Original Paper

The TaqI Gene Polymorphisms of VDR and the Circulating 1,25-Dihydroxyvitamin D Levels Confer the Risk for the Keloid Scarring in Chinese Cohorts

Dongmei Yu Yong Shang Sai Luo Lijun Hao

Plastic and Cosmetic Center, The First Affiliated Hospital of Harbin Medical University, 23 Youzheng Str. Nangang District, 150001, Harbin, P.R. China

Key Words

Vitamin D receptor • 25-Hydroxyvitamin D levels • Keloid scar • Risk

Abstract

Aim: to investigate the association between vitamin D receptor polymorphisms and circulating 1,25-Dihydroxyvitamin D (1,25(OH)₂D) levels with keloid scar (KS) risk. **Methods:** A total of 261 patients with KS and 261 normal healthy individuals were enrolled. VDR gene polymorphisms were determined. Circulating 1,25(OH)₂D levels were detected. **Results:** In this study, we investigated the role of four loci of VDR gene polymorphisms in determining the risk of KS in a Chinese cohort. We found that the TaqI C>T polymorphism was closely associated with the KS incidence. Carriers with CC genotype of TaqI had a higher chance of developing KS. Stratification analyses by sex showed that this trend exists only in female subjects but not in male subjects. Furthermore, the TaqI C>T polymorphism affects the circulating 25-Hydroxyvitamin D levels. The CC carriers had a significantly lower mean circulating 1,25(OH)₂D level than TT carriers and CT carriers. KS subjects had significantly lower mean serum circulating 1,25(OH)₂D level than controls. The receiver operating characteristic curve analysis revealed that the serum circulating 1,25(OH)₂D level at the cut-off point of 16.1 ng/ml could discriminate KS subjects from controls. **Conclusion:** Collectively, these data provide evidence that the vitamin D/VDR pathway plays an important role in the development of KS. The TaqI gene polymorphisms of VDR and circulating 1,25(OH)₂D levels may thus be used as potential markers for prediction of KS development.

Copyright © 2013 S. Karger AG, Basel

Introduction

Keloid scar (KS) is a benign hyperproliferative growth of dermal fibroblasts characterized by the excessive deposition of extracellular matrix components. The incidence of KS has been estimated to be approximately 4–6 % in the general population and up to 16 % in people of African cohorts [1–3]. Research in the past decades attempted to identify genetic, cellular, and molecular factors responsible for the incidence and development of KS, yet the mechanism of KS formation is largely unknown [4, 5]. Increased familial aggregation, a higher prevalence in certain races, parallelism in identical twins, and alterations in gene expression favor the contribution of genetic risk factors to the development of KS [6]. Some genetic markers have been reported to have significant importance in diagnosis, prognosis, and development of KS.

Vitamin D and its metabolites play an important role in calcium homeostasis, bone remodeling, hormone secretion, cell proliferation and differentiation [7]. Recent studies also suggest a beneficial role of vitamin D in slowing the progression of tissue fibrosis. A previous study *in vivo* revealed vitamin D supplements increase promoter activity of cultured keloid fibroblasts and decrease fibrosis, suggesting that vitamin D supplementation could be a preventive early treatment strategy for keloid and related fibrotic disorders [8].

The effect of vitamin D is mediated by vitamin D receptor (VDR), a member of the nuclear receptor super-family of ligand-inducible transcription factors involved in many pathological processes [9–11]. The expression of VDR in cultured keloid fibroblasts has been confirmed [8]; however, there is no study regarding the association between VDR level and KS in a clinical setting. In addition, whether the genetic variants of VDR are associated with the risk for KS has not been investigated. In this study, we enrolled KS patients and healthy controls to testify this possible association.

Materials and Methods

Subjects

A total of 261 patients between the ages of 19–55 years old with KS were recruited from our hospital. Patients who had KS due to any syndrome, skin disorder, hypertrophic scars, or genetic or chromosomal disorders were excluded from the study. The controls comprised 261 normal healthy individuals who were devoid of any keloid. The study was approved by the ethics committees of our hospital and written informed consent was obtained from each participant.

Sample Collection and Genotyping

Venous blood (10 ml) was collected from each patient into tubes containing 50 mmol of EDTA per liter, and genomic DNA was isolated with DNA blood kit, according to manufacturer's instructions. Four diallelic polymorphisms of the VDR were genotyped: FokI C>T (rs10735810) and TaqI T>C (rs10735810) polymorphic sites on the coding sequence, BsmI A>G (rs1544410) and Apal G>T (rs7975232) on the last intron. The polymerase chain reaction (PCR) technique was applied and followed by restriction fragment length polymorphism assays. The primer and conditions for VDR gene polymorphisms were listed in Table 1. All PCR products were sized by electrophoresis on a 2% agarose gel stained with ethidium bromide.

