Original Article

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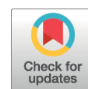

KIDNEY RESEARCH AND CLINICAL PRACTICE

Genetic variants of interferon lambda-related genes and chronic kidney disease susceptibility in the Korean population

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Background: Chronic kidney disease (CKD) is a common condition leading to renal dysfunction and is closely related to increased cardiovascular and mortality risk. CKD is an important public health issue, and recent genetic studies have verified common CKD susceptibility variants. This research examines the interrelationship between candidate genes polymorphisms of interferon lambda (*IFNL*) induction, its signaling pathway, and CKD.
Methods: Seventy-five patients with advanced CKD and 312 healthy subjects (as controls) participated in this research. A replication set composed of 172 patients with advanced CKD and 365 controls was used for additional analysis. The genotype of single nucleotide polymorphisms (SNPs) was determined by the Axiom Genome-Wide Human Assay and SNaPshot assay.
Results: The SNP of *IFNL3* was significantly associated with CKD in the codominant (p = 0.02) and dominant models (p = 0.02). In addition, the SNPs of *IFNL2* were significantly associated with CKD in the dominant model (p = 0.03), and the SNP of interferon alpha receptor 2 (*IFNAR2*) was significantly associated with CKD in the log-additive model (p = 0.03). Concerning rs148543092, in the *IFNL3* gene, a significant association was observed after pooling the original and replication sets.
Conclusion: These results indicate that SNPs in the *IFNL* induction and signal pathway may be associated with CKD risk in the Korean population. Finally, our results also show that the *IFNL3* gene variant may be associated with CKD risk.

Keywords: Chronic renal insufficiency, DNA replication, Interferon type III, Single nucleotide polymorphism

Introduction

Chronic kidney disease (CKD) is a worldwide health problem. The overall prevalence of CKD globally is estimated to be 11% to 13% [1,2], and CKD is a major risk factor for cardiovascular diseases and all-cause mortality [3]. Addi-

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tionally, CKD has become a socioeconomic and medical issue for global healthcare [4]. Therefore, it is paramount to identify individuals that are at risk for the development and progression of CKD.

A significant association between large numbers of genes, their polymorphisms, and kidney function was observed in genetic studies. Therefore, it can be concluded that a strong genetic component exists in CKD [5,6]. The pathogenesis of CKD is complex and dependent on a broad spectrum of diverse etiologies. A major pathophysiology of CKD is persistent, chronic inflammation [7]. In the active phase of inflammation, immune cells migrate to the injury site, resolve the damage, and initiate the healing process. However, persistent inflammation is problematic, as it can lead to tissue damage and fibrosis. In addition, chronic inflammation is associated with various diseases including CKD [8].

Interferon (IFN), a marker of inflammation, may play a role in CKD development. However, the role of IFN in CKD is not well understood. Type I IFNs are central mediators of antiviral immunity and kidney inflammation [9]. Although type III IFN, known as IFN lambda (IFNL), has several similarities in function with type I IFNs, little is known about the role of IFNL in CKD.

The IFNL signaling pathway is initiated as IFNL binds to the heterodimeric IFNL receptor. The IFNL receptor is composed of interleukin 28 receptor alpha (IL28RA) and interleukin 10 receptor beta (IL10RB) subunits, where the Janus kinase (JAK)-signal transducer and activator of the transcription (STAT) signaling cascade induces hundreds of IFN-stimulated genes (RIG-1-like receptor, toll-like receptor [*TLR*], nuclear factor kappa-light-chain-enhancer of activated B cells, *IL-28RA*, *IL-10RB*, *JAK1*, tyrosine kinase 2, *STAT*, IFN regulatory factor [*IRF*], IFN-stimulated response element, IFN-induced GTP-binding protein Mx1, and 2′-5′-oligoadenylate synthetase) [10].

This study explores the association between IFNL induction and signaling pathway candidate genes consisting of *IFNL3*, *IFNL2*, IFN alpha receptor 1 (*IFNAR1*), *IFNAR2*, *TLR9*, *IL22*, *IL-10RB*, *IRF7*, *JAK2*, and *STAT3* polymorphisms and CKD.

