Association between *VDR* gene *FokI* polymorphism and renal function in patients with IgA nephropathy

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Background . Studies have shown that the occurrence and development of IgA nephropathy (IgAN) are genetically susceptible, but the relationship between vitamin D receptor (*VDR*) gene polymorphisms and renal function in IgAN patients is unclear. **Methods.** We investigated the relationship between *VDR FokI* (rs2228570) single nucleotide polymorphism (SNP) and renal function and related clinicopathologic parameters in IgAN patients. Clinical and pathological data of 282 IgAN patients treated at the First Affiliated Hospital of Guangxi Medical University were collected, and *FokI* genotypes were determined by PCR and direct sequencing. Patients were divided into the renal dysfunction group and normal renal function (control) group by estimated glomerular filtration rate (eGFR) and serum creatinine level. **Results.** Frequencies of TT genotype and T allele in the renal dysfunction group were higher than those of the control group. Blood urea nitrogen, serum phosphorus (P), proportions of mesangial cell proliferation, interstitial fibrosis/tubular atrophy and crescents in T allele carriers were higher than those in non-T allele carriers, while eGFR and 25-Dihydroxyvitamin D3 were lower in T allele carriers than non-T allele carriers. Multiple linear regression analysis showed that eGFR was affected by *FokI* genotypes in IgAN patients. Logistics regression analysis showed that middle and elderly age, elevated P, intact parathyroid hormone and TT genotype were independent risk factors for renal dysfunction in IgAN patients; the odds ratio of carrying the TT genotype was as high as 84.77 (P<0.05 for all). **Conclusion s.** IgAN patients carrying the *VDR FokI* TT genotype have an increased risk of renal dysfunction. *VDR FokI* SNP is closely related to renal function, calcium-phosphate metabolism and related pathological damage in IgAN patients.

1 **Association between *VDR* gene *FokI* polymorphism and renal function in**

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14 **Abstract**

15 **Background.** Studies have shown that the occurrence and development of IgA nephropathy

16 (IgAN) are genetically susceptible, but the relationship between vitamin D receptor (*VDR*) gene

17 polymorphisms and renal function in IgAN patients is unclear.

18 **Methods.** We investigated the relationship between *VDR FokI* (rs2228570) single nucleotide

19 polymorphism (SNP) and renal function and related clinicopathologic parameters in IgAN

20 patients. Clinical and pathological data of 282 IgAN patients treated at the First Affiliated

21 Hospital of Guangxi Medical University were collected, and *FokI* genotypes were determined by

22 PCR and direct sequencing. Patients were divided into the renal dysfunction group and normal

23 renal function (control) group by estimated glomerular filtration rate (eGFR) and serum

24 creatinine level.

25 **Results.** Frequencies of TT genotype and T allele in the renal dysfunction group were higher

26 than those of the control group. Blood urea nitrogen, serum phosphorus (P), proportions of

27 mesangial cell proliferation, interstitial fibrosis/tubular atrophy and crescents in T allele carriers

28 were higher than those in non-T allele carriers, while eGFR and 25-Dihydroxyvitamin D3 were

29 lower in T allele carriers than non-T allele carriers. Multiple linear regression analysis showed

30 that eGFR was affected by *FokI* genotypes in IgAN patients. Logistics regression analysis

31 showed that middle and elderly age, elevated P, intact parathyroid hormone and TT genotype

32 were independent risk factors for renal dysfunction in IgAN patients; the odds ratio of carrying

33 the TT genotype was as high as 84.77 (P<0.05 for all).

34 **Conclusions.** IgAN patients carrying the *VDR FokI* TT genotype have an increased risk of renal

35 dysfunction. *VDR FokI* SNP is closely related to renal function, calcium-phosphate metabolism

36 and related pathological damage in IgAN patients.

37

38 Introduction

39 As one of the most common primary glomerular diseases, IgA nephropathy (IgAN) results in a

40 high incidence of renal dysfunction, and more than 40% of patients progress to end-stage renal

41 disease (ESRD) within 20 years (Li et al. 2015). Many studies have shown that genetic cause is

42 one of the primary factors affecting IgAN (Magistroni et al. 2015). Recently, genome-wide

43 association studies (GWAS) have identified 20 genetic susceptibility genes of IgAN, which are

44 closely related to the occurrence, progression and prognosis of IgAN.

45 The vitamin D receptor (*VDR*) gene is a crucial mediator of active vitamin D biological activity.

46 Active vitamin D binds to VDR to regulate target gene transcription, thus realizing biological

47 functions such as regulation of calcium-phosphorus metabolism, immunity and inflammatory

48 state (Christensen et al. 2013). *VDR* polymorphisms are associated with occurrence and

49 prognosis of certain diseases, such as chronic kidney disease (CKD), ischemic stroke and

50 malignant tumors (Cho et al. 2018; Prabhakar et al. 2015; Santoro et al. 2013). *FokI* is one locus

51 located in the initiation codon region of *VDR*, which is the only functional locus known to affect

52 VDR protein peptide chain structure (Hu et al. 2017). Previous studies have indicated that *FokI*

53 polymorphism was associated with diabetic nephropathy and lupus nephritis, which increased the

54 susceptibility of chronic renal failure (CRF) (Imam et al. 2017; Razi et al. 2019). However, the

55 relationship between *VDR* polymorphisms and renal function of IgAN patients is unclear. Hence,

56 we investigated the association between *VDR FokI* single nucleotide polymorphism (SNP) and

57 renal function and related clinicopathological damage of IgAN.