Measurement of serum 1,25(OH)₂D

A radioimmunoassay kit from Immunodiagnostic system, Boldon, UK, was used to analyze the 1,25(OH)₂D levels. This assay had a sensitivity of 100% for 1,25(OH)₂D₃ and 91% for 1,25(OH)₂D₂. Detection limit was 8 pmol/L for 1,25(OH)₂D and the inter-assay variations were 13.6%, 9.6% and 9.6% for concentrations of 20.5 pmol/L, 60.8 pmol/L and 135.2 pmol/L, respectively [12].

Statistical Analyses

χ^2 tests were used to compare genotype frequency and demographic distributions between cases and controls. Multiple logistic regression analyses were used to evaluate if each polymorphism was indepen-

VDR gene polymorphisms	Primer	Base change	Annealing temperature
FokI	5'AGCTGGCCCTGGCACTGACTCTGCTCT3'(F)	C/T	61°C
	5'ATGGAAACACCTTGCTTCTTCTCCCTC 3'(R)		
BsmI	5'CAACCAAGACTACAAGTACCGCGTCAGTGA3'(F)	G/A	57°C
	5'AACCAGCGGGAAGAGGTCAAGGG3'(R)		
ApaI	5'CAGAGCATGGACAGGGAGCAA3'(F)	G/T	60°C
	5'GCAACTCCTCATGGCTGAGGTCTC3'(R)		
TaqI	5'CAGAGCATGGACAGGGAGCAA3'(F)	T/C	60°C
	5'GCAACTCCTCATGGCTGAGGTCTC3'(R)		

Table 1. PCR-RFLP primers and conditions for VDR gene polymorphisms

Characteristic	KS=261	Control=261	P
Sex (female, n)	181	130	<0.001
Age(mean±SD)	24.7 ± 6.24	24.6 ± 8.18	0.569
Tobacco users (n)	80	75	0.351
Alcohol users (n)	63	59	0.378
Serum1,25(OH)2D(mean±SD, ng/ml)	15.1±3.4	20.4±5.7	<0.001

Table 2. The clinical characteristics of all subjects. SD: stand deviation

dently associated with KS while adjusting for the potential confounding effects of important clinical variables. The odds ratios (OR) and 95% confidence intervals (CIs) were calculated. The receiver operating characteristic curve (ROC) analyses was performed to determine whether serum circulating 1,25(OH)2D can discriminate KS subjects from controls. $P < 0.05$ was considered statistically significant. All analyses were performed by using SPSS software (Statistical Package for the Social Sciences, version 16.0, SPSS Inc, Chicago, IL, USA).

Results

Patient Characteristics

The clinical characteristics of all subjects are showed in Table 2. There were no significant differences in mean age, tobacco and alcohol use ($P > 0.05$). The KS group was found to include more females than the control group ($P < 0.001$). In addition, KS subjects had significantly lower serum 1,25(OH)2D than controls ($P < 0.001$).

Genotype frequencies of VDR polymorphisms between KS and controls were found to be in Hardy-Weinberg equilibrium (all $P > 0.05$). The genotype and the allele frequencies of Fok I, Bsm and ApaI were not significantly different between the KS and controls (all $P > 0.05$). However, the genotypic and allelic frequencies of TaqI T>C were significantly different between KS and controls. KS subjects had markedly higher CC genotype of TaqI than controls (29% vs. 16%, overall $P < 0.001$, Table 3). With TT of as reference, multivariate logistic regression analysis showed the CC genotype carriers had a higher chance of having KS (adjusted OR=3.334, 95% CI: 2.042-5.276, adjusted $P < 0.001$) after adjusting for age, sex, tobacco and alcohol use and serum 1,25(OH)2D. Being a carrier of the C allele represented a higher risk for KS after adjustment with the above mentioned clinical variables (adjusted OR=1.658, adjusted $P < 0.001$) compared with T allele carriage. However, multivariate logistic regression analysis did not reveal any association between of Fok I, Bsm and ApaI polymorphisms and KS risk in these patients (all $P > 0.05$).

