No association of vitamin D binding protein gene polymorphisms with ulcerative colitis in Chinese patients

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Abstract

Object Vitamin D (VD) deficiency has been reported in patients with ulcerative colitis (UC), and polymorphism in the gene encoding the vitamin D binding protein (DBP) can affect the characteristics of DBP, thus affecting the level and function of VD in vivo . Previous studies have rarely reported on the potential relationship between DBP polymorphisms and UC. To investigate the associations between genetic variants in DBP genes and UC susceptibility in the Han Chinese population, in order to discern whether any differences exist between this population and those of other countries.

Methods In this case-control study, the genotyping of DBP rs4588 and rs7041 polymorphisms was conducted using polymerase chain reaction (PCR)-ligase detection reactions, and the rs4588 and rs7041 genotypes were detected by PCR-restriction fragment length polymorphism.

Results In our case-control cohort, no significant difference was observed in the UC risk for either of the two SNPs (rs4588 and rs7401) in the DBP genes ($P > 0.05$). No association between UC susceptibility and the DBP gene haplotypes was found either.

Conclusions Our results suggest that the two SNPs (rs4588 and rs7401) in the DBP genes may have no correlation with susceptibility to UC in the Chinese Han population. But interestingly, haplotype GC, which contains the rs4588 and rs7041 variants in the DBP gene, may affect the level of oxidative stress in UC patients, especially the level of MDA.

Introduction

Ulcerative colitis (UC) is a chronic, non-specific inflammatory disease of the colon and rectum with unknown aetiology, the incidence of which has been increasing worldwide over time[1]. The lesions, which are mainly localized in the colonic mucosa and submucosa, appear most commonly in the sigmoid colon and rectum. The pathogenesis of UC is multifactorial, involving genetic predisposition, environmental factors, dietary changes, immune disorders, and intestinal mucosal barrier dysfunction[2, 3]. The main clinical manifestations of UC are diarrhoea, mucus-like bloody stools, abdominal pain, a prolonged course with repeated attacks, and this affects each patient's physical and mental health and quality of life[4].

Vitamin D (VD), which comprises a group of fat-soluble molecules, is converted into $1,25(OH)_2D_3$ in the body by two hydroxylation reactions, after which the converted form plays a biological function[5]. In addition to its well-known roles in calcium absorption and phosphorus metabolism, VD is also associated with the occurrence and development of UC[6]. In fact, VD deficiency has been reported in 60% of patients with inflammatory bowel disease[7]. This vitamin also plays a role in intestinal health mainly by resisting infection and regulating the immune response, including maintaining the balance of intestinal flora and the barrier function of the intestinal epithelium[8].

In the process of VD metabolism, the vitamin D binding protein (DBP) binds and transports VD and its metabolites to target organs, while also regulating the level of VD in the body[9–11]. Polymorphisms in the DBP gene can affect the characteristics of the protein it encodes, thus affecting the level and function of VD *in vivo.* DBP is an α2-glycosylated globulin[12] whose encoding gene, which is highly polymorphic and contains 13 exons and 12 introns, is located at positions 12 to 13 of the long arm of chromosome 4. The DBP is 458 amino acids long, and the two most functionally important single nucleotide polymorphisms (SNPs) in it are rs7041 and rs4588. Rs7041 is a missense mutation of GAT-GAG, which changes aspartic acid at position 416 to glutamic acid[9], and rs4588 is a missense mutation of ACG-AAG, which changes threonine at position 420 to lysine. There is linkage disequilibrium between these two loci, and their haplotype forms the most important three polymorphisms in the DBP gene, namely Gc1F, Gc1S, and Gc2(Table 1)[13, 14]. It is also known that different genotypes of the DBP and VD affect their binding affinities, with the affinity of VD metabolites to Gc1F being the strongest, and *vice versa* for Gc2. The stronger the affinity, the more VD exists in its combined form, a consequence of which is that free VD is reduced, and active VD entering the target tissue is reduced accordingly.

However, to the best of our knowledge, previous studies on VD have rarely reported on the potential relationship between DBP polymorphisms and UC. We herein conducted a case-control study to identify associations between DBP gene variants and UC susceptibility in the Han Chinese population to determine whether any differences exist between this population and those from other countries.