58

59 Materials & Methods

60   *Subjects*

61   All subjects were patients treated in the Department of Nephrology at the First Affiliated

62   Hospital of Guangxi Medical University from August 2014 to December 2016, who were

63   diagnosed with IgAN by renal biopsy. Inclusion criteria were patients who met diagnostic

64   criteria of the 2014 edition of the IgAN Guidelines issued by the Japanese Society of Nephrology

65   (Yuzawa et al. 2016) and age ≥16 years. Exclusion criteria were secondary IgAN (secondary to

66   autoimmune diseases such as allergic purpura, systemic lupus erythematosus, dry syndrome,

67   arthritis and psoriasis or secondary to hepatobiliary and gastrointestinal diseases, respiratory

68   diseases, viral infections and tumors) (Saha et al. 2018), patients with severe liver failure,

69   patients with acute cardiovascular diseases or acute cerebrovascular diseases and patients with a

70   history of medications such as steroid hormones, immunosuppressants and active vitamin D

71   preparations/analogs for the past 3 months. The study was approved by the Ethics Committee of

72   the First Affiliated Hospital of Guangxi Medical University (Approval Number: 2019KY-E-006),

73   and all participants were aware of the purpose of this study and provided written informed

74   consent. Ultimately, 282 subjects were included in this study.

75   *Clinical data*

76   Basic information of subjects was collected by questionnaires. Height, weight and vital signs

77   were recorded. Body mass index (BMI) was calculated by the following formula: BMI = weight

78   (kg)/height^2 (m^2). Pulse pressure was calculated by the following formula: pulse pressure =

79   systolic blood pressure (SBP) − diastolic blood pressure (DBP).

80   Peripheral venous blood (4 mL) was obtained from each participant and used to examine liver

81   and kidney function, electrolytes, iPTH, 25-Dihydroxyvitamin D3 [25(OH)$_2$D3] and 25-

82   Dihydroxyvitamin D2 [25(OH)$_2$D2]. We also collected 24-hour urine volume for urine protein

83   quantitation. Morning urine samples (approximately 5 mL) were used for routine urine testing.

84   *Clinical grouping*

85   eGFR was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation

86   according to serum creatinine (Scr), sex and age (Levey et al. 2017). eGFR <60 mL/min/1.73 m^2

87   and/or Scr >104 μmmol/L in males or Scr >84 μmmol/L in females was considered to indicate

88   renal dysfunction. Subjects were then divided into the renal dysfunction group (n=156) and the

89   control group (normal renal function group, n=126) for analyses.

90   ***DNA amplification, sequencing and genotyping***

91   Genomic DNA was extracted from peripheral blood leukocytes using the phenol-chloroform

92   method (Nan et al. 2016). Based on gene sequences provided by the National Center for

93   Biotechnology, specific primers were devised and aligned by Primer 5.0 software (Premier

94   Company, Canada). The primer pair rs2228570-3F: 5′-TGGGTGGCACCAAGGATG-3′ and

95   rs2228570-3R: 5′-CCTTCATGGAAACACCTTGC-3′ was synthesized by Shanghai Sangon

96   Biological Engineering Technology & Services Co., Ltd., China. PCR was performed in 40-μL

97   volume reactions comprising 20 μL of master mix, 17 μL of water, 1 μL of each upstream and

98   downstream primer and 1 μL of DNA template. PCR cycle conditions were as follows: initial

99   denaturing at 94°C for 5 min, followed by 35 cycles of denaturing at 94°C for 30 s, annealing at

100   55°C for 30 s and elongation at 72°C for 30 s. Amplification was completed with a final

101   extension at 72°C for 5 min. PCR products were visualized on 2.0% agarose gels (Fig. 1). *FokI*

102   genotype was determined by direct sequencing of all PCR products and performed by Shanghai

103   Sangon Bioengineering Company (partial nucleotide sequences of CC, CT and TT genotypes are

104   shown in Fig. 2).

105   ***Diagnosis and staging criteria of renal pathology***

106   IgAN was diagnosed according to renal pathology. IgAN is characterized by proliferation of

107   mesangial cells, and IgA is dominant or co-dominant in glomerular deposition, which is usually

108   accompanied by complement C3 and varying amounts of IgG and/or IgM (Kiryluk & Novak

109   2014).

110   Renal pathology staging was based on the Oxford classification and crescent scores. Scores were

111   defined as follows: M1, >50% of the mesangial area consisting of more than 3 mesangial cells,

112   otherwise scored as M0. E1, narrowed glomerular capillary cavities due to cell proliferation,

113   otherwise scored as E0. S1, any degree of injury in capillary fistula excluding injury to the entire

114   glomerulus or adhesion, otherwise scored as S0. Interstitial fibrosis/tubular atrophy (IFTA) was

115   defined as follows: T0, 0%–25%; T1, 26%–50%; and T2, >50%. Crescents were scored as

116   follows: C0, no crescent; C1, crescents in <25% of the glomerulus; and C2, crescents in ≥25% of

117   the glomerulus (Trimarchi et al. 2017).

