HK J Paediatr (new series) 2016;**21**:162-167

Vitamin D Metabolism-related Gene Polymorphisms in Crohn's Disease

YY ***Luo***, S ***Chen***, JD ***Yu***, XL ***Shu***, Q ***Cao***, J ***Chen***

Abstract ***Objectives:*** To observe single nucleotide polymorphisms (SNPs) of vitamin D metabolism-related factors, vitamin D receptor (VDR) and vitamin D binding protein (DBP), in Crohn's disease (CD). ***Methods:*** 121 patients with CD and 381 controls were enrolled. SNP (rs731236) in VDR gene and SNPs (rs4588, rs7401 and rs2282679) in DBP gene were typed in patients with CD and controls by gene sequencing. ***Results:*** In our case-control cohort, no significant difference was observed on CD risk for any of the four SNPs (rs731236, rs4588, rs7401 and rs2282679) in vitamin D metabolism-related genes (P>0.05). Furthermore, there was no association between CD susceptibility and the haplotypes of DBP gene (P>0.05). ***Conclusions:*** Our study suggests that the four SNPs (rs731236, rs4588, rs7401 and rs2282679) in vitamin D metabolism-related genes may have no correlation with susceptibility of CD in Chinese Han population. SNP (rs731236) in VDR gene may play a role in the aetiology of CD among affected males. However, our findings need to be confirmed in multi-centre studies.

Key words Crohn's disease; Gene polymorphism; Vitamin D; Vitamin D binding protein; Vitamin D receptor

Introduction

Crohn's disease (CD), one of the common forms of inflammatory bowel disease (IBD), is known as a chronic relapsing inflammatory disorder of the whole gastrointestinal tract. The aetiology includes a series of complex interactions of numerous genetic, immunomodulatory and environmental factors. Recently, 163 IBD susceptibility polymorphisms were confirmed by genome wide association studies (GWAS). Some immune-mediated diseases, such as psoriasis and ankylosing spondylitis, share a large number of loci with IBD.[1] Taking the importance of immune and genetic issues in CD pathogenesis, further investigations on IBD susceptibility genes associated with immunity have been paid more attention.

Vitamin D plays an important role in calcium/bone homeostasis and immune modulation. Serum 25-hydroxy vitamin D_3 [25(OH)D] is transported to macrophages by vitamin D binding protein (DBP) and then turned to 1,25-dihydroxy vitamin D_3 [1,25$(OH)_2$D], the biologically active form of vitamin D, 1,25$(OH)_2$D interacts with vitamin D receptor (VDR), regulates cell maturation and inflammation, modulates the function of activated T- / B-lymphocytes and macrophages.[2]

DBP is a multifunctional serum glycoprotein, encoded by the GC (group-specific component) gene located on chromosome 4q12-q13. The DBP gene is a member of a

Department of Gastroenterology, The Children's Hospital, School of Medicine, Zhejiang University, Hangzhou 310003, China

YY **Luo** (羅優優) *MD*
JD **Yu** (余金丹) *MD*
XL **Shu** (舒小莉) *MD*
J **Chen** (陳潔) *MD*

Department of Gastroenterology, Surrunrun Shao Hospital, School of Medicine, Zhejiang University, Hangzhou 310003, China

Q **Cao** (曹倩) *MD*

Department of Internal Medicine, The Third Affiliated Hospital of Zhejiang Chinese Medical University, Hangzhou, China

S **Chen** (陳矢) *MD*

Correspondence to: Dr J **Chen**

Received May 23, 2015

multigene family that includes albumin, α-fetoprotein and α-albumin/afamin.[3] Most of the variants are rare but two single nuclear polymorphisms (SNPs), rs4588 encoding Thr436Lys and rs7041 encoding Asp432Glu. These two common variants change the amino acid and alter the protein function. In a large Chinese cohort, SNPs (rs4588 and rs2282679) at the DBP gene were found to be significantly associated with lower plasma 25(OH)D.[4] Several studies found that polymorphisms of the DBP gene were associated with immune-mediated diseases,[5-7] including IBD. So was the VDR gene. The VDR gene is an IBD candidate gene that located on chromosome 12. There are four common polymorphisms of the VDR gene, named by the restriction enzymes ApaI, TaqI, BsmI and FokI. Among those four SNPs, SNP of TaqI (rs731236) was reported to increase the risk of CD in UK population.[8] However, there are some controversies for the association between VDR gene and IBD susceptibility in different races and different countries.[9,10]

Since rare studies indicated the associations between VDR, DBP genes and the risk of CD in Asian populations, we aimed to investigate the associations between variants of these genes and CD susceptibility in southern Han Chinese population, in order to discuss the differences between China and other countries.