Materials And Methods

Study population

In this case-control study, we recruited 70 patients with UC and 84 healthy controls from December 2016 to December 2018 in Shanxi Province, China (The calculation formula of sample size obtained that 70 samples were needed for each group). The specific exclusion and inclusion criteria are shown in (Table 2). Informed consent was obtained from all participants before the study commenced. The protocol, which was approved by the Ethical Committee of Shanxi Medical University, China, is in accordance with the Declaration of Helsinki statements. The study questionnaire (which included demographic data and any factors affecting VD levels *in vivo,* such as milk consumption and outdoor sunlight exposure) was collected from the recruited volunteers, and the 5 ml of fasting venous blood collected from each volunteer into a heparinized tube was centrifuged. The plasma, red blood cells and white blood cells from each sample were collected and stored at −80°C for later use.

Genomic DNA extraction and genotype analysis

Peripheral blood DNA was extracted strictly according to the instructions from the QIAamp DNA mini kit (QIAGEN, Germany), and the recovered samples were stored at −20°C for preservation. DBP rs4588 and rs7041 characterisation was conducted using polymerase chain reaction-ligase detection reactions (PCR-

LDRs) with TaqMan genotyping assays on the 3730XL DNA analyser (ABI Co., USA). The sequences of the forward and reverse primers were 5'-GTT TTT CAG ACT GGC AGA GCG−3' and 5'-ACA CCA GGA AAA GCC TGT CAC−3', respectively, and the annealing temperature of the reactions was 60°C. The length of the amplified product was 259 bp.

Index detection

The plasma levels of $25(OH)D_3$, $1,25(OH)_2D_3$, DBP, C-reactive protein, and various inflammatory biomarkers (tumour necrosis factor (TNF)-α, interleukin (IL)−1β, IL−6, IL−17, IL−23) in the two groups were measured by enzyme-linked immunosorbent assay kits according to the manufacturer's (Shanghai Bioswamp Co., China) instructions. The levels of myeloperoxidase (MPO), malondialdehyde (MDA) and superoxide dismutase (SOD) in the two groups were detected using biochemical kits (Nanjing Jiancheng Biological Co., China), the instructions of which were strictly followed.

Statistical analysis

All statistical analyses were performed using the SPSS 22.0 statistical package. The measurement data were expressed as mean±SD, and the counting data were expressed as frequencies (%). Analysis of variance (ANOVA) and the chi-square test were used for continuous variables and categorical variables, respectively. For continuous variables that did not satisfy the test for normality or homogeneity of variance, the Mann-Whitney U test in the nonparametric test was adopted and the results were expressed as median values and upper and lower quartiles [median(Q_{25}-Q_{75})]. Hardy-Weinberg equilibrium was tested using the chi-square test and SHEsis software to analyse linkage imbalance and haplotypes. When the haplotype distribution frequency was less than 0.03, the software would automatically ignore the comparison between the two groups. Logistic regression analysis was used for the multivariate analysis, the results of which were considered statistically significant at $P < 0.05$ (two-tailed).

Results

- Study population and VD influencing factors

Our comparison of the baseline characteristics and VD influencing factors between the case and control groups is shown in Table 3. There were 70 patients in the case group and 84 in the control group. The median age was 49 years (range, 40.00−59.25 years) in the case group, and 46.50 years (range, 36.25−54.75 years) in the control group. There were 31 (44.3%) males and 39 (55.7%) females (23 females were postmenopausal women) in the case group and 32 (38.1%) males and 52 (61.9%) females (31 females were postmenopausal women) in the control group. Excluding body mass index (BMI) ($P < 0.05$), there was no significant differences in the values shown in Table 3 ($P > 0.05$).

- Serum VD, DBP, inflammatory factors and oxidation indexes

The analysis results for serum VD, DBP, inflammatory factors and antioxidant indicators in the case and control groups are shown in Table 4. Among these results, the levels of the case group's serum DBP, TNF-α and IL−1β were higher than those of the control group ($P < 0.05$), whereas the serum 25(OH)D$_3$, 1,25(OH)$_2$D$_3$, IL−23, MPO and SOD levels of the control group were higher than those of the case group ($P < 0.05$).

- DBP gene sequencing results and Hardy-Weinberg equilibrium testing

Sequence analysis of the PCR product from the rs4588 variant in the DBP gene revealed three sequence types (AA, CC and AC), with AA and CC being single peaks, and AC the overlapping peak (Figure 1 A, B and C). The gene sequence for rs7041 was found to be GG, TT, and GT (Figure 1 D, E and F).