118 *Statistical analysis*

119   Statistical analyses were performed using SPSS version 21.0 (SPSS, Chicago, IL, USA). Data

120   with a normal distribution were expressed as mean ± standard deviation (SD) or percentages.

121   Data with a non-normal distribution were presented as the median and quartile. The chi-squared

122   test was used to determine Hardy–Weinberg equilibrium (HWE). The t-test and chi-squared test

123   were used to determine differences of indicators between two groups. Comparison of clinical

124   indicators among different genotypes was analyzed by variance analysis; distribution of

125   pathological staging among different genotypes was compared by the chi-squared test. Multiple

126   linear regression analysis and binary logistic regression analysis were used to analyze risk factors

127   associated with renal dysfunction in IgAN. P<0.05 was considered to be statistically significant

128   in all analyses.

Results

130   We enrolled 282 IgAN patients; the sex ratio (male:female) was 1:1.19 (129/153), mean age (±

131   SD) was 33.67±10.75 years and 156 patients had renal dysfunction (55.32%). There were no

132   significant differences in age and sex between renal dysfunction and control groups. Compared

133   with the control group, SBP, DBP, pulse pressure, BUN, Scr, UA, 24-hour urine protein

134   quantitation (24hUPro) and iPTH levels of the renal dysfunction group were higher, while eGFR,

135   Hb, $25(OH)_2D3$ and $25(OH)_2D2$ were lower (P<0.05 for all). Detailed information is shown in

136   Table 1.

137   Distribution of *FokI* genotype and allele frequencies was consistent with HWE in enrolled IgAN

138   patients (P>0.05, Table 1). Frequencies of CC, CT and TT genotypes were 19.15%, 50.35% and

139   30.50%, and frequencies of C and T alleles were 44.33% and 55.67%, respectively. Frequencies

140   of TT genotype and T allele in the renal dysfunction group were significantly higher than those

141   of the control group (P<0.0001).

142   Comparison of clinical and pathological indicators between different genotypes is shown in

143   Table 2. There were significant differences in BUN, UA, P, eGFR, $25(OH)_2D3$, $25(OH)_2D2$,

144   extent of mesangial cell hyperplasia, capillary intravascular hyperplasia, IFTA and crescents

145   among different genotypes. BUN and P of T allele carriers (TT+CT) were higher than those of

146   non-T allele (CC) carriers, while eGFR, $25(OH)_2D3$ and $25(OH)_2D2$ levels of T allele carriers

147 were lower than those of non-T allele carriers. Furthermore, proportions of M1, T2 and C2 of T

148 allele carriers were higher than those of non-T allele carriers (P<0.05 for all).

149 Multiple linear regression analysis showed that eGFR was closely associated with *FokI*

150 genotypes (P<0.05, Table 3). Additionally, eGFR was also affected by various factors such as

151 age, BMI, Hb, 25(OH)$_2$D3 and pathological stage (P<0.05 for all). Logistic regression analysis

152 showed that TT genotype may be a significant factor of renal dysfunction in IgAN after adjusting

153 for sex, age, P, iPTH and other factors [odds ratio (OR)=84.77, P<0.0001], and the risk of renal

154 dysfunction in TT genotype carriers was 84.77-fold higher than that in CC genotype carriers with

155 IgAN. Furthermore, age >40 years (OR=9.60, P=0.005), serum P (OR=15.68, P=0.001) and

156 iPTH (OR=1.13, P<0.0001) were independent risk factors of renal dysfunction in IgAN patients.

157 Detailed information is shown in Table 4.

158 **Discussion**

159 IgAN accounts for 40%–47.2% of primary glomerular nephritis in China and is one of the most

160 common primary glomerular diseases worldwide and an important cause of CRF (Wang et al.

161 2015; Wyatt & Julian 2013). Our results indicated that 55.32% of IgAN patients have renal

162 dysfunction, which is similar to the proportion of primary IgAN patients with renal failure in

163 India (47%) (Chowdry et al. 2018). We also found that the *VDR* gene was closely related to

164 elevated serum iPTH and P, and elevated iPTH and P were independent risk factors for renal

165 dysfunction in IgAN. Similar to our results, studies showed that secondary hyperparathyroidism

166 (SHPT) and hyperphosphatemia not only affect renal progression but also increase the mortality

167 of CKD, especially in ESRD (Ritter & Slatopolsky 2016). Therefore, the *VDR* gene may be

168 closely related to renal function and prognosis of IgAN patients.