Methods

Study Population

The study cohort consisted of 381 controls and 121 patients with CD. The adult patients and controls were recruited from the Surrunrun Shao Hospital, Zhejiang University (Hangzhou, China), and the third Affiliated Hospital of Zhejiang Chinese Medical University (Hangzhou, China), while the paediatric patients and controls were collected from the Children's Hospital, Zhejiang University. Cases were sex-matched and age-matched with healthy subjects. The diagnosis of CD was confirmed by standard endoscopic, histopathological and radiological criteria. The median age at diagnosis was 28 years (range 2-72), with 46% females in CD group. This study was approved by the Ethics Committee of Zhejiang University, in Hangzhou, China. All adult participants provided their informed consent, while paediatric informed consents were provided by participants' parents.

Single Nucleotide Polymorphism Genotyping

Genomic DNAs were extracted from EDTA-blood using AXYGEN DNA extraction kit (AxyPrep Blood Genomic DNA Miniprep Kit, USA), according to the manufacture's recommended protocol. Four known variants in DBP and VDR genes previously reported to be associated with IBD / immune-related diseases were investigated in this study. One of the four loci, rs731236(T→C), is located at the 3' end of the VDR gene in linkage disequilibrium(LD), while the other three variants lies in the DBP gene. Rs7041(T→G) and rs4588 (C→A) are both located in exon 11 in complete LD. Rs 2282679 (A→C) lies in intron 12. SNPs genotyping was analysed using an ABI 3730XL Genetic analyser. SNP calling was performed with ABI Variant Reporter. Quality control procedure was carried out by repeating a random 10% samples to assess genotyping reproducibility.

Statistic Analysis

Statistical analysis was performed using SPSS 17.0 (SPSS Inc., USA). The genotyping results and the Hardy-Weinberg equilibrium of the variants were analysed using χ^2 test. The LD structure was examined by D' and r^2, using Haploview 4.2 with the Gabriel method.[11] Haplotype interaction analysis of the genes containing the significant SNPs was performed in the same block. SNPs were removed from analysis if they were in Hardy-Weinberg disequilibrium ($P<0.05$), or had a minor allele frequency (MAF) <0.05.

Results

Descriptive Analysis of Study Population

Detailed demographic data of the study group were shown in Table 1. Five hundred and two Han Chinese participants were enrolled, comprising of 121 patients with CD and 381 healthy controls with similar sex and age profiles. Phenotypes of CD patients were shown in Table 2.

Table 1 Demographic data of study groups

	CD	Controls	P
N	121	381	
Age (mean±SD; years)	28.0±14.7	29.0±19.1	0.60
Adults [n(%)]	93 (76.9)	260 (68.2)	0.07
Children [n(%)]	28 (23.1)	121 (31.8)	
Sex			0.16
Female [n(%)]	55 (45.5)	137 (36.0)	0.06
Male [n(%)]	66 (54.5)	244 (64.0)	

CD: Crohn's disease

Association Between Different Genetic Variants and CD

The four variants selected for the study are single nucleotide substitutions. The genotype frequencies in all groups were in the Hardy-Weinberg equilibrium. The obtained allele/genotype frequencies are given in Table 3 and Table 4.

Association between individual SNPs and CD risk was assessed by logistic regression. P values were adjusted by age and sex. No significant association between any of the four genotypes and alleles, rs2282679, rs7401, rs4588, and rs731236, and the risk of CD in the 121 cases and 381 controls was found. For the three SNPs in DBP gene, gender stratification showed no significant difference in MAF in CD patients compared with healthy controls. However, a higher MAF was found for rs731236 in male CD patient than that in male controls (P<0.05).

Association Between DBP Haplotypes and CD

We found LD between the rs2282679, rs7401, and rs4588, especially between rs2282679 and rs4588 (rs2282679 and rs4588, D'=0.98, r^2=0.96; rs2282679 and rs7401, D'=0.97, r^2=0.16; rs7401 and rs 4588, D'=1.0, r^2=0.17). The haplotype prediction analysis of DBP gene was carried out. The frequency distribution of the DBP haplotypes and association with CD were shown in Table 5. No significant difference was observed in the frequency of the DBP haplotypes in cases and controls.