Methods

Study subjects

This study enrolled 90 patients with CKD who were distributed by the Keimyung Human Bio-Resource Bank in 2012. In addition, 312 control subjects who participated in health checkup programs from the health promotion center from July to October 2008 participated in this study. The control group was defined as those with no clinical evidence for kidney impairment, cancer, hypertension, diabetes mellitus, dyslipidemia, and cardiovascular diseases. Among the 90 patients with CKD, 75 (83.3%) had an estimated glomerular filtration rate (eGFR) of less than 15 mL/min/1.73 m^2. Since these patients could not represent the entire CKD group, we excluded patients with eGFR values above 15 mL/min/1.73 m^2. A replication set consisting of 172 patients with advanced CKD and 365 controls was used for additional analysis.

Samples from 172 patients with advanced CKD were consecutively distributed by the Keimyung Human Bio-Resource Bank in 2018, and the controls were collected at the health promotion center of the Keimyung University Dongsan Medical Center (Daegu, Korea). Written informed consent was obtained from all the subjects. The approved protocol from the Institutional Review Board of the Keimyung University Dongsan Medical Center was used for this study (No. 2018-02-029).

Clinical characteristics and biomedical measurement

Participants' clinical characteristics, such as systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured. The body mass index (BMI) was calculated by weight divided by the square of the height (kg/m^2).

Biochemical markers were measured using samples in the fasted state. The levels of fasting blood sugar (FBS), triglyceride, total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, aspartate aminotransferase, alanine aminotransferase, albumin, blood urea nitrogen (BUN), creatinine, and uric acid were measured using an auto-analyzer (ADVIA2400 Chemistry System; Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA). eGFR was calculated using the simplified prediction equation derived from chronic

kidney epidemiology collaboration (modification of diet in renal disease): eGFR = 175 × standardized $Scr^{-1.154}$ × $age^{-0.203}$ × 0.742 [if female], where GFR is expressed as mL/min/1.73 m^2 of the body surface area and serum creatinine is expressed in mg/dL [11]. CKD was defined as eGFR of <60 mL/min/1.73 m^2 for 3 months or more.

Single nucleotide polymorphism selection and genotyping of the interferon lambda-related gene single nucleotide polymorphisms

Seventeen single nucleotide polymorphisms (*IFNL3* gene 2SNPs, *IFNL2* gene 2SNPs, *IFNAR2* gene 2SNPs, *TLR9* gene 2SNPs, *IL-22* gene 2SNPs, *IL10RB* gene 2SNPs, *IFNAR1* gene 1SNP, *IRF7* gene 1SNP, *JAK2* gene 1SNP, and *STAT3* gene 1SNP) of the IFNL-related gene were selected based on database searches (http://ncbi.nlm.nih.gov/SNP). SNPs with <0.05 minor allele frequency, <0.1 heterozygosity, and unknown genotype frequencies in Asian populations were excluded. Human genomic DNA was extracted from peripheral blood samples using the Qiagen DNA Extraction Kit (Qiagen, Tokyo, Japan) and then stored at 20°C. The SNPs of the *IFNL3*, *IFNL2*, *IFNAR2*, *TLR9*, *IL-22*, *IL-10RB*, *IFNAR1*, *IRF7*, *JAK2*, and *STAT3* genes were genotyped by direct sequencing. The following primers for the 17 SNPs were used to amplify the genomic DNA (Table 1). Polymerase chain reaction (PCR) conditions included 32 cycles at 92°C for 30 seconds, 60°C for 50 seconds, and 70°C for 40 seconds. PCR products were identified on 1.5% agarose gel by electrophoresis. Furthermore, the PCR products were sequenced by the DNA analyzer (ABI Prism 3730XL; Applied Biosystems, Foster City, CA, USA) to analyze the genotypes of each SNP. Finally, the genotypes were determined using SeqManII software (DNASTAR Inc., Madison, WI, USA).

Genotyping of replication SNPs was screened using the single base primer extension assay with ABI PRISM SNaPShot Multiplex kit (ABI, Foster City, CA, USA) according to the manufacturer's protocol. Analysis was conducted using the Genemapper software (version 4.0; Applied Biosystems).