169 Many studies have confirmed that IgAN has ethnic and regional differences and family

170 aggregation tendencies (Cox et al. 2017; Schena & Nistor 2018). Genetic causes are important

171 mechanisms of IgAN occurrence and progression. A study from China including 613 IgAN adult

172 patients created the best predictive model of IgAN progression consisting of four loci

173 (rs11150612, rs7634389, rs2412971 and rs2856717). According to the genetic risk score, the risk

174 of disease progression in IgAN patients with moderate and high genetic risk was 2.12- and 3.61-

175 fold higher than that of IgAN patients with low genetic risk, respectively (Shi et al. 2018). Our

176 study indicated that the frequency of TT genotype in the renal dysfunction group was higher than

177   that in the control group, and eGFR of T allele carriers was lower than that of non-T allele

178   carriers. Thus, TT genotype is an independent risk factor of renal dysfunction. *VDR FokI* SNP is

179   closely associated with renal dysfunction in IgAN patients, which also supported the concept that

180   genetic factors play an important role in IgAN renal function and progression. A previous study

181   has shown that *VDR FokI* polymorphism is associated with risk of CRF in Asians (Li et al. 2018),

182   which is also supported by our findings.

183   The *VDR* gene is located on the long arm of chromosome 12, with a total length of about 75 kb,

184   and contains 11 exons and several introns. *VDR* polymorphisms can encode different proteins

185   and cause various physiological effects. Studies have confirmed that *VDR* polymorphisms affect

186   CKD occurrence and progression such as diabetic nephropathy, lupus nephritis and hypertensive

187   renal damage (Mahto et al. 2018; Yang et al. 2017). Our study also showed that *VDR FokI* is

188   associated with renal dysfunction in IgAN patients. Renal function of IgAN may be influenced

189   by *VDR* polymorphisms through the following reasons and mechanisms. First, *VDR* SNPs affect

190   mRNA quality and stability by interfering with *VDR* mRNA expression and splicing,

191   subsequently affecting VDR protein number and/or activity, and transactivates VDR protein and

192   target gene(s) (Karonova et al. 2018). Second, *VDR* polymorphisms may affect CKD occurrence

193   and development such as IgAN by inhibiting the anti-inflammatory activity of active vitamin D,

194   protecting endothelial cells and promoting mesangial cell proliferation, podocyte loss and

195   tubulointerstitial fibrosis (Yang et al. 2012). The *FokI* locus is located on the transcription

196   initiation site, and SNPs in this region can change the length of the amino acid sequence. When

197   the genotype is T/T, the initiation codon in *VDR* is mutated from ATG to ACG, which results in

198   loss of translation function and ultimately changes its biological functions such as proliferation

199   and division (Beysel et al. 2018). This may be one of the potential molecular mechanisms by

200   which *FokI* TT genotype carriers are more likely to suffer from renal dysfunction in IgAN

201   patients. Ethnic differences in *VDR FokI* polymorphism and the influence of environmental,

202   geographic, dietary or occupational factors must be further elucidated.

203   Recently, with the development of molecular genetics and the completion of the Human Genome

204   Project, greater attention has been paid to the pathogenesis of genetic factors in IgAN patients.

205   To date, several GWAS have been carried out in IgAN patients of different ancestries, and at

206   least 20 susceptibility loci have been identified, including human leukocyte antigen gene,

207 complement factor H-related protein genes and tumor necrosis factor superfamily member 13

208 (Han et al. 2016; Yang et al. 2018; Zhu et al. 2015). However, the clinical application value is

209 rarely found. Our study found that VDR gene polymorphism was associated with renal

210 insufficiency of IgAN. Recent studies have shown that active vitamin D and its preparation can

211 significantly alleviate renal dysfunction in CKD patients, reduce proteinuria, inhibit the secretion

212 of parathyroid hormone and delay the progression to end-stage kidney disease and all-cause

213 mortality (Gluba-Brzozka et al. 2018; Melamed et al. 2018). Furthermore, active vitamin D and

214 its preparation are commonly administered to treat CKD-related mineral and bone metabolism

215 (MBD), which improve the prognosis in CKD patients. Active vitamin D is not only an effective

216 therapy for CKD but also a drug that can be safely administered for prolonged periods. As the

217 necessary receptor of active vitamin D, VDR should have significant clinical applying prospects.

218 Our study found serum P of T allele carriers was higher than that of non-T allele carriers. *VDR*

219 *FokI* polymorphism was shown to be associated with MBD (Bouksila et al. 2018). The T (or F)

220 site of FokI protein is three amino acids shorter than the f (or C) site. Therefore, the affinity for

221 ligand, mRNA stability and transport activity of F are higher than those of f (Elias et al. 2018),

222 subsequently increasing intestinal calcium absorption, promoting calcium and phosphorus

223 deposition in bone and affecting serum Ca and P levels. Simultaneously, SHPT can also inhibit

224 phosphate reabsorption in renal proximal tubules. Studies have shown that *VDR* expression may

225 be affected by calcium homeostasis and adequate calcium intake may offset the influence of

226 genetic factors of *VDR* on bones (Moran et al. 2015). This may explain why we observed no

227 significant difference in calcium levels between different genotypes. Our study also found that

228 25(OH)$_2$D3 of T allele carriers was lower than that of non-T allele carriers. Previous studies have

229 shown that 25(OH)D deficiency is significantly associated with renal pathology severity and

230 increases the risk of kidney progression (Li et al. 2016). Thus, the T allele of *VDR FokI* may be a

231 susceptive allele for renal dysfunction and progression of IgAN.