We next analyzed the association between KS risk and the circulating serum 1,25(OH)2D levels. For TaqI polymorphism, we found that the TaqI genetic variants distributions were

		KS		Control		adjusted OR	95%CI		adjusted P
		N	%	N	%				
ApaI	TT	90	30%	86	28%	1.000			
	TG	124	41%	129	43%	0.919	0.614	1.317	0.668
	GG	47	16%	46	15%	0.976	0.552	1.637	0.926
	T	304	51%	301	50%	1.000			
	G	295	49%	304	50%	0.961	0.764	1.214	0.747
BsmI	AA	62	23%	63	23%	1.000			
	AG	113	41%	116	42%	0.990	0.640	1.531	0.963
	GG	86	31%	82	29%	1.066	0.671	1.693	0.788
	A	237	43%	242	44%	1.000			
	G	312	57%	314	56%	1.015	0.800	1.287	0.905
FokI	TT	68	24%	57	20%	1.000			
	CT	120	42%	125	44%	0.805	0.522	1.240	0.324
	CC	73	26%	79	28%	0.775	0.482	1.245	0.291
	T	256	45%	239	42%	1.000			
	C	313	55%	329	58%	0.888	0.702	1.123	0.322
TaqI	TT	49	17%	94	32%	1.000			
	TC	129	45%	119	40%	1.280	0.858	2.184	0.051
	CC	83	29%	48	16%	3.334	2.042	5.276	<0.001
	T	227	40%	307	52%	1.000			
	C	341	60%	286	48%	1.657	1.248	2.253	<0.001

Table 3. The genotype and the allele frequencies of VDR gene polymorphism between the KS and controls. OR and P were adjusted by age, sex, tobacco and alcohol use and serum 1,25(OH)2D levels

		KS		Control		adjusted OR	95%CI		adjusted P value
TaqI T>C		N=261	%	N=261	%				
Male	TT	18	26%	21	29%	1.000			
	TC	27	39%	31	43%	1.065	0.443	2.523	0.958
	CC	20	29%	10	14%	2.333	0.858	6.228	0.074
	T	63	46%	73	50%	1.000			
	C	74	54%	72	50%	1.191	0.746	1.937	0.472
Female	TT	32	15%	64	28%	1.000			
	TC	98	46%	97	42%	1.021	0.785	3.374	0.922
	CC	66	31%	38	17%	3.474	1.940	6.220	<0.001
	T	162	38%	225	49%	1.000			

Table 4. The genotype and the allele frequencies of TaqI gene polymorphism between the KS and controls stratified by sex. OR and P were adjusted by age, sex, tobacco and alcohol use and serum 1,25(OH)2D levels

only significant in the female groups but not in the male groups. Multivariate logistic regression analysis showed the CC genotype of TaqI to be associated with a significantly higher risk for KS in female subjects (adjusted OR=3.474, $P<0.001$, compared with TT carriage) but not in male subjects ($P=0.059$, compared with TT carriage, Table 4). The KS risk associated with Fok I, Bsm and ApaI polymorphisms were similar between male and female groups (data not shown).

KS patients had a significantly lower mean serum 1,25(OH)2D level than controls. We next analyzed the influence of the VDR genetic polymorphism on the serum 1,25(OH)2D levels. When stratified by the individual genotypes, we found that the TaqI was the only polymorphism that affected the serum 1,25(OH)2D levels. The CC carriers had a significantly lower serum 1,25(OH)2D level (12.9 ± 3.6 ng/ml) compared with TT carriers (17.4 ± 3.4 ng/ml, $P=0.011$ vs. CC) and CT carriers (16.9 ± 4.1 ng/ml, $P=0.007$, vs. CC, Fig. 1).

Fig. 1. The serum 1,25(OH)2D levels stratified by TaqI gene polymorphism. vs. CC $P=0.011$ ‡ vs. CC $P=0.007$.

Fig. 2. The ROC analyses of serum 1,25(OH)2D level in discrimination of KS from controls. AUC=0.911, $P=0.018$

We further performed the ROC analyses to determine serum 1,25(OH)2D level as a biomarker to discriminate the KS subjects from controls. We found that the serum 1,25(OH)2D level at the cut-off point of 16.1 ng/ml distinguished KS subjects from controls. (area under curve=0.911, $P=0.018$, sensitivity=87.2% and specificity=78.3%) (Fig. 2).