Statistical analysis

IBM SPSS version 24 (IBM Corp., Armonk, NY, USA) and R version 3.2.2 (R Foundation for Statistical Computing, Vienna, Austria) were used for statistical analysis. The results were considered statistically significant when $p < 0.05$. Student t test was used for comparisons between the two groups among continuous variables. Additionally, con-

Table 1. Polymerase chain reaction primers of the SNPs in the interferon lambda-related genes

Gene	SNP	Forward	Reverse	Product size
IFNL3	rs148543092	5'-GAGGATATGGTGCAGGGTGT-3'	5'-CTCTATCCTCCTCCCCCAAC-3'	201 bp
IFNL3	rs150748693	5'-GAAGGGTCAGACACACAGGT-3'	5'-GAGCCCAGAACCCAGACAG-3'	152 bp
IFNL2	rs8103362	5'-CCCTCACCTGCTCTTTCTCA-3'	5'-GAGGATATGGTGCAGGGTGT-3'	163 bp
IFNL2	rs59746524	5'-CCCACAGATCCAGCCTCAG-3'	5'-TGTAGGGAGGAGGGGATGG-3'	183 bp
IFNAR2	rs2229207	5'-CAAAGATGCTTTTGAGCCAGA-3'	5'-TTGCTTTCCACTTAACTCCTGA-3'	208 bp
IFNAR2	rs1051393	5'-TTGATCACCTAATGTTGATTTCAGA-3'	5'-AGGGTGGTACTGGGTCCTCT-3'	234 bp
TLR9	rs187084	5'-GCTGGGTGTACATAATTCAGCA-3'	5'-GAGCTCCTTTGCCTGGTCTA-3'	220 bp
TLR9	rs5743836	5'-GGGGTGGGAGGTTTGTAAGA-3'	5'-CTGTTCCCCTGAGTGCTCT-3'	217 bp
IL-22	rs2227513	5'-CTTCTACCTTCCCCGTCACA-3'	5'-GGTCCCCATAAGGAAAGAGC-3'	218 bp
IL-22	rs2227484	5'-GATATATTTACTTCTGCCTTAATTG-3'	5'-GGACCCATGTCCTATATCCTC-3'	220 bp
IL-22	rs2227485	5'-TCCGTGACCAAAATGCTTACTC-3'	5'-ACGTCACTATTAGAGCCCGG-3'	165 bp
IL-10RB	rs8178562	5'-TCAGAAGTTGGCCACTGAGA-3'	5'-CGCCATCATGCCTAGCTAAT-3'	231 bp
IL-10RB	rs2834167	5'-CTCTCTACCTCTTCCGCCGTCTACA-3'	5'-GGTCCCCATAAGGAAAGAGC-3'	223 bp
IFNAR1	Affx-52347487	5'-GAGAAACTGGGGGTCCCCCA-3'	5'-GCTCCGGTGGTAAGGTGC-3'	104 bp
IRF7	Affx-52325648	5'-GCTACACGGAGGAACTGCTG-3'	5'-GCCTCACTGACCTTGGAAGA-3'	218 bp
JAK2	rs77375493	5'-AGCAAGTATGATGAGCAAGCT-3'	5'-ACCTAGCTGTGATCCTGAAACT-3'	163 bp
STAT3	rs113994139	5'-TTCCTTCCCATGTCCTGTGA-3'	5'-CTGGCCGACAATACTTTCCG-3'	203 bp

SNP, single nucleotide polymorphism.

tinuous variables were presented as the mean ± standard deviation. The chi-square test was used for comparison between the two groups among the categorical variables. Categorical variables were presented as the frequency, percentage, and odds ratio (OR) and 95% confidence intervals (CIs) were calculated. To estimate the Hardy-Weinberg equilibrium (HWE), logistic regression analysis for the genetic data was used for SNPStats (http://bioinfo.iconcologia.net/index.php) and IBM SPSS version 24. Allele frequency comparison between the two groups was used for the chi-square test. The associations between SNPs and CKD were estimated by computing the OR and their 95% CI with logistic regression analyses adjusted for age and sex as covariates. In the CKD group, multivariate logistic regression analysis of the gene data and clinical variables was used with R version 3.2.2. Age, sex, BMI, hypertension, diabetes mellitus, and dyslipidemia were adjusted as covariates in multivariate logistic regression.