The distributions results for the rs4588 and rs7041 genotypes are shown in Table 5. The genotypes for these two gene loci in the case and the control groups were evaluated using a Hardy-Weinberg equilibrium test, the results of which generated a value of P < 0.05, indicating that the selected population met the study requirements and was representative of the population as a whole (Table 5).

- DBP polymorphisms and UC in the study groups

The genotype distributions and allele frequencies of the DBP gene variants rs4588 and rs7041 in the control and case groups are shown in Table 6, the differences of which were not statistically significant ($P > 0.05$).

- Linkage disequilibrium and haplotype analyses

The linkage imbalance analysis showed that D' = 1.000 and r^2 = 0.162 (Figure 2) between rs4588 and rs7041. We speculated that there is a chain disequilibrium reaction between rs4588 and rs7041. Haplotype analysis showed that there was no significant difference in frequency among the three main haplotypes between the case group and the control group ($P > 0.05$, Table 7).

- Haplotype analysis results

The results of the analysis on VD, DBP, inflammatory factors and antioxidant indexes for the different haplotypes in the case and control groups are shown in Table 8. In the case group, the different haplotypes differed in their MDA distributions ($P = 0.014$), but there was no significant difference in the other indicators ($P > 0.05$).

- Logistic regression analysis

Grouping was used as the dependent variable in the logistic stepwise regression analysis, with the cutoff value of α$_{in}$ = 0.05, α$_{out}$ = 0.10, a model likelihood ratio of ^{2} = 270.097, and statistical significance set at $P < 0.001$. Age, DBP, VD levels, haplotypes, serum inflammation markers and oxidative antioxidant levels

were examined. Finally, a model with 6 independent variables showed statistical significance, and gender and $1,25(OH)_2D_3$ were found to be protective factors. The results from the model are shown in Table 9.

Discussion

UC, one of the main manifestations of inflammatory bowel disease (IBD), most commonly presents with abdominal pain, diarrhoea, and purulent mucus discharge with blood in the stools from patients. A few patients with this condition present with severe clinical disease of rapid inset, the progression of which develops quickly. [15]The clinical signs can include the frequent passage of bloody stools, up to 30 times a day, as well as high fever, anaemia, nutritional disorders and weight loss. In addition to these signs, the present study also found that the BMI of patients in the case group was lower than that of the control group. In healthy people, the higher the BMI, the lower the VD level, and the lower the BMI, the higher the VD level, because of the fat dilution effect[16].

However, the morbid weight loss seen in patients with UC will reduce the VD level. One study found that more than half of its UC patients were low in VD[17]. Our investigations found that the levels of $25(OH)D_3$ and $1,25(OH)_2D_3$ in patients with UC were lower than those in healthy people, but the opposite was found for DBP levels. The relationship between DBP and vitamin D concentration is unclear[18]. We hypothesized that the level of DBP needed for VD to function in the body is small when VD levels are low (as in the case group); consequently, the amount of free DBP is large when VD levels are low. When normal VD levels are present, as in the control group, more DBP is needed and there is less free DBP in the serum. Hence, people with UC will have higher DBP levels than healthy individuals, but lower VD levels.

Modern studies have argued that the pathogenesis of UC is multifactorial, and that imbalanced cytokine regulation is an important cause of this condition[19, 20]. TNF-α is produced by a variety of immune cells, epidermal cells, endothelial cells and fibroblasts, and is involved in inflammatory and immune responses[21]. IL−1β, which is produced by activated macrophages as proproteins, is an important mediator of inflammatory responses and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis. It has been shown that IL−1β is elevated in patients with UC and Crohn's disease and is also associated with disease severity[22]. A number of studies have reported that IL−23 is a key mediator of intestinal inflammation, which can cause a cascade reaction of inflammatory factors in the intestinal tract, resulting in increased expression levels of IL−17, IL−6 and TNF-α in the intestinal tract[23, 24]. TNF-α, IL−1β, and IL−23 are key factors in the pathogenesis of UC, and we found higher levels of TNF-α and IL−1β in the case group than in the control group, but lower levels of IL−23[25−28].

Many studies have shown that MPO expression in the intestinal mucosa is positively correlated with the severity of intestinal inflammation in UC, which can be used as a monitoring index for disease severity in UC patients[29]. MPO is abundant in neutrophils, which use hydrogen peroxide as an oxidant to oxidize tyrosine to tyrosyl. Hypochlorous acid and tyrosyl are both cytotoxic and are used by neutrophils to kill bacteria and other pathogens. MPO defence as an endogenous enzyme may delay this type of oxidative

damage, but exogenous antioxidants are required[30]. SOD, a catalytic enzyme, is also anti-inflammatory and can decompose reactive oxygen into oxygen and hydrogen peroxide, thereby preventing cell aging and reducing damage to the intestinal mucosa[31]. The levels of MPO and SOD in the control group were higher than those in the case group. This finding suggests that patients with UC have higher levels of oxidative stress.