232 Our study also found that there are different degrees of change in mesangial cell proliferation,

233 capillary proliferation, IFTA and crescent lesions between genotypes; proportions of M1, T2 and

234 C2 of patients with TT genotype were higher than those of non-TT carriers. Several studies have

235 indicated a significant correlation between mesangial hyperplasia, IFTA, crescent and renal

236 dysfunction and progression in IgAN patients (Bao et al. 2014; Xie et al. 2018). Combined with

237 our findings, IgAN patients carrying *FokI* TT genotype or T allele likely have more serious renal

238   pathological damage. The loss of VDR can lead to renal fibrosis by affecting perirenal

239   inflammation and epithelial-mesenchymal transition (EMT) (Ito et al. 2013). *VDR FokI* SNP

240   may influence renal pathology by affecting the quantity or activity of active vitamin D and VDR,

241   which could inhibit EMT, mesangial proliferation and podocyte loss.

242   Several limitations exist in this study. First, the study was a cross-sectional study with small

243   sample size. Thus, we cannot verify the direct causal relationship between *FokI* polymorphism

244   and renal function. Second, we only evaluated one locus of the *VDR* gene and did not investigate

245   the influence of inheritance of other *VDR* SNPs. Finally, the study did not conduct a dietary

246   questionnaire survey and could not exclude potential effects of diet on indicators related to

247   calcium-phosphorus metabolism. Therefore, large-scale cohort studies should be conducted to

248   confirm our findings and explore additional *VDR* SNPs.

249

250   Conclusions

251   IgAN patients have a higher prevalence of renal dysfunction. Patients with the *FokI* TT genotype

252   are likely to have renal dysfunction, calcium-phosphorus metabolism disorder, mesangial

253   proliferation, IFTA and crescents in IgAN. *VDR* may be a susceptibility gene for renal

254   dysfunction in IgAN, and *VDR* SNP is closely related to clinical symptoms and pathological

255   damage in IgAN patients.

256

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260

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395

Figure 1

Representative electrophoresis gel of PCR for *VDR FokI* (rs2228570) polymorphism

Lane M: 100-bp marker ladder; Lanes 1–12: PCR products from 12 different DNA samples (292 bp).

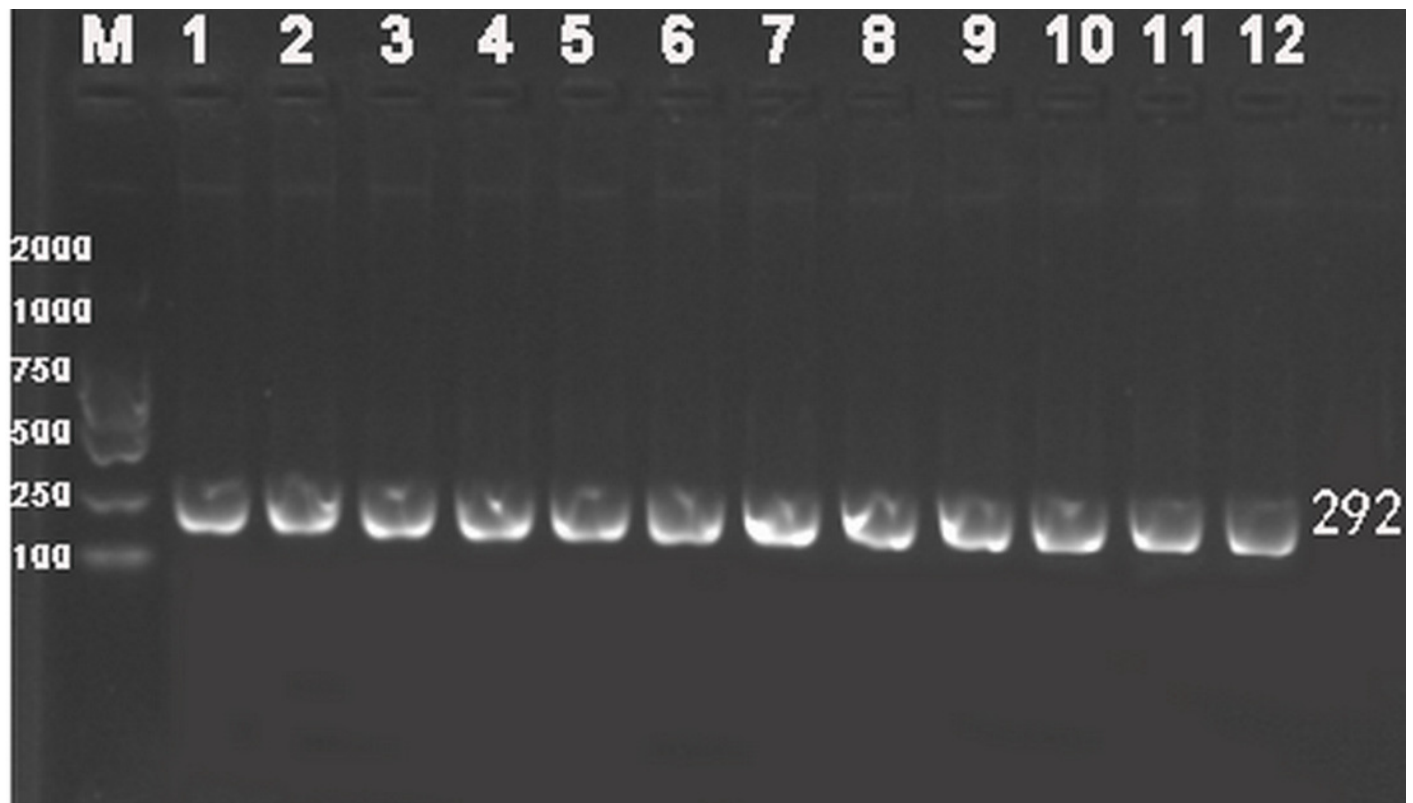
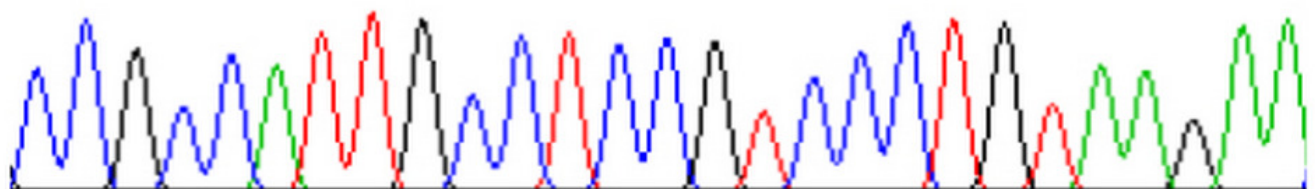
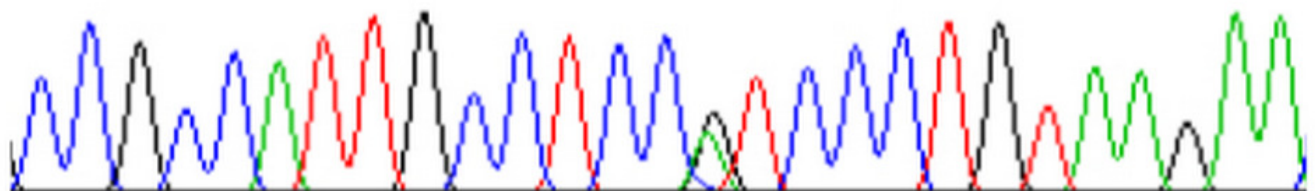
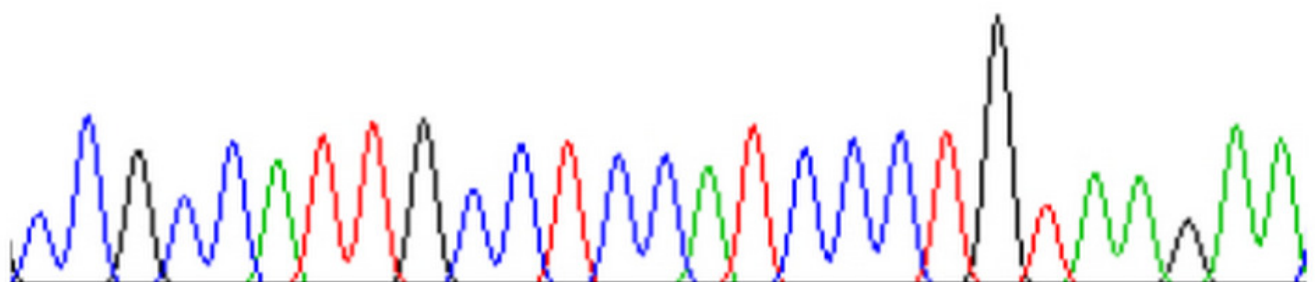



Figure 2

Chromatograms of direct sequencing for *VDR FokI* (rs2228570) polymorphism

CC: CC genotype; CT: CT genotype; TT: TT genotype.

Clinical characteristics and genotype and allele frequencies of IgAN patients

25(OH)$_2$D2, 25-Dihydroxyvitamin D2; 25(OH)$_2$D3, 25-Dihydroxyvitamin D3; 24hUpro, quantitative 24-hour urinary protein; BMI, body mass index; BUN, blood urea nitrogen; Ca, calcium; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; iPTH, intact parathyroid hormone; P, phosphorus; SBP, systolic blood pressure; Scr, serum creatinine; UA, uric acid.