Results

Demographic and clinical characteristics of the participants

The demographic characteristics and clinical parameters of the study subjects are summarized in Table 2. The original set consisted of 312 control subjects included 157 males and 155 females with a mean age of 46.7 ± 10.3 years. The CKD group was composed of 75 adults and involved 36 males and 39 females with a mean age of 50.2 ± 12.1 years. In the replication set, 365 control subjects included 177 males and 188 females with a mean age of 50.6 ± 13.8 years. The CKD group was composed of 172 adults and included 92 males and 80 females with a mean age of 59.2 ± 15.1 years. In the original and replication sets, the sex distribution of the subjects was not significantly different in the two groups. Additionally, in the original and replication sets, BMI, SBP, DBP, BUN, creatinine, uric acid, FBS, and triglyceride levels in the CKD group were significantly higher compared to the control group. Conversely, eGFR, total protein, albumin, TC, HDL cholesterol, and LDL cholesterol levels in the CKD group were significantly lower compared to the control group. In the control and CKD groups, the genotype distribution of the 17 polymorphic SNPs was in the HWE.

Genotype and allele frequencies of the *IFNL3*, *IFNL2*, *IFNAR2*, *TLR9*, *IL-10RB*, *IL-22*, *IFNAR*, *IRF7*, *JAK2*, and *STAT3* genes' single nucleotide polymorphisms

The SNPs of *IFNL3*, rs148543092 (T > C), were significantly associated with CKD in the codominant and dominant models (T/T vs. T/C and T/T vs. T/C + C/C, p = 0.02, OR = 2.61, 95% CI = 1.28–5.80). The SNPs of *IFNL2*, rs8103362 (A > G), were significantly associated with CKD in the codominant, dominant, and log-additive models (A/A vs. A/G, p = 0.06, OR = 2.61, 95% CI = 1.18–5.80; A/A vs. A/G + G/G, p = 0.03, OR = 2.45, 95% CI = 1.11–5.40; A/A vs. A/G vs. G/G, p = 0.05, OR = 2.17; 95% CI = 1.02–4.63, respectively). The SNP of *IFNAR2*, rs1051393 (G > T), was significantly associated with CKD in the codominant and log-additive models (G/G vs. T/T, p = 0.05, OR = 2.10, 95% CI = 1.00–4.40; G/G vs. G/T vs. T/T, p = 0.047, OR = 1.42, 95% CI = 1.01–2.00, respectively) (Table 3). There was no significant difference in the genotype and allele frequencies between the control and CKD group in the SNP of *TLR9*, rs187084 (T > C), SNP of *IL-22*, rs2227484 (G > A), *IL-10RB* gene polymorphisms (rs8178562 G > A, rs 2834167 A > G), and *IRF7* gene polymorphism (Affix-52325648 T/del). Finally, there were no polymorphisms, but only major allele homozygotes in *IFNAR1* (Affix-52347487), *JAK2* (rs77375493), and *STAT3* (rs113994139) (data not shown). Genotype and allele frequencies of the *IFNL*-related genotype in the replication set are shown in Supplementary Table 1 (available online).

Replication of the *IFNL3*, *IFNL2*, and *IFNAR2* genes' single nucleotide polymorphisms

Comparing genotypic frequencies between cases and controls for all SNPs analyzed achieved a significant nominal value in three polymorphisms located in three genetic regions. We attempted to replicate associations involving *IFNL3*, *IFNL2*, and *IFNAR2* using a second sample set (Table 4).

No significant associations involving *IFNL2* and *IFNAR2* were observed in the replication set. Regarding rs148543092, in the *IFNL3* gene, a significant association was observed after pooling the original and replication sets (p = 0.02, OR = 2.50, 95% CI = 1.14–5.47; p < 0.001, OR = 0.92, 95% CI, 0.89–0.95) (Table 4).