It is well known that VD is widely involved in a variety of biological activities in the body, especially chronic diseases. Polymorphisms in VD pathway-related genes have been extensively studied in cancer, chronic inflammation, autoimmune diseases, and lipid metabolism disorders[9, 32, 33]. In recent years, some studies have highlighted the importance of VD in the pathogenesis of IBD[34, 35]. DBP is an important component in the vitamin D signalling pathway and has multiple functions in the body, including transport of VD, regulation of inflammatory reactions *in vivo* and immune regulation[36].It plays a key role in signal transduction, and its GC coding gene polymorphism exists in more than 2000 SNP loci, and polymorphisms in these loci may affect the structure and concentration of the protein, leading to disease onset[10, 37].

In the present study, we investigated the association between DBP variants and UC disease using a case-control design with 84 controls and 70 cases from the Chinese Han population. We found no significant association between the polymorphisms and risk of UC for genotype or haplotype. Our findings are consistent with a previous report by Luo et al[38]. Additionally, our logistic regression analysis further confirmed that the DBP gene haplotypes were independently correlated with UC after adjustment for other confounders.

A variety of factors affect the level and activity of VD during its activation, including the DBP, and most VD in the circulation is combined with the DBP. The biological activity of VD may depend mainly on this free component[12]. Therefore, the gene encoding DBP is a key candidate for the VD pathway and could play an important role in maintaining the overall level of VD and regulating the amount of free VD available. Mutations in the DBP gene cause changes in serum DBP levels, which are associated with changes in plasma VD concentrations[39–41]. A recent study reported that two SNPs in the DBP gene (rs7041 and rs4588) can produce three common variants affecting the amount of free VD metabolites. In addition, the same study also determined that SNPs in DBP (rs7041, rs4588 and rs2282679) were the genetic determinants of reduced 25(OH)D levels[42].

More than 120 DBP gene variants have been found through gene sequencing, and the existence of these variants affect the function of DBP, thus affecting the affinity between $25(OH)D_3$ and DBP, making the ability of the DBP to bind VD differ significantly in the variants[43]. Previous studies have shown that DBP gene variants are associated with the risk of autoimmune diseases, such as thyroid autoimmune diseases, diabetes and asthma[44–46]. In a European case-control study, the DBP gene with a homozygous rs4588 SNP was significantly associated with the risk of Crohn's disease. Similarly, haplotype Gc2, which consists of rs4588 and rs7401 variants, appears to be a protective factor in the UC group[47]. There are few reports on DBP gene polymorphism and UC susceptibility in the Asian

population. The association between genetic variation in the DBP and IBD has been reported mainly in a European cohort[47]. Our results suggest that there is no association between these variants and the risk of UC. As there is little evidence of a link between DBP variation and IBD susceptibility, multicentre studies will be needed to confirm these results.

In the present study, we found an interesting result. Specifically, in the patients with UC, the haplotypes are significantly associated with the MDA level, and the highest level of MDA is found when the haplotype is CG. Studies have shown that UC is often accompanied by a certain degree of oxidative stress injury, which stimulates the excessive generation of oxygen free radicals, leading to a decline in the activity of SOD, and inducing an increase in MDA concentration and aggravating the disease[48]. At the same time, MDA can stimulate the production of arachidonic acid, cyclooxygenase and other proinflammatory substances, and induce the release of TNF-α. MDA levels can indirectly indicate the degree of damage caused by oxygen free radicals to tissue cells[49]. TNF-α does not only target tumours, but also releases inflammatory mediators and promotes the necrosis of intestinal epithelial cells[50, 51]. Therefore, one possible explanation for our results is that when DBP gene polymorphism occurs, oxygen free radicals in the body are induced to act on MDA, and the peroxides produced by MDA can cause DBP gene variation[52–54]. Consequently, haplotype CG, which comprises rs4588 and rs7401 variants, may be a risk factor for the UC group.

Conclusion

In summary, our study is the first to reveal the relationship between the DBP gene and UC in the Han Chinese population. The results show that haplotype GC, which comprises rs4588 and rs7041 variants of the DBP gene, may affect the level of oxidative stress in UC patients, especially their MDA levels. However, the results need to be replicated and confirmed in a multi-centre study.