Discussion

Evidence of the importance of vitamin D in the pathogenesis of IBD has been suggested in recent years.[12,13]

Table 2 Phenotypes of Crohn's disease patients

		Children (n=28)	Adults (n=93)
Clinical manifestations	Abdominal pain [n (%)]	12 (42.8%)	39 (41.9%)
	Diarrhoea [n (%)]	4 (14.3%)	13 (14.0%)
	Coexistence of abdominal pain and diarrhoea [n (%)]	12 (42.9%)	27 (29.0%)
	Other manifestations [n (%)]	0 (0%)	14 (15.1%)[a]
Disease locations	Small intestine and colon involved [n (%)]	18 (64.3%)	28 (30.1%)
	Ileocolonic disease [n (%)]	1 (3.6%)	32 (34.4%)
	Only small intestine involved [n (%)]	4 (14.3%)	19 (20.4%)
	Colonic disease [n (%)]	5 (17.9%)	11 (11.8%)
	Other gastrointestinal parts involved [n (%)]	0 (0%)	3 (3.2%)[b]
Behaviour	Stricture [n (%)]	1 (3.6%)	12 (12.9%)
	Penetration [n (%)]	1 (3.6%)	8 (8.6%)
	Stricture and penetration [n (%)]	0 (0%)	1 (1.1%)
Perianal disease	Perianal fistula [n (%)]	2 (7.2%)	26 (28.0%)
	Perianal abscess [n (%)]	1 (3.6%)	4 (4.3%)

[a]Other clinical manifestations, including fever (4 cases), melena (3 cases), bloody stool (3 cases), perianal pain (3 cases) and vomiting (1 case), were found in adult patients.

[b]Two of adult patients had whole gastrointestinal disease. One adult patient had isolated upper gastrointestinal disease.

Table 3 Associations between allele frequencies of VDR and DBP SNPs in cases and controls

Genes	SNP	MAF Cases / Controls	OR (95%CI)[a]	P[a]
DBP	rs2282679	0.34/0.33	1.139 (0.836-1.551)	0.409
	rs7401	0.26/0.25	1.057 (0.950-1.175)	0.308
	rs4588	0.33/0.33	1.040 (0.839-1.289)	0.721
VDR	rs731236	0.05/0.05	0.925 (0.782-1.095)	0.367
	rs731236 (male)	0.09/0.05	0.781 (0.626-0.975)	0.029
	rs731236 (female)	0.06/0.06	1.228 (0.905-1.666)	0.187

DBP, vitamin D binding protein; VDR, vitamin D receptor; SNP, single nucleotide polymorphism; MAF, minor allele frequency.

[a]All listed ORs and P values referred to the adjusted age and sex estimations.

VDR and DBP, the two vitamin D metabolism related factors, play important roles in vitamin D homeostasis. The association between VDR/DBP genetic variations and IBD has been reported mostly in European cohort. However, the results remain conflicting.[9,10] Herein, we investigated the association between 3 DBP variants and 1 VDR variant of CD using a hospital-based case-control design with 381 controls and 121 cases from Chinese Han population. We found no significant association between any of the polymorphisms and risk of CD for either genotype or haplotype.

Prior investigations have demonstrated different results

Table 4 Genotype frequencies of VDR and DBP SNPs in cases and controls

Genes	SNPs	Genotypes	Cases / Controls[a]	OR (95%CI)[b]	P[b]
DBP	rs2282679	AA	54/162	1.00 (-)	–
		CC	14/35	1.270 (0.633-2.554)	0.500
		AC	53/184	0.925 (0.601-1.425)	0.725
	rs7401	TT	69/206	1.00 (-)	–
		GG	7/15	1.253 (0.487-3.223)	0.640
		GT	45/160	0.809 (0.528-1.240)	0.331
	rs4588	CC	54/163	1.00 (-)	–
		AA	15/36	1.337 (0.676-2.646)	0.404
		AC	52/182	0.927 (0.601-1.430)	0.731
VDR	rs731236	TT	107/348	1.00 (-)	–
		CC	2/2	3.193 (0.444-22.933)	0.249
		TC	12/31	1.339 (0.677-2.649)	0.402

DBP, vitamin D binding protein; VDR, vitamin D receptor; SNP, single nucleotide polymorphism.

[a]The case and control entries refer to the number of successfully genotyped individuals.

[b]All listed ORs and P values referred to the adjusted age and sex estimations. OR, 95% CI, and Pvalues were calculated for each SNP analysed using logistic regression. For each SNP, ORs were first presented for the genotype analysis with reference to the most frequent homozygous genotype.

Table 5 Predicted haplotype frequencies of the DBP SNPs in cases and controls

Haplotype sequence	Frequency in controls	Frequency in cases	χ^2	Individual P value	P_{per} value	OR (95%CI)
Rs7401 - rs4588 - rs2282679						
TAC	0.331	0.328	3.674	0.056	0.208	1.001 (0.648-1.545)
TCA	0.416	0.416	0.127	0.721	1.000	1.006 (0.664-1.524)
GCA	0.248	0.244	3.008	0.083	0.377	0.993 (0.619-1.596)
Rs7401 - rs2282679						
TA	0.420	0.426	0.045	0.831	0.982	0.961 (0.635-1.453)
TC	0.331	0.331	3.737	0.053	0.116	1.001 (0.648-1.545)
GA	0.247	0.242	3.187	0.074	0.162	1.039 (0.644-1.676)
Rs2282679 - rs4588						
AC	0.664	0.660	3.687	0.054	0.204	1.013 (0.657-1.561)
CA	0.331	0.328	3.884	0.048	0.198	1.001 (0.648-1.545)
Rs7401 - rs4588						
TC	0.417	0.420	0.102	0.750	0.137	0.983 (0.650-1.488)
TA	0.333	0.336	4.159	0.041	0.205	0.976 (0.633-1.504)
GC	0.249	0.244	3.485	0.062	0.990	1.008 (0.628-1.618)

P_{per} value, p value with 10,000 times permutation test; OR, odds ratio; 95% CI, 95% confidence interval.

The haplotypes with frequencies lower than 5% were excluded from analysis.

regarding the association between polymorphisms in VDR and risk of IBD. Most studies have only focused on Western populations. In a UK study with 245 CD patients and only 164 controls, homozygous SNP carriers in rs731236 had nearly doubled risk of CD, comparing with controls.[8] However, an Irish hospital-based case-control study showed no significant association between rs731236 SNP and risk of CD.[10] In Asian populations, rs731236 SNP was only found to be associated with susceptibility of UC. In our study, the interesting result is that a variant in VDR gene was strongly associated with susceptibility to male CD. Similar results can be obtained from Caucasian IBD cohort.[14] One possible explanation of sex-specific association is that the underlying genetic modulators for CD may differ with gender. Previous studies showed that the DBP gene variants were associated with the risk of autoimmune disease, such as diabetes, asthma and thyroid autoimmune disease.[15-17] In a case-control study of European population, DBP gene homozygous in rs4588 showed significant association with the risk of CD. Meanwhile, the haplotype Gc2, consisting of rs4588 and rs7401 variants, seemed to be a protective factor for UC group.[7] Rare data of DBP gene polymorphisms and CD susceptibility in Asian populations was reported. The results of our study suggest that there was no association between these variants and the risk of CD. It remains possible that the same disease in different descents may have different genetic causes. For example, CARD15/NOD2 defects in Caucasians were reported to be associated with CD susceptibility, but not in East Asian populations.[18-20] Since evidences of the association between DBP variants and IBD susceptibility are rare, well-designed, large sample, multi-centre studies are needed to confirm the results.

In a large Han Chinese cohort, three SNPs (rs7401, rs4588 and rs2282679) at the DBP locus were found to be significantly associated with lower plasma 25(OH)D.[4] Meanwhile, lower plasma 25(OH)D was found in CD patients.[21,22] Our study has the limitation that we did not have available data to get the plasma 25(OH)D concentrations in order to see whether there were some associations between lower plasma 25(OH)D and DBP gene polymorphisms in CD patients. It will be of interest to further explore whether the CD-associated low plasma 25(OH)D Concentration is related to VDR or DBP gene polymorphisms.

In summary, the present study revealed that SNP (rs731236) in VDR gene may play a role in the aetiology of CD among affected males in Chinese Han population. However, the results need replication and confirmation in multi-centre studies.

References

1. Jostins L, Ripke S, Weersma RK, et al. Host-microbe interactions have shaped the genetic architecture of inflammatory bowel disease. Nature 2012;491:119-24.
2. Bikle DD. Vitamin D regulation of immune function. Vitam Horm 2011;86:1-21.
3. Song YH, Naumova AK, Liebhaber SA, Cooke NE. Physical and meiotic mapping of the region of human chromosome 4q11-q13 encompassing the vitamin D binding protein DBP/Gc-globulin and albumin multigene cluster. Genome Res 1999;9:581-7.
4. Lu L, Sheng H, Li H, et al. Associations between common variants in GC and DHCR7/NADSYN1 and vitamin D concerntration in Chinese Hans. Hum Genet 2012;131:505-12.
5. Yoshida S, Ikari K, Furuya T, et al. A GC polymorphism associated with serum 25-hydroxyvitamin D level is a risk factor for hip fracture in Japanese patients with rheumatoid arthritis: 10-year follow-up of the Institute of Rheumatology, Rheumatoid Arthritis cohort study. Arthritis Res Ther 2014;16:R75.
6. Jung KH, Kim TH, Sheen DH, et al. Associations of vitamin D binding protein gene polymorphisms with the development of peripheral arthritis and uveitis in ankylosing spondylitis. J Rheumatol 2011;38:2224-9.
7. Eloranta JJ, Wenger C, Mwinyi J, et al. Association of a common vitamin D-binding protein polymorphism with inflammatory bowel disease. Pharmacogenet Genomics 2011;21:559-64.
8. Wang L, Wang ZT, Hu JJ, Fan R, Zhou J, Zhong J. Polymorphisms of the vitamin D receptor gene and the risk of inflammatory bowel disease: a meta-analysis. Genet Mol Res 2014;13:2598-610.
9. Simmons JD, Mullighan C, Welsh KI, Jewell DP. Vitamin D receptor gene polymorphism: association with Crohn's disease susceptibility. Gut 2000;47:211-4.
10. Hughes DJ, McManus R, Neary P, O'morain C, O'sullivan M. Common variation in the vitamin D receptor gene and risk of inflammatory bowel disease in an Irish case-control study. Eur J Gastroenterol Hepatol 2011;23:807-12.
11. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005; 21:263-5.
12. Ghaly S, Lawrance I. The role of vitamin D in gastrointestinal inflammation. Expert Rev Gastroenterol Hepatol 2014;22:1-15.
13. Reich KM, Fedorak RN, Madsen K, Kroeker KI. Vitamin D improves inflammatory bowel disease outcomes: basic science and clinical review. World J Gastroenterol 2014;20:4934-47.
14. Bentley RW, Keown D, Merriman TR, et al. Vitamin D receptor gene polymorphism associated with inflammatory bowel disease in New Zealand males. Aliment Pharmacol Ther 2011;33:855-6.
15. Malecki MT, Klupa T, Wanic K, Cyganek K, Frey J, Sieradzki J. Vitamin D binding protein gene and genetic susceptibility to type 2 diabetes mellitus in a Polish population. Diabetes Res Clin Pract 2002;57:99-104.
16. Li F, Jiang L, Willis-Owen SA, Zhang Y, Gao J. Vitamin D binding protein variants associate with asthma susceptibility in the Chinese Han population. BMC Med Genet 2011;12:103.
17. Kurylowicz A, Ramos-Lopez E, Bednarczuk T, Badenhoop K. Vitamin D-binding protein(DBP) gene polymorphism in associated with Graves' disease and the vitamin D status in a Polish population study. Exp Clin Endocrinol Diabetes 2006;114:329-35.

18. Nagy Z, Karadi O, Rumi G, et al. Crohn's disease is associated with polymorphism of CARD15/ NOD2 gene in a Hungarian population. Ann N Y Acad Sci 2005;1051:45-51.
19. Yamazaki K, Takazoe M, Tanaka T, Kazumori T, Nakamura Y. Absence of mutation in the NOD2/CARD15 gene among 483 Japanese patients with Crohn's disease. J Hum Genet 2002;47: 469-72.
20. Zheng CQ, Hu GZ, Zeng ZS, Lin LJ, Gu GG. Progress in searching for susceptibility gene for inflammatory bowel disease by positional cloning. World J Gastroenterol 2003;9:1646-56.
21. Ham M, Longhi MS, Lahiff C, Cheifetz A, Robson S, Moss AC. Vitamin D levels in adults with Crohn's disease are responsive to disease activity and treatment. Inflamm Bowel Dis 2014;20:856-60.
22. Pappa HM, Grand RJ, Gordon CM. Report on the vitamin D status of adult and pediatric patients with inflammatory bowel disease and its significance for bone health and disease. Inflamm Bowel Dis 2006;12:1162-74.