School of Medicine, in Shanghai Jiaotong University were enrolled in the study. The study was approved by the ethical committee of Renji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200127, China. Written informed consent was obtained from each patient.

Study of peritoneal transport

A standard PET was performed for each of the enrolled patients. Dialysate as well as plasma

creatinine and glucose levels were measured at 4 h using 2 L of 2.5% glucose dialysis fluid. Creatinine dialysate to plasma ratios at 4 h (D/P Cr) were calculated. Patients were classified into four types based on the D/P Cr value: high (D/P Cr > 0.8), high average (D/P Cr 0.66 to 0.8), low average (D/P Cr 0.5 to 0.65), and low transporters (D/P Cr < 0.5). Then, they were divided into two groups: group 1 consisted of low/low average transporters, and group 2 consisted of high/high average transporters. The residual urine volume was assessed after 24 h of urine collection. Weekly peritoneal Kt/V (peritoneal Kt/V) and residual urine Kt/V (urine Kt/V) were calculated and presented as total weekly Kt/V (total Kt/V).

DNA extraction and genotyping

DNA was extracted from whole blood using a DNA purification kit (Promega, USA). The SNPs of IL-6 and TIE2 were genotyped by a single base primer extension assay. The genomic DNA flanking the SNP was amplified by polymerase chain reaction (PCR) using forward and reverse primer pairs (Tables 1 and 2), and standard PCR reagents in a 10-μL reaction volume containing 20 ng DNA sample, 0.4 μmol of each primer, 10X PCR buffer, 0.4 μmol dNTPs (Generay Biotech, China), 10 mmol $MgCl_2$, and 0.25 units HotStarTaq DNA Polymerase (QIAGEN, Germany). After 40 cycles of PCR (MJ Research PT-100), the products were purified by 2 U SAP and 2 U Exonuclease I (Epicentre, USA). The purified amplification products (2 μL) were added into a SNaPShot Multiplex Ready reaction mixture (Applied Biosystems, USA) containing 1 μL of genotyping primer for the primer extension reaction (Table 1, Table 2). The primer extension reaction was carried out with 25 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 30 s. The reaction products were purified by 0.5 U SAP. The final reaction samples (0.5 μL) were added into 9.25 μL Hi-Di formamide (Applied Biosystems) and 0.25 μL GS-120 LIZ (Applied Biosystems). The mixture was incubated at 95°C for 5 min and then analysed by electrophoresis using the ABI Prism 3730xl DNA analyser (Applied Biosystems). Results were analysed using GeneScan analysis software

(Applied Biosystems).

Statistical analysis

Statistical analysis was conducted using SPSS version 17.0. All categorical data were presented as absolute counts or percentages, mean and standard deviation (SD) were provided for continuous data. To compare the differences between two baseline transport groups, categorical data were analysed by Fisher's exact test and continuous variables were analysed by an unpaired t-test. Logistic regression analysis was applied to determine whether polymorphism of IL-6 and TIE2 affected the baseline peritoneal transport status. P-values of < 0.05 were considered statistically significant.

Results

Clinical parameters between different transport groups

In total, 220 patients were enrolled in this study. The average age of the patients was 52.54 ± 14.56 years; the male to female ratio was 118:102 and the average body mass index was 21.83 ± 3.47 kg/m^2. Residual renal function was 3.98 ± 3.47 mL/min. The causes of ESRD were as follows: chronic glomerulonephritis (n = 71; 32.3%), diabetic nephropathy (n = 32; 14.5%), hypertensive nephropathy (n = 9; 4.1%), and other/unknown (n = 107; 48.6%). Based on the first PET results, there were 97 patients (44.1%) in the H/HA group, and 123 patients (55.9%) in the L/LA group. Comparisons of clinical characteristics between the two groups are shown in Table 3.