Discussion

In this study, we investigated the role of four loci of VDR gene polymorphisms in determining the risk of KS in a Chinese cohort. We found that the TaqI C>T polymorphism was closely associated with KS incidence. Carriers with CC genotype of TaqI had a higher chance to develop KS. Stratification analyses by sex showed that this trend existed only in female subjects but not in male subjects. The TaqI C>T polymorphism also affected the serum 1,25(OH)2D levels. The CC carriers had a significantly lower serum 1,25(OH)2D level compared with TT carriers and CT carriers. KS subjects had a significantly lower mean serum 1,25(OH)2D level than controls. ROC analyses revealed that the serum 1,25(OH)2D level at the cut-off point of 16.1 ng/ml could discriminate KS subjects from controls with an area under curve. Collectively, these data provide evidence that the Vit D/VDR plays an important role in the development of KS. Moreover, the TaqI gene polymorphisms of VDR and the circulating 1,25(OH)2D levels may be used as potential marker for prediction of KS incidence.

Vitamin D has been shown to have anti-proliferative effects in a wide variety of pathological conditions, such as cancers and fibroproliferative diseases. The anticancer effects of vitamin D are mediated primarily by its active metabolite, 1,25-dihydroxyvitamin D (calcitriol), through vitamin D receptor (VDR) signaling [13]. Vitamin D has been shown to play an important role in collagen synthesis and degradation. In the rat glomerulonephritis model and heart fibrosis, Vitamin D was shown to inhibit collagen synthesis in interstitial fibrosis

and the accumulation of interstitial matrix components by suppressing transforming growth factor (TGF)- β 1 gene expression or by up-regulating antifibrotic HGF gene expression [14-16]. Clinical studies also provide evidence that the deficiency in vitamin D and its active metabolites is closely associated with fibroproliferative diseases, and the administration of vitamin D is an effective treatment for skin fibrosis diseases such as scleroderma [17]. The imbalance between collagen synthesis and degradation plays an important role in the KS process, implicating the influence of vitamin D in the development of KS [18-20].

The vitamin D receptor (VDR), expressed in normal and malignant prostate cells, mediates the biological actions of 1,25(OH) $_2$ D [21]. VDR is located on the long arm of 12. chromosome (3q11 locus) and consists of 11 exons [22]. Several common polymorphisms in the VDR gene have been described [23]. The FokI polymorphism is a T>C transition polymorphism (ATG to ACG) at the first of two potential translation initiation sites in exon II has been defined using the FokI restriction endonuclease. The TaqI polymorphism is a T/C nucleotide substitution (ATT to ATC) leading to a synonymous change at codon 352 (isoleucine) in exon IX. Bsm I and ApaI restriction site polymorphisms occur in the intron separating exons VIII and IX. When the genotypes were analyzed with respect to 25-OH-Vitamin D levels in an Indian population, a significant association was seen for the Taq I SNP but not with the Fok I [24]. In the present study, we observed the similar result that the TaqI, rather than the other three loci, affected the circulating 1,25(OH) $_2$ D.

Several limitations in this study need to be addressed. This study was a single-center cohort investigation on a relatively small scale, and thus, replication studies with large independent cohorts are warranted. Secondly, we did not detect the VDR expression in KS tissue. The detection of VDR protein will help to clarify the effect of the genetic variants of VDR gene on KS formation.

Conflict of Interest

None

Acknowledgements

We thank Dr. Xuwei Hou for his help in statical analysis. We thank Dr.Liu Jin for her help in the genotyping of OPN polymorphisms. We thanks Ms. Elisa Miguel for her help in language revisions.

References

- 1 Lee JY, Yang CC, Chao SC, Wong TW: Histopathological differential diagnosis of keloid and hypertrophic scar. *Am J Dermatopathol* 2004;26:379-384.
- 2 Emami A, Halim AS, Salahshourifar I, Yussof SJ, Khoo TL, Kannan TP: Association of TGF β 1 and SMAD4 variants in the etiology of keloid scar in the Malay population. *Arch Dermatol Res* 2012;304:541-547.
- 3 Tsao SS, Dover JS, Arndt KA, Kaminer MS: Scar management: keloid, hypertrophic, atrophic, and acne scars. *Semin Cutan Med Surg* 2002;21:46-75.
- 4 Ehrlich HP, Desmouliere A, Diegelmann RF, Cohen IK, Compton CC, Garner WL, Kapanci Y, Gabbiani G: Morphological and immunochemical differences between keloid and hypertrophic scar. *Am J Pathol* 1994;145:105-113.
- 5 Rossiello L, D'Andrea F, Grella R, Signoriello G, Abbondanza C, De Rosa C, Prudente M, Morlando M, Rossiello R: Differential expression of cyclooxygenases in hypertrophic scar and keloid tissues. *Wound Repair Regen* 2009;17:750-757.