Study limitations

Our study has some limitations. The number of participants included was small, especially the patients. The source range of the population was also narrow, so its representativeness is not ideal. In addition, the limited economic conditions meant that we only examined two loci in the DBP gene, and the confounding factors were not considered comprehensively. Therefore, a large-scale study, detection of more disease indicators, and comprehensive consideration of the impact of confounding factors are all needed to reach a decisive conclusion.

Abbreviations

VD: vitamin D

UC: ulcerative colitis

DBP: vitamin D binding protein

PCR: polymerase chain reaction

SNPs: single nucleotide polymorphisms

PCR-LDRs: polymerase chain reaction-ligase detection reactions

TNF: tumour necrosis factor

IL: interleukin

MPO: myeloperoxidase

MDA: malondialdehyde

SOD: superoxide dismutase

ANOVA: analysis of variance

IBD: inflammatory bowel disease

Declarations

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Consent for publication

Agree to published.

ACKNOWLEDEGMENTS

Ethical approval and consent to participate: The protocol, which was approved by the Ethical Committee of Shanxi Medical University, China. All procedures performed in studies involving human participants

were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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Tables

Table 1 Relationship between common DBP genotypes, single nucleotide polymorphisms and corresponding amino acids

4209(rs4588)	416(rs7041)	
	Asp(T)	Glu(G)
Thr(C)	Gc1F	Gc1s
Lys(A)	Gc2	rare

Table 2 Inclusion and exclusion criteria for the case-control group

Variable	Inclusion criteria	Exclusion criteria
Control group	a. Male or female over 18 years old, volunteer to join the study, and sign the informed consent; b. Those who do not meet the criteria for case diagnosis.	a. Those who are currently participating in other clinical trials or have participated in other clinical trials in the past 3 months b. Pregnant or lactating women; c. taking any medicine for nearly three months.
Case group	a. Men or women over the age of 18 years old who volunteer to join the study and sign informed consent; b. UC has been confirmed by colonoscopy, pathology, laboratory examination and clinical manifestations.	a. Accompanied by heart, liver, kidney and hematopoietic system and other serious primary diseases and mental illness patients; b. Those who are currently participating in other clinical trials or have participated in other clinical trials in the past 3 months; c. Pregnancy or lactation women; d. Taking any vitamin preparations and hormones in the past three months.

Table 3 Comparison of baseline characteristics and VD influencing factors between the case and control groups

Factors	Control group(N=84)	Case group(N=70)	t / c2/Z	P
Age[median(Q25-Q75);years]	46.50(36.25-54.75)	49.00(40.00-59.25)	-1.324	0.187
Gender[n(%)]	32□38.1%□	31□44.3%□	0.605	0.437
	52□61.9%□	39□55.7%□		
BMI(mean±SD)	23.62±2.44	22.08±3.39	-2.948	0.003*
Female menopause[n(%)]	31□59.6%□	23□59.0%□	0.004	0.951
	21□40.4%□	16□41.0%□		
Seasonal situation 3 months before sampling[†][n(%)]	60□71.4%□	46□65.7%□	0.581	0.446
	24□28.6%□	24□34.3%□		
Outdoor exposure time in the sun[n(%)]	30□35.7%□	25□35.7%□	-0.088	0.930
	37□44.0%□	30□42.9%□		
	17□20.3%□	15□21.4%□		
Egg intake[n(%)]	52□61.9%□	32□45.7%□	-1.860	0.063
	23□27.3%□	29□41.4%□		
	4□4.8%□	2□2.9%□		
	5□6.0%□	7□10.0%□		
Intake of fish and shrimp[n(%)]	1□1.2%□	0□0.0%□	-0.548	0.583
	10□11.9%□	8□11.4%□		
	61□72.6%□	50□71.4%□		
	12□14.3%□	12□17.2%□		
Intake of milk and milk products[n(%)]	17□20.2%□	14□20.0%□	-0.398	0.691
	43□51.2%□	35□50.0%□		
	14□16.7%□	7□10.0%□		
	10□11.9%□	14□20.0%□		
The amount of intake of milk and milk products[n(%)]	10□11.9%□	5□7.1%□	-0.743	0.457
	39□46.4%□	34□48.6%□		
	23□27.4%□	18□25.7%□		
	12□14.3%□	13□18.6%□		
Liver uptake in animals[n(%)]	5□6.0%□	2□2.8%□	-1.557	0.119
	35□41.6%□	23□32.9%□		
	44□52.4%□	45□64.3%□		

Note:†:The low VD season was from October to March of the next year, and the high VD season was from April to September; *$P < 0.05$.

Table 4 Comparison of serum VD, VDR, DBP, inflammatory factors and oxidation indexes between case group and control group

Indexes	Control group(N=84)	Case group(N=70)	t /Z	P
25(OH)D$_3$(ng/ml)	21.03±5.74	17.76±7.86	2.894	0.004*
1,25(OH)$_2$D$_3$(pg/ml)	24.64±16.77	15.71±9.54	-4.681	<0.001**
DBP(ng/mL)	223.90±41.97	276.49±50.49	-6.942	<0.001**
CRP(mg/L)	6.44±2.69	7.08±2.80	-1.440	0.152
TNF-α(pg/ml)	214.42±70.47	264.59±106.97	-3.024	0.002*
IL-6(ng/L)	11.26±3.13	11.17±3.28	0.172	0.864
IL-1β(ng/L)	109.53±28.64	124.97±32.86	-3.076	0.003*
IL-17(pg/ml)	302.49±96.11	290.74±68.99	-0.265	0.791
IL-23(pg/ml)	178.58±56.39	160.68±44.96	2.190	0.030*
MPO(U/L)	10.44±2.33	9.24±2.64	2.956	0.004*
MDA(nmol/ml)	9.61±1.67	9.78±1.81	-0.584	0.560
SOD(U/L)	83.70±27.40	65.78±21.06	-4.097	<0.001**

Table 5 DBP genotype distributions and Hardy-Weinberg equilibrium test results

rs4588	N	genotype frequency			c^2 value	P value
		AA	AC	CC		
Control group						
Actual frequency	84	10	27	47	1.510	0.470
Expected frequency	84	7	34	43		
Case group						
Actual frequency	70	5	34	31	0.646	0.724
Expected frequency	70	7	30	33		
rs7401	N	genotype frequency			c^2 value	P value
		GG	GT	TT		
Control group						
Actual frequency	84	5	36	43	0.160	0.923
Expected frequency	84	6	34	44		
Case group						
Actual frequency	70	6	28	36	0.000	1.000
Expected frequency	70	6	28	36		

Table 6 Genotype and allele frequency distribution of the DBP gene variants and correlations with UC risk

Gene loci	Genotypes and alleles	Case group frequency(%)	control group frequency(%)	c2 value	P value	OR(95%CI)
rs4588	AA	5 (7.1)	10 (11.9)	4.517	0.105	Ref.
	AC	34 (48.6)	27 (32.1)			2.519（0.769-8.249）
	CC	31 (44.3)	47 (56.0)			1.319（0.411-4.231）
	A	44(31.4)	47 (28.0)	0.437	0.508	Ref.
	C	96(68.6)	121 (72.0)			0.841(0.515-1.373)
rs7041	GG	6 (8.6)	5 (6.0)	0.442	0.802	Ref
	GT	28 (40.0)	36 (42.9)			1.312（0.415-4.148）
	TT	36 (51.4)	43 (51.2)			1.256（0.408-3.864）
	G	40 (28.6)	46 (27.4)	0.054	0.816	Ref.
	T	100 (71.4)	122 (72.6)			0.935(0.568-1.540)

Table 7 Haplotype analysis of rs4588 and rs7041

Haplotype	Case group n(%)	Control group n(%)	c^2 value	P value	OR(95%CI)
AG	0.00（0.000）	0.00（0.000）	—	—	—
AT	44.00（0.3000）	47.00（0.280）	0.437	0.509	1.180(0.722-1.927)
CG	40.00（0.286）	46.00（0.274）	0.054	0.817	1.061(0.644-1.748)
CT	56.00（0.414）	75.00（0.446）	0.673	0.412	0.827(0.525-1.303)

Table 8 Serum VD, VDR, DBP and inflammatory cytokine levels and the oxidative index differ among the various haplotypes

Factors	Haplotype	Control group [median(Q_{25}-Q_{75})]	H	P	Case group [median(Q_{25}-Q_{75})]	H	P
DBP(ng/ml)	AT	222.27(194.80-264.23)	1.905	0.386	222.27（194.80-264.23）	0.725	0.696
	CG	209.78(186.84-253.41)			209.78（186.84-253.41）		
	CT	230.46(194.18-254.22)			230.46（194.18-254.22）		
25(OH)D$_3$(ng/ml)	AT	19.79(16.35-24.66)	1.931	0.381	19.79（16.35-24.66）	1.146	0.564
	CG	21.21(15.65-26.12)			21.21（15.65-26.12）		
	CT	21.73(16.20-26.06)			21.73（16.20-26.06）		
1,25(OH)$_2$D$_3$（pg/ml）	AT	17.59(13.39-27.09)	1.538	0.463	17.59（13.39-27.09）	0.222	0.895
	CG	15.93(13.05-25.47)			15.93（13.05-25.47）		
	CT	18.25(13.82-39.13)			18.25（13.82-39.13）		
CRP（mg/L）	AT	6.03(4.26-7.63)	1.370	0.504	6.03（4.26-7.63）	0.338	0.844
	CG	5.67(3.73-7.37)			5.67（3.73-7.37）		
	CT	6.26(4.52-8.31)			6.26（4.52-8.31）		
TNF-α（pg/ml）	AT	221.34(156.90-273.87)	0.516	0.772	221.34（156.90-273.87）	3.205	0.201
	CG	221.02(158.40-279.52)			221.02（158.40-279.52）		
	CT	221.34(153.95-262.75)			221.34（153.95-262.75）		
IL-6（ng/L）	AT	10.54(9.27-13.40)	0.775	0.679	10.54（9.27-13.40）	0.731	0.694
	CG	11.24(9.68-13.00)			11.24（9.68-13.00）		
	CT	10.60(8.48-13.06)			10.60（8.48-13.06）		
IL-1β（ng/L）	AT	112.59(95.57-124.89)	2.756	0.252	112.59（95.57-124.89）	4.973	0.083
	CG	122.05(97.51-139.20)			122.05（97.51-139.20）		
	CT	106.92(92.73-129.62)			106.92（92.73-129.62）		
IL-17（pg/ml）	AT	276.86(202.33-364.47)	0.354	0.838	276.86（202.33-364.47）	0.293	0.864
	CG	298.01(235.73-345.16)			298.01（235.73-345.16）		
	CT	302.17(224.83-382.00)			302.17（224.83-382.00）		
IL-23（pg/ml）	AT	159.73(122.40-207.14)	0.707	0.702	159.73（122.40-207.14）	0.699	0.705
	CG	193.52(147.37-213.19)			193.52（147.37-213.19）		
	CT	175.87(140.56-220.27)			175.87（140.56-220.27）		
MPO（U/L）	AT	11.02(8.63-12.08)	1.129	0.569	11.02（8.63-12.08）	1.837	0.399
	CG	10.34(8.00-11.44)			10.34（8.00-11.44）		
	CT	10.88(8.45-12.74)			10.88（8.45-12.74）		
MDA（nmol/ml）	AT	9.88(8.32-10.64)	0.826	0.662	9.88（8.32-10.64）	8.564	0.014*
	CG	10.27(8.24-10.99)			10.27（8.24-10.99）		
	CT	9.87(7.75-11.11)			9.87（7.75-11.11）		
SOD（U/ml）	AT	84.77(65.03-101.81)	0.247	0.884	84.77（65.03-101.81）	0.821	0.663
	CG	85.36(66.19-104.36)			85.36（66.19-104.36）		
	CT	85.94(57.80-101.42)			85.94（57.80-101.42）		

Note: *$P<0.05$.

Table 9 Logistic regression analysis of the factors influencing UC disease

Factor	B	S.E.	Wald	P	OR	OR(95% CI)
Age(year)	0.042	0.014	9.308	0.002	1.043	1.015-1.071
Male				Ref		
Female	-0.965	0.328	8.640	0.003	0.381	0.200-0.725
DBP(ng/ml)	0.034	0.005	55.579	0.000	1.034	1.025-1.043
$1,25(OH)_2D_3$(pg/ml)	-0.080	0.014	32.913	0.000	0.923	0.898-0.949
CRP(mg/L)	0.159	0.065	6.006	0.014	1.172	1.032-1.331
MDA(nmol/ml)	0.179	0.090	3.998	0.046	1.196	1.004-1.426
Constant	-11.171	1.783	39.265	0.000		

Figures
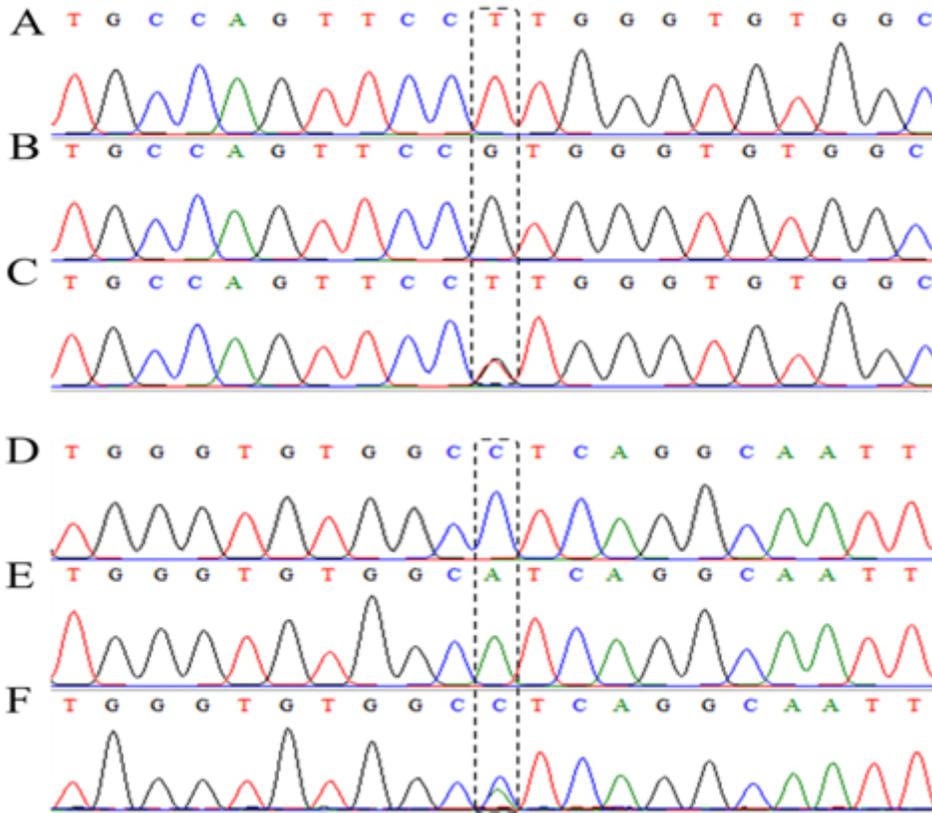
Figure 1
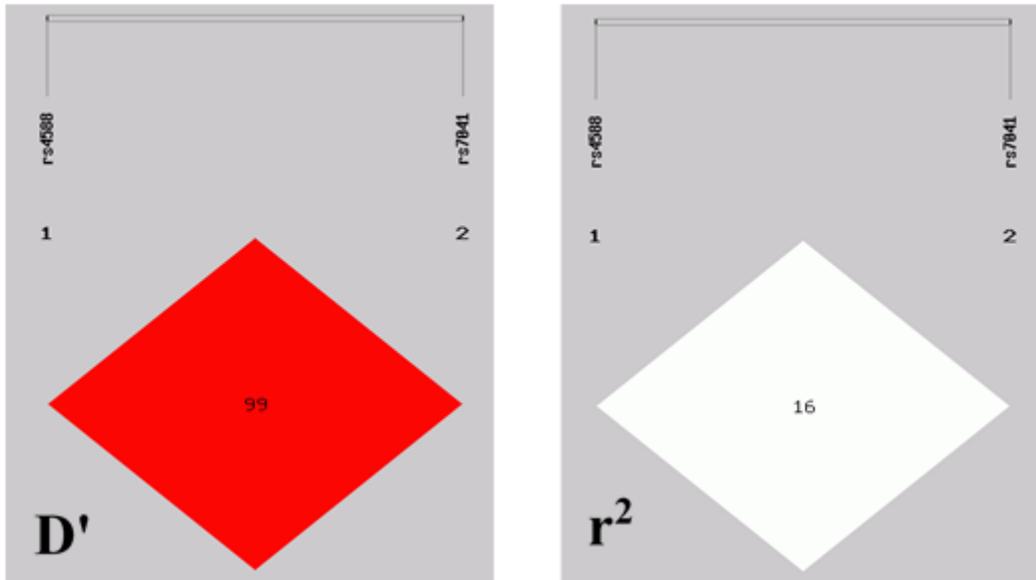
SNP sequencing result of DBP gene rs4588 and rs7041

Figure 2

linkage unbalance analysis D' value and r2 value