Distribution of IL-6 and TIE2 polymorphisms in different transport groups

The distribution of IL-6 and TIE2 genotypes in the peritoneal transport groups are summarized in Tables 4 and 5. Distributions of the 24 alleles (12 polymorphisms) were within the Hardy–Weinberg equilibrium. For the IL-6 polymorphism, there was a statistically

significant correlation between AT genotype of rs13306435 and peritoneal transport group (p = 0.023). For the TIE2 polymorphism, the distribution of rs10967789 and rs639225 genotypes differed significantly between the two groups (p = 0.039 and 0.047, respectively).

Parameters for peritoneal solute transport rate for different genotypes

We further compared the peritoneal solute transport rate among groups with the three SNPs (rs13306435, rs10967789, and rs639225). We found no statistically significant difference in the data of D/P creatinine and D/D0 glucose among different genotypes (Table 6).

Roles of IL-6 and TIE2 gene polymorphisms in predicting initial peritoneal high transport status

With possible clinical factors controlled in a multiple logistic regression model, the genotype AT in IL-6 Rs13306435 and CC in TIE2 Rs639225 were both negatively associated with a higher initial peritoneal transport status (IL-6 Rs13306435: OR=0.408, 95% CI, 0.227~0.736; TIE2 Rs639225: OR = 0.188, 95% CI, 0.044~0.806) (Table 7).

Discussion

The relationship between gene polymorphisms and disease has been gaining greater attention by researchers. It has been shown that SNPs of IL6 may influence the development of cardiovascular disease[28], cancer[29,30], fractures[31], and autoimmune diseases[32-34]. In contrast, there is less available research regarding TIE2 gene polymorphisms, except for a study on the relationship between rs638203/ rs639225 and vascular malformations[35].

In this study, we investigated the effect of genetic polymorphisms of IL-6 and TIE2 on the baseline peritoneal transport property. Results showed that IL-6 and TIE2 gene

polymorphisms were both negatively associated with initial high transport status. The genotypes of rs13306435 and rs639225 were shown to be independent predictors of initial high transport status in PD patients.

Initial transport status can determine the patients' dialysis prescription, which may influence the outcome for PD patients. Previous studies have shown an association between initial high transport status and poor outcome. Although icodextrin and APD have been shown to improve the prognosis of high transporters, most of the PD patients in developing countries are unable to use them. In China, for instance, icodextrin has not been approved for sale yet, and few patients can afford the cost of APD therapy. Therefore, predicting the baseline transport status is important to select a better treatment strategy. Our study provided a potential solution to predict initial high transport status before beginning PD.

There have been several genetic studies of peritoneal solute transport rate in PD patients. Polymorphisms of endothelial nitric oxide synthase[16], receptor of advanced glycation end products[17], and transforming growth factor –βl[18] were reported to be involved in baseline transport status. In 2005, Gillerot et al. showed that the SNP of IL-6 (rs1800795) influenced baseline peritoneal permeability in Caucasian PD patients[15]. Additionally, Hwang et al. reported that the rs1800795 polymorphism was associated with dialysate IL-6 concentration and baseline peritoneal transport status in Korean PD patients[19]. However, for the Chinese Han population, the minor allele frequency (MAF) of rs1800795 was reported to be very low (MAF = 0.02). Thus, it might not be appropriate to directly apply these results to this population. Our finding similar to those of the aforementioned studies. This supports the role of the IL-6 polymorphism in predicting baseline peritoneal transport.

Angiogenesis in the peritoneum could directly increase the effective solute exchange area and influence the transport characteristics. Previous studies have found that polymorphisms of vascular endothelial growth factor were not associated with initial peritoneal transport type[15,

[17]. Ang/Tie2 has recently been confirmed to play an important role in angiogenesis in the peritoneum[26]. Whether the polymorphisms of TIE2 are involved in initial peritoneal transport status remains unknown. In this study, we carefully selected nine SNPs with MAF > 0.05 in the Chinese Han population to investigate their possible role in predicting high transport status. Results showed that TIE2 could be a new factor in predicting the baseline transport type. This study is the first to explore the possible association between TIE2 gene polymorphisms and characteristics of peritoneal transport. Rs639225 is located in exon 13 of TIE2. A study reported its association with venous malformation and presumed that this polymorphism might cause abnormal splicing of *TIE2* into a defective protein[28]. Functional validation of this polymorphism is warranted in the future.

There are some limitations to our study. The TA genotype of rs13306435 presents in only 7% of the total population; therefore, it is not the main determinant of peritoneal transport in most patients. Additionally, this study was conducted at a single centre and the number of cases was limited; increasing the sample size would improve this study. Research has shown that the IL-6/TIE2 concentration was associated with baseline transport status. We hypothesized that the SNPs may participate in the formation of high transport status by influencing the dialysate IL-6/TIE2 concentration. We did not examine the dialysate IL-6/TIE2 concentration in this study. As shown in Table 6, we did not find any statistically significant difference in the data of D/P creatinine and D/D0 glucose between the different genotypes. We believe this may be due to the limited sample size.

In conclusion, IL-6 and TIE2 polymorphisms are associated with baseline peritoneal transport property. Functional study of the polymorphisms is required in the future.

Author contributions statement

Li Ding, Xinghua Shao and Zhaohui Ni contributed to conception and design, Li Ding, Xinghua Shao, Liou Cao1, Wei Fang, Hao Yan, Jiaying Huang, Aiping Gu, Zanzhe Yu, Chaojun Qi, Xinbei Chang and Zhaohui Ni contributed to acquisition of data, or analysis and interpretation of data; Li Ding, Xinghua Shao, Liou Cao1, Wei Fang, Hao Yan, Jiaying Huang, Aiping Gu, Zanzhe Yu, Chaojun Qi, Xinbei Chang and Zhaohui Ni contributed to drafting the manuscript or revising it critically for important intellectual content. All authors reviewed the manuscript.

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Competing interests

The authors declare that they have no competing interests.

Data sharing statement

Technical appendix, statistical code and dataset are available from the corresponding author.

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Tables

Table 1 Primer Sequences of IL6 for Genotyping Using SNapShot Assay

SNP	PCR-L	PCR-R	Target
rs1800795	AACCTCCTCTAAGTGGGCTG	GGTGGGGCTGATTGGAAAC	TCCCCCTAGTTGTGTCTTGC
rs1800796			CCAGGCAGTTCTACAACAGCC
rs13306435	GAAGGGTCCTACTCAGAGCA	GTTGGGTCAGGGGTGGTTAT	TTCCTTCAGGCAAAGAATCTAGA

Table 2 Primer Sequences of TIE2 for Genotyping Using SNapShot Assay

SNP	PCR-L	PCR-R	Target
rs10967717	TTTCCTCTGGTGGGTAGGAA	GGGCTACTGGGATCTCTGAC	GAGGAGTATAATGATTTCCTCAGGC
rs667867			CCACATGGTTTGAATTGGGA
rs639225	TTCTTCCTCCTCAACCAGAAA	TCACATCAACGTGCTGGTCT	CAATATTGTCCAAGAAATCACAGC
rs542913	ACGGGTGGGTCTGTTTCTC	GAGGCTTGCCTAAGGGAAAT	CATTCTCCTTTGCACATTTGC
rs3737188	GATTGTCCCGAGGTCAAGAG	TTTCCCAGGGCACACAGTAT	TTGTCCCGAGGTCAAGAGGTGTA
rs2273719	TGGCACTGTTTGTCTTCCAG	ACCGGCTGACTTTGCTAGAG	AGGCACACCCTACTGCGG
rs2273718			AGTCTGTAGCCCTGGGGCA
rs10967789	ATGGGCTGAAATCAGAATGC	AACCTGTACTATCAGGGTCATTG	AATGCTATTAAATGTTTTCCTGTGT
rs9987817	CTGGGTGACATTTGGGAGAC	CACTCCTGGATGAGACGTGA	GATGAGACGTGAGTAGGCAAGA

Table 3 Comparison of Clinical Characteristics and Peritoneal Parameters between the PeritonealTransport Groups of 220 Peritoneal Dialysis Patients