1 **Table 1. Clinical characteristics and genotype and allele frequencies of IgAN patients**

Parameters	Total	Renal dysfunction	Control	$t\ (\chi^2/Z)$	p
Number	282	156(55.32%)	126(44.68%)		
Male/female	129/153	69/87	60/66	2.406	0.121
Age(year)	33.67±10.75	34.58±9.89	32.55±11.66	1.585	0.114
BMI(kg/m^2)	22.06±3.39	21.75±3.42	22.44±3.32	-1.707	0.089
SBP(mmHg)	132.5±17.97	140.85±13.89	122.36±17.20	9.764	**<0.0001**
DBP(mmHg)	81.33±12.69	86.64±11.19	74.76±11.32	8.816	**<0.0001**
Pulse pressure(mmHg)	51.25±10.92	54.21±10.56	47.60±10.28	5.289	**<0.0001**
eGFR(ml/min/1.73 m^2)	76.49±35.91	53.63±21.11	104.79±29.79	-16.260	**<0.0001**
Hb(g/L)	120.4±20.58	116.86±23.46	124.79±15.30	-3.414	**0.001**
BUN(mmol/L)	5.87±2.46	6.74±2.76	4.78±1.41	7.687	**<0.0001**
Scr(μmol/L)	116.2±99.27	153.2±120.95	70.50±13.88	8.476	**<0.0001**
UA(μmol/L)	400.06±112.81	452.85±97.83	334.71±94.66	10.264	**<0.0001**
Ca(mmol/L)	2.19±0.14	2.18±0.13	2.21±0.16	-1.215	0.226

P(mmol/L)	1.36±0.48	1.34±0.39	1.38±0.58	-0.665	0.507
iPTH(mmol/L)	51.45(16.26)	55.76(25.72)	41.75(14.67)	-6.891	**<0.0001**
24hUPro(g/d)	1.71±1.54	2.08±1.20	1.26±1.78	4.426	**<0.0001**
25(OH)$_2$D3(nmol/L)	41.89±19.61	38.91±18.39	45.68±20.52	-2.759	**0.006**
25(OH)$_2$D2(nmol/L)	4.39±2.04	4.08±1.93	4.77±2.12	-2.693	**0.008**
Genotypes					
CC[n(%)]	54(19.15)	15(9.62)	39(30.95)		
CT[n(%)]	142(50.35)	76(48.72)	66(52.38)		
TT[n(%)]	86(30.50)	65(41.66)	21(16.67)	31.042	**<0.0001**
Alleles				HWE(P)	0.734
C[n(%)]	250(44.33)	106(33.97)	144(57.14)		
T[n(%)]	314(55.67)	206(66.03)	108(42.86)	30.322	**<0.0001**

2 25(OH)$_2$D2, 25-Dihydroxyvitamin D2; 25(OH)$_2$D3, 25-Dihydroxyvitamin D3; 24hUpro,

3 quantitative 24-hour urinary protein; BMI, body mass index; BUN, blood urea nitrogen; Ca,

4 calcium; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; Hb,

5 hemoglobin; iPTH, intact parathyroid hormone; P, phosphorus; SBP, systolic blood pressure; Scr,

6 serum creatinine; UA, uric acid.

Comparison of clinical and pathological characteristics between IgAN patients by genotypes

25(OH)$_2$D2, 25-Dihydroxyvitamin D2; 25(OH)$_2$D3, 25-Dihydroxyvitamin D3; 24hUpro, quantitative 24-hour urinary protein; BUN, blood urea nitrogen; Ca, calcium; DBP, diastolic blood pressure; E, capillary hyperplasia; eGFR, estimated glomerular filtration rate; IFTA, interstitial fibrosis/tubular atrophy; iPTH, intact parathyroid hormone; M, mesangial cell proliferation; P, phosphorus; S, segmental glomerular sclerosis; SBP, systolic blood pressure; Scr, serum creatinine; UA, uric acid.

1 **Table 2. Comparison of clinical and pathological characteristics between IgAN patients by**

2 **genotypes**

Parameters	CC	CT	TT	$F(\chi^2)$	p
SBP(mmHg)	134.22±22.15	129.23±17.27	137.09±15.03	5.579	**0.004**
DBP(mmHg)	79.78±12.23	81.71±13.07	81.69±12.41	0.500	0.607
Pulse pressure (mmHg)	54.44±11.80	47.52±9.55	55.41±10.44	18.958	**<0.0001**
BUN(mmol/L)	5.50±2.41	6.31±2.90	6.20±2.14	3.343	**0.037**
Scr(μmol/L)	100.94±49.37	111.01±79.61	134.59±141.45	2.330	0.099
UA(μmol/L)	370.44±100.86	399.88±132.50	418.97±75.41	3.115	**0.046**
eGFR(ml/min/1.73 m^2)	81.30±37.37	80.72±36.13	66.47±32.87	4.954	**0.008**
24hUPro(g/d)	1.59±1.96	1.81±1.59	1.61±1.08	0.697	0.499
Ca(mmol/L)	2.16±0.19	2.20±0.14	2.21±0.09	1.879	0.155
P(mmol/L)	1.20±0.32	1.44±0.54	1.33±0.44	5.254	**0.006**
iPTH(mmol/L)	50.73±15.48	55.40±28.37	58.16±18.33	0.763	0.468