Table 2. Demographic characteristics and clinical parameters for the study population

	Original set			Replication set		
	Control	CKD	p-value	Control	CKD	p-value
No. of patients	312	75	NA	365	172	NA
Age (yr)	46.7 ± 10.3	50.2 ± 12.1	0.01[a]	50.6 ± 13.8	59.2 ± 15.1	<0.001[a]
Male	47.2 ± 10.5	51.8 ± 9.6	NA	50.3 ± 13.6	60.5 ± 14.1	NA
Female	46.2 ± 10.1	48.9 ± 13.9	NA	50.9 ± 14.1	58.8 ± 16.1	NA
Sex						
Male/female	157 (50.3)/155 (49.7)	36 (48.0)/39 (52.0)	0.79[b]	177 (48.5)/188 (51.5)	92 (53.5)/80 (46.5)	0.16[b]
Etiology						
Diabetes mellitus	NA	24 (32.0)	NA	NA	70 (40.7)	NA
Hypertension	NA	4 (5.3)	NA	NA	22 (12.8)	NA
Chronic glomerulonephritis	NA	46 (61.3)	NA	NA	74 (43.0)	NA
Others	NA	1 (1.3)	NA	NA	6 (3.5)	NA
Renal replacement therapy	NA	69 (92.0)	NA	NA	151 (87.8)	NA
Left ventricular hypertrophy	NA	16 (21.3)	NA	NA	34 (19.5)	NA
Body mass index (kg/m^2)	22.5 ± 2.6	23.8 ± 3.6	0.003[a]	22.5 ± 2.9	23.6 ± 4.0	0.003[a]
Systolic blood pressure (mmHg)	109.0 ± 7.2	143.7 ± 21.7	<0.001[a]	109.0 ± 9.0	138.6 ± 22.7	<0.001[a]
Diastolic blood pressure (mmHg)	68.7 ± 5.9	84.7 ± 12.9	<0.001[a]	68.6 ± 6.1	79.1 ± 12.6	<0.001[a]
Blood urea nitrogen (mg/dL)	13.9 ± 3.6	72.8 ± 26.4	<0.001[a]	14.3 ± 3.8	78.1 ± 29.1	<0.001[a]
Creatinine (mg/dL)	0.9 ± 0.2	8.4 ± 3.0	<0.001[a]	0.9 ± 0.2	7.9 ± 3.1	<0.001[a]
eGFR (mL/min/1.73 m^2)	76.7 ± 11.4	6.8 ± 2.7	<0.001[a]	75.9 ± 11.1	7.2 ± 2.7	<0.001[a]
Uric acid (mg/dL)	4.6 ± 1.3	8.5 ± 2.6	<0.001[a]	4.6 ± 1.3	8.7 ± 2.6	<0.001[a]
Fasting blood sugar (mg/dL)	85.8 ± 6.6	128.5 ± 63.6	<0.001[a]	86.6 ± 8.0	125.7 ± 80.1	<0.001[a]
Total protein (g/dL)	7.4 ± 0.4	6.3 ± 0.8	<0.001[a]	7.4 ± 0.4	6.2 ± 0.8	<0.001[a]
Albumin (g/dL)	4.4 ± 0.2	3.5 ± 0.5	<0.001[a]	4.4 ± 0.2	3.5 ± 0.6	<0.001[a]
AST (IU/L)	21.5 ± 5.2	18.6 ± 15.6	0.11[a]	22.0 ± 5.5	20.3 ± 34.8	0.52[a]
ALT (IU/L)	17.6 ± 6.7	18.9 ± 23.7	0.62[a]	17.6 ± 6.7	18.1 ± 29.6	0.81[a]
Total cholesterol (mg/dL)	186.3 ± 25.3	164.2 ± 45.4	<0.001[a]	186.0 ± 26.1	158.7 ± 47.2	<0.001[a]
Triglyceride (mg/dL)	88.2 ± 35.8	123.1 ± 79.7	0.004[a]	91.8 ± 37.9	159.0 ± 127.7	<0.001[a]
HDL cholesterol (mg/dL)	55.4 ± 11.1	42.1 ± 15.2	<0.001[a]	55.2 ± 11.1	44.5 ± 23.2	0.001[a]
LDL cholesterol (mg/dL)	113.0 ± 25.0	99.4 ± 37.9	0.02[a]	113.3 ± 24.9	95.8 ± 41.4	0.006[a]

Data are expressed as number only, mean ± standard deviation or number (%).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NA, not applicable.
The p-values were analyzed using [a]the t test or [b]the chi-square test.

Association of *IFNL3* single nucleotide polymorphism with clinical characteristics

After adjustments for age, sex, BMI, hypertension, diabetes mellitus, and dyslipidemia as covariates, we examined whether the genotype distribution of *IFNL3* gene polymorphism, rs148543092, is associated with clinical characteristics (creatinine, eGFR, uric acid, total protein, and albumin) in the original and replication sets of the CKD group. In addition, in the original and replication sets, creatinine, eGFR, uric acid, total protein, and albumin levels exhibited no significant difference in the genotype distribution (Table 5).

Discussion

This study examines the association between the polymorphisms of *IFNL3* (rs148543092 T > C), *IFNL2* (rs8103362 A > G), *IFNAR2* (rs1051393 G > T), *TLR9* (rs187084 T > C), *IL-22* (rs2227513 T > C), and CKD development in patients

Table 3. Distribution of frequencies of the interferon lambda-related genotype in controls and chronic kidney disease patients in the model of inheritance

Gene	SNP number	Function	Codominant genetic model OR (95% CI)	Codominant p-value	Dominant genetic model OR (95% CI)	Dominant p-value	Recessive genetic model OR (95% CI)	Recessive p-value	Log-additive genetic model OR (95% CI)	Log-additive p-value
IFNL3	rs148543092	Missense	2.61 (1.28–5.80)	0.02	2.61 (1.28–5.80)	0.02	NA	NA	NA	NA
	Thr108Ala		NA	NA	NA	NA	NA	NA	NA	NA
IFNL2	rs8103362	Missense	2.61 (1.18–5.80)	0.06	2.45 (1.11–5.40)	0.03	NA	NA	2.17 (1.02–4.63)	0.05
	Thr112Ala		NA	NA	NA	NA	NA	NA	NA	NA
IFNAR2	rs1051393	Missense	1.53 (0.82–2.88)	0.18	1.68 (0.92–3.06)	0.08	1.59 (0.87–2.90)	0.14	1.42 (1.01–2.00)	0.047
	Phe10Ile		2.10 (1.00–4.40)	0.05	NA	NA	NA	NA	NA	NA
TLR9	rs187084	NearGene-5’	1.38 (0.73–2.62)	0.32	1.56 (0.86–2.84)	0.13	1.61 (0.90–2.86)	0.11	1.41 (0.98–2.01)	0.06
	T-1486C		1.98 (0.97–4.05)	0.06	NA	NA	NA	NA	NA	NA
IL-22	rs2227513	NearGene-5’	1.97 (0.32–12.28)	0.48	1.97 (0.32–12.28)	0.48	NA	NA	NA	NA
	T-111C		NA	NA	NA	NA	NA	NA	NA	NA

CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism; NA, not applicable.

Table 4. Distribution of the frequencies of the *IFNL3* genotype

IFNL3 rs148543092 genotype	Original set[a] CKD (n = 75)	Original set[a] Control (n = 312)	Replication set[b] CKD (n = 172)	Replication set[b] Control (n = 365)
TT	64 (85.3)	291 (93.6)	172 (100)	338 (92.9)
TC	11 (14.7)	20 (6.4)	0 (0)	26 (7.1)
CC	0 (0.0)	0 (0)	0 (0)	0 (0)

Data are expressed as number (%).
TT vs. TC + CC.
CKD, chronic kidney disease.
The p-values were analyzed using the chi-square test: [a]p = 0.02, odds ratio (OR), 2.50 (95% confidence interval [CI], 1.14–5.47); [b]p < 0.001, OR, 0.92 (95% CI, 0.89–0.95).

Table 5. Association of *IFNL3* SNP with the clinical characteristics

SNP	Parameter	Genotype T/T (n = 64)	Genotype T/C + C/C (n = 11)	p-value[a]	Genotype T/T (n = 172)	Genotype T/C + C/C (n = 0)	p-value[b]
rs148543092	Creatinine (mg/dL)	8.32 ± 2.95	8.78 ± 3.64	0.64	7.86 ± 3.14	NA	NA
	eGFR (mL/min/1.73 m^2)	6.79 ± 2.74	6.74 ± 2.89	0.95	7.18 ± 2.68	NA	NA
	Uric acid (mg/dL)	8.53 ± 2.76	8.55 ± 1.86	0.98	8.69 ± 2.62	NA	NA
	Total protein (g/dL)	6.36 ± 0.71	5.90 ± 1.09	0.21	6.25 ± 0.78	NA	NA
	Albumin (g/dL)	3.53 ± 0.45	3.35 ± 0.67	0.25	3.53 ± 0.56	NA	NA

Data are expressed as mean ± standard deviation.
eGFR, estimated glomerular filtration rate; SNP, single nucleotide polymorphisms; NA, not applicable.
The p-values were analyzed using the t test: [a]original set and [b]replication set.

with advanced CKD. The SNPs of *IFNAR2* (rs1051393), *IFNL2* (rs8103362), and *IFNL3* (rs148543092) were significantly associated with CKD. Among them, the frequency of rs148543092 in *IFNL3* was significantly higher in CKD than the control group in the original and replication sets.

Persistent, low-grade inflammation is considered an essential component of CKD, playing an important role in its pathophysiology [12]. Patients with CKD exhibit elevated cytokine levels and dysregulated cytokine metabolism, leading to increased circulating acute-phase proteins [13]. In addition, IFN, an inflammatory cytokine, may play a regulatory role in the development and progression of CKD. However, the role of IFN in CKD is not well understood, especially in IFNL.

Additionally, type III IFN (IFNL) is associated with a cytokine family that has several similarities in functions with type I IFNs (either IFN-α, IFN-β, or IFN-α/β). The four IFNL proteins (IFNL1, IFNL2, IFNL3, and IFNL4) and 17 IFN-α/β proteins (13 IFN-α subtypes, IFN-β, IFN-ω, IFN-ε, and IFN-κ) are encoded by genes in humans [14]. Located in human chromosome 19, genes encoding *IFNL* have a similar gene structure with the 5-exon gene of the IL-10 cytokine family [15]. IFNL has several biological features, which begin with IFNL effectiveness. The efficacy of IFNL is most pronounced in epithelial cells where it explicitly strengthens the immune systems that protect the surface of the upper skin that is exposed to general and pathogenic microorganisms [16]. IFNL is involved in inflammation, one of the main pathophysiologies of CKD, and is expected to affect CKD development. This is the first study to identify the association between IFNL and CKD to the best of our knowledge.

IFNL has emerged as a new immune control cytokine with a particular function controlling damage to maintain an immune balance and limit immunology. In addition, IFNλ limits inflammation to prevent damage to the host by chronic illnesses including asthma, auto-immune diseases, and colitis [17]. The genetic association of *IFNL* gene polymorphisms among humans expands to various illnesses such as allergies, nonalcoholic fatty liver disease, and several other viral diseases caused by human immunodeficiency virus and hepatitis C virus infections [18]. The difference in expression levels by the *IFNL3* genotype was shown in numerous studies. For example, recent research outcomes verified this result in *ex/in vivo* conditions. These results demonstrate that differences in *IFNL3* expression levels by the alleles at the three functional SNPs (rs28416813, rs4803217, and rs59702201) may play a role in the disease [19–21]. Furthermore, a recent study revealed that genetic variants of *IFNL3/4* play an essential role in developing lupus nephritis and systemic lupus erythematosus in the Taiwanese population [22]. However, little is known about the association between *IFNL* and CKD. In the present study, we demonstrate that the SNP of *IFNL3* (rs148543092) is significantly associated with CKD development in patients with advanced CKD. Furthermore, these results are consistent with the entire CKD cohort (Supplementary Table 2, available online).

Additionally, several researchers have reported SNPs of *IFNAR2* in hepatitis B virus (HBV) infections. Specifically, *IFNAR2* polymorphisms may be involved in chronic HBV infection susceptibility among the Thai population [23]. It may also be involved when determining IFN response and predictive markers of HBV infections among the Chinese Han population [24]. Ma et al. [25] reported that the polymorphism of *IFNAR2* (rs1051393 G > T) is a missense changing from phenylalanine to valine. This SNP may be important in the risk of HBV infection by influencing the expression of IFNAR2 protein on the cell's surface, resulting in an antiviral response and damaged signal transduction. Our result also suggests that *IFNAR2* polymorphisms (rs1051393 G > T) are associated with CKD. This research found that the T allele of *IFNAR2* (rs1051393 G > T) was higher in the CKD group compared with the control. The interrelationship of this SNP may be a codominant effect shown by the inheritance analysis model (major allele homozygotes vs. minor allele homozygotes). Therefore, this study indicates that the mechanism underlying the association between *IFNAR2* SNP (rs1051393 G > T) and CKD may control *IFNAR2* expression, which affects the type I IFN effect.

CKD and end-stage kidney disease are featured by increased proinflammatory cytokine levels and inflammatory labeling. Cytokines may control the risk of developing kidney disease [13] and induce resident cells to proliferate and influence metalloproteinases, bioactive lipids, the expression of adhesion receptors, reactive oxygen/nitrogen species, procoagulant activity of the endothelium, and aberrant matrix metabolism. In addition, these molecules may be the action mediators of the renin-angiotensin sys-

tem and hemodynamic factors [26–33]. IL-10, an anti-inflammatory cytokine with numerous functions, is primarily secreted by monocytes and lymphocytes. *IL-22*, an IL-10–related cytokine, activates the upward adjustment of the acute-phase reactor. It also guides JAK/STAT activation in several cell lines, including hepatomas, intestinal epithelial cells, and mesangial cells [34]. Meta-analysis outcomes have shown that the *IL-22* gene rs1179251 polymorphism (but not rs2227485 polymorphism) may be a cancer risk factor [35]. The rs2227485 SNP of *IL-22* may have a connection with the risk and multifocality of primary thyroid cancers according to Eun et al. [36]. However, this research did not show that the association between polymorphisms (rs2227513 T > C; rs2227485 G > A) of the *IL-22* gene and CKD development exhibited an association with rs2227484 polymorphisms.

Furthermore, the second sample set was used to analyze replicate associations involving *IFNL3*, *IFNL2*, *IFNAR2*, *TLR9*, and *IL22*. No significant associations involving *IFNL2*, *IFNAR2*, *TLR9*, and *IL22* were observed in the replication set. Whereas concerning rs148543092, in the *IFNL3* gene, a significant association was observed after pooling the original and replication sets. These results suggest that *IFNL3* polymorphisms are associated with CKD. However, there were no significant differences between the clinical characteristics and genotypes of *IFNL3*.

There are several limitations to this study. First, this study was a single-center study and the sample size was relatively small. However, we performed a genetic analysis of the association between IFNL induction and signal pathway genes, such as *IFNL3*, *IFNL2*, *IFNAR2*, *TLR9*, *IL-22*, and *IL-10RB* and CKD, for the first time. Second, we analyzed advanced CKD rather than entire CKD patients due to the characteristics of our study cohort. However, even when entire CKD patients were analyzed, the same SNP of *IFNL3* was associated with CKD. Third, homozygous genotypes were observed in CKD patients in the replication set. However, the heterozygous genotypes were observed in the original set which indicated that CKD had *IFNL3* polymorphisms.

In conclusion, the outcome of this study indicates the possibility of an association between *IFNL* induction polymorphisms and signal pathway genes with CKD in the Korean population. Furthermore, our results indicate that the *IFNL3* gene variant may be associated with CKD risk. Therefore, early interventions in patients with high-risk genotypes may delay CKD progression. However, further large-scale prospective studies are necessary to establish the role of *IFNL* in CKD.

Conflicts of interest

All authors have no conflicts of interest to declare.

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Authors' contributions

Conceptualization: JHK, DHS, KJ
Data curation: JHK, JHP
Formal analysis: JHK, JHP, GIY
Funding acquisition: KJ
Investigation: GIY
Writing–original draft: JHK, DHS, GIY, SH, WYP, YK
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