	L/LA (n=123)	H/HA (n=97)	*P*
Age (y)	52.85±14.94	52.27±14.42	0.761
Male (%)	68(55.3)	60(61.8)	0.291
BMI (kg/㎡)	21.82±3.58	21.85±3.03	0.939
DM (%)	22(17.8)	23(23.7)	0.266
Hypertension (%)	94(68.1)	75(70.1)	0.740
Periods between operation and initial PET(d)	48(39,69)	47(35,68)	0.924
Hemoglobin (g/L)	104.60±22.67	99.13±21.29	0.056
Serum albumin (g/L)	36.37±4.7	34.8±5.33	0.017
hs-CRP (mg/L)	2.65(0.71,3.83)	2.58(1,3.86)	0.657
ACEI/ARB(%)	64(52)	53(54.6)	0.624
D4/D0 (glu)	0.44±0.08	0.34±0.08	＜0.01
D/P Cr	0.55±0.08	0.74±0.07	＜0.01
RRF(ml/min)	4±2.92	3.95±2.63	0.901
nPCR[g/(kg.d)]	1.05±0.6	0.96±0.26	
UF(ml)	160(-200,520)	80(-45,500)	0.004
Urine volume (ml)	1000(560,1400)	1000(500,1500)	0.217
Kt/V	2.23±0.58	2.20±0.56	0.647

Table 4 IL-6 Gene Polymorphisms in Two Groups

Rs13306435	L/LA (n=123)	H/HA (n=97)	P
AT	14	3	0.023
TT	109	94	
Rs1800796			
CC	77	61	0.986
CG	37	28	
GG	9	8	
Rs1800795			
GG	121	96	0.506
GC	2	1	

Table 5 TIE2 Gene Polymorphisms in Two Groups

	L/LA (n=123)	H/HA (n=97)	P
Rs10967717			
AA	15	16	0.652
AG	56	47	
GG	52	34	
Rs10967789			
CC	90	83	0.039
CG	33	14	
Rs2273718			
AG	14	4	0.081
GG	109	93	
Rs2273719			
AA	4	2	0.868
AG	36	27	
GG	83	68	
Rs3737188			
CC	6	1	0.373
CT	44	31	
TT	73	65	
Rs542913			
CC	100	80	0.403
CT	22	17	
TT	1	0	
Rs639225			
CC	40	18	0.047
CT	53	46	
TT	30	33	
Rs657867			
AA	112	87	0.732
AG	11	10	
Rs9987817			
CC	97	74	0.730
CT	22	22	
TT	4	1	

Table 6 The parameters for peritoneal solute transport rate according to different genotypes

IL-6 Rs13306435	AT	TT	P
D/P creatinine	0.61±0.99	0.63±0.12	0.357
D/D0 glucose	0.41±0.092	0.39±0.085	0.424
TIE2 Rs639225	CC	CT/TT	P
D/P creatinine	0.61±0.11	0.64±0.11	0.056
D/D0 glucose	0.40±0.09	0.39±0.08	0.518
TIE2 Rs10967789	CC	CG	P
D/P creatinine	0.64±0.12	0.61±0.09	0.187
D/D0 glucose	0.39±0.09	0.40±0.06	0.839

Table 7 Multivariate Logistic Regression Model to Identify Factors Associated with High/High-Average Transport Status

	OR	95%CI	P
TIE2 Rs639225 (CC vs. CT/TT)	0.188	0.044~0.806	0.024
IL-6 Rs13306435 (AT vs. TT)	0.408	0.227~0.736	0.043
Age	0.966	0.930~1.004	0.081
Male	1.401	0.519~3.788	0.506
DM	3.28	0.952~11.360	0.060
Periods between operation and initial PET(d)	0.996	0.987~1.005	0.401
hs-CRP(mg/L)	1.081	0.964~1.212	0.182
Serum albumin (g/L)	0.898	0.796~1.014	0.083
TIE2 Rs10967789 (CC vs. CG)	1.061	0.371~1.632	0.197
Hemoglobin (g/L)	0.984	0.796~1.014	0.192