$25(OH)_2D3$(nmol/L)	47.88±8.59	39.88±21.56	39.61±22.91	4.187	**0.016**
$25(OH)_2D2$(nmol/L)	5.02±0.90	4.17±2.23	4.16±2.40	4.380	**0.014**
M					
M0[n(%)]	39(72.22)	106(74.65)	36(41.86)		
M1[n(%)]	15(27.78)	36(25.35)	50(58.14)	26.925	**<0.0001**
E					
E0[n(%)]	42(77.78)	136(95.77)	83(96.51)		
E1[n(%)]	12(22.22)	6(4.23)	3(3.49)	21.198	**<0.0001**
S					
S0[n(%)]	24(44.44)	48(33.80)	24(27.91)		
S1[n(%)]	30(55.56)	94(66.20)	62(72.09)	4.048	0.132
IFTA					
T0[n(%)]	39(72.22)	69(48.59)	77(89.53)		
T1[n(%)]	15(27.78)	64(45.07)	6(6.98)		
T2[n(%)]	0(0)	9(6.34)	3(3.49)	43.907	**<0.0001**

Crescent					
C0[n(%)]	45(83.33)	127(89.44)	83(96.51)		
C1[n(%)]	9(16.67)	12(8.45)	0(0)		
C2[n(%)]	0(0)	3(2.11)	3(3.49)	15.326	**0.004**

3 25(OH)$_2$D2, 25-Dihydroxyvitamin D2; 25(OH)$_2$D3, 25-Dihydroxyvitamin D3; 24hUpro,
4 quantitative 24-hour urinary protein; BUN, blood urea nitrogen; Ca, calcium; DBP, diastolic
5 blood pressure; E, capillary hyperplasia; eGFR, estimated glomerular filtration rate; IFTA,
6 interstitial fibrosis/tubular atrophy; iPTH, intact parathyroid hormone; M, mesangial cell
7 proliferation; P, phosphorus; S, segmental glomerular sclerosis; SBP, systolic blood pressure; Scr,
8 serum creatinine; UA, uric acid.

Relationship between eGFR and relative risk factors in IgAN patients

25(OH)$_2$D3, 25-Dihydroxyvitamin D3; Alb, albumin; BMI, body mass index; BUN, blood urea nitrogen; DBP, diastolic blood pressure; E, capillary hyperplasia; eGFR, estimated glomerular filtration rate; Hb, hemoglobin; IFTA, interstitial fibrosis/tubular atrophy; iPTH, intact parathyroid hormone; M, mesangial cell proliferation; P, phosphorus; S, segmental glomerulosclerosis; SBP, systolic blood pressure; UA, uric acid.

1 **Table 3. Relationship between eGFR and relative risk factors in IgAN patients**

Parameters	Risk factors	B	Std.error	Beta	*t*	*p*
eGFR	BUN	-5.108	0.011	-0.423	-463.547	**<0.0001**
	SBP	-4.668	0.012	-2.271	-398.449	**<0.0001**
	UA	0.088	0.000	0.292	206.940	**<0.0001**
	Age>40	94.215	0.180	0.885	524.790	**<0.0001**
	BMI	-4.498	0.036	-0.447	-124.938	**<0.0001**
	M	24.518	0.073	0.369	335.974	**<0.0001**
	S	-22.264	0.072	-0.277	-311.333	**<0.0001**
	Alb	-7.961	0.013	-1.234	-628.707	**<0.0001**
	IFTA	31.241	0.146	0.542	213.292	**<0.0001**
	$25(OH)_2D3$	1.036	0.002	0.567	483.723	**<0.0001**
	P	-58.854	0.123	-0.574	-480.129	**<0.0001**
	iPTH	-1.946	0.004	-0.862	-553.134	**<0.0001**
	DBP	5.674	0.013	1.860	439.998	**<0.0001**

E	-10.019	0.109	-0.062	-91.619	**<0.0001**
Hb	0.065	0.002	0.041	38.457	**<0.0001**
FokI genotypes	9.469	0.067	0.181	142.337	**<0.0001**
Crescent	-20.396	0.130	-0.264	-156.677	**<0.0001**

2   25(OH)$_2$D3, 25-Dihydroxyvitamin D3; Alb, albumin; BMI, body mass index; BUN, blood urea
3   nitrogen; DBP, diastolic blood pressure; E, capillary hyperplasia; eGFR, estimated glomerular
4   filtration rate; Hb, hemoglobin; IFTA, interstitial fibrosis/tubular atrophy; iPTH, intact
5   parathyroid hormone; M, mesangial cell proliferation; P, phosphorus; S, segmental
6   glomerulosclerosis; SBP, systolic blood pressure; UA, uric acid.

Analysis of risk factors related to renal dysfunction in IgAN patients

iPTH, intact parathyroid hormone; P, phosphorus.

1 **Table 4. Analysis of risk factors related to renal dysfunction in IgAN patients**

Risk factors	Renal dysfunction	
	OR (95% CI)	*P*
FokI genotypes		
CC	1	
CT	2.79(0.72-10.80)	0.136
TT	84.77(13.61-528.01)	**<0.0001**
Age>40	9.60(1.98-46.47)	**0.005**
P	15.68(3.20-76.83)	**0.001**
iPTH	1.13(1.08-1.19)	**<0.0001**

2 iPTH, intact parathyroid hormone; P, phosphorus.