- 6 Halim AS, Emami A, Salahshourifar I, Kannan TP: Keloid scarring: understanding the genetic basis, advances, and prospects. *Arch Plast Surg* 2012;39:184-189.
- 7 Muller DN, Kleinewietfeld M, Kvakan H: Vitamin D review. *J Renin Angiotensin Aldosterone Syst* 2011;12:125-128.
- 8 Zhang GY, Cheng T, Luan Q, Liao T, Nie CL, Zheng X, Xie XG, Gao WY: Vitamin D: a novel therapeutic approach for keloid, an in vitro analysis. *Br J Dermatol* 2011;164:729-737.
- 9 Subramaniam N, Leong GM, Cock TA, Flanagan JL, Fong C, Eisman JA, Kouzmenko AP: Cross-talk between 1,25-dihydroxyvitamin D3 and transforming growth factor-beta signaling requires binding of VDR and Smad3 proteins to their cognate DNA recognition elements. *J Biol Chem* 2001;276:15741-15746.
- 10 Kato S, Yoko Y: [VDR function in skin]. *Clin Calcium* 2004;14:116-119.
- 11 Slattery ML: Vitamin D receptor gene (VDR) associations with cancer. *Nutr Rev* 2007;65:S102-104.
- 12 Christensen MH, Apalset EM, Nordbo Y, Varhaug JE, Mellgren G, Lien EA: 1,25-dihydroxyvitamin d and the vitamin d receptor gene polymorphism apa1 influence bone mineral density in primary hyperparathyroidism. *PLoS One* 2013;8:e56019.
- 13 Srinivasan M, Parwani AV, Hershberger PA, Lenzner DE, Weissfeld JL: Nuclear vitamin D receptor expression is associated with improved survival in non-small cell lung cancer. *J Steroid Biochem Mol Biol* 2011;123:30-36.
- 14 Li Y, Spataro BC, Yang J, Dai C, Liu Y: 1,25-dihydroxyvitamin D inhibits renal interstitial myofibroblast activation by inducing hepatocyte growth factor expression. *Kidney Int* 2005;68:1500-1510.
- 15 Tan X, Li Y, Liu Y: Paricalcitol attenuates renal interstitial fibrosis in obstructive nephropathy. *J Am Soc Nephrol* 2006;17:3382-3393.
- 16 Koleganova N, Piecha G, Ritz E, Gross ML: Calcitriol ameliorates capillary deficit and fibrosis of the heart in subtotaly nephrectomized rats. *Nephrol Dial Transplant* 2009;24:778-787.
- 17 Humbert PG, Dupond JL, Rochefort A, Vasselet R, Lucas A, Laurent R, Agache P: Localized scleroderma--response to 1,25-dihydroxyvitamin D3. *Clin Exp Dermatol* 1990;15:396-398.
- 18 Diao JS, Xia WS, Yi CG, Wang YM, Li B, Xia W, Liu B, Guo SZ, Sun XD: Trichostatin A inhibits collagen synthesis and induces apoptosis in keloid fibroblasts. *Arch Dermatol Res* 2011;303:573-580.
- 19 Gao Z, Wang Z, Shi Y, Lin Z, Jiang H, Hou T, Wang Q, Yuan X, Zhao Y, Wu H, Jin Y: Modulation of collagen synthesis in keloid fibroblasts by silencing Smad2 with siRNA. *Plast Reconstr Surg* 2006;118:1328-1337.
- 20 Wang Z, Gao Z, Shi Y, Sun Y, Lin Z, Jiang H, Hou T, Wang Q, Yuan X, Zhu X, Wu H, Jin Y: Inhibition of Smad3 expression decreases collagen synthesis in keloid disease fibroblasts. *J Plast Reconstr Aesthet Surg* 2007;60:1193-1199.
- 21 Kato S: The function of vitamin D receptor in vitamin D action. *J Biochem* 2000;127:717-722.
- 22 Whitfield GK, Remus LS, Jurutka PW, Zitzer H, Oza AK, Dang HT, Haussler CA, Galligan MA, Thatcher ML, Encinas Dominguez C, Haussler MR: Functionally relevant polymorphisms in the human nuclear vitamin D receptor gene. *Mol Cell Endocrinol* 2001;177:145-159.
- 23 Thakkestian A, D'Este C, Attia J: Haplotype analysis of VDR gene polymorphisms: a meta-analysis. *Osteoporos Int* 2004;15:729-734.
- 24 Bhanushali AA, Lajpal N, Kulkarni SS, Chavan SS, Bagadi SS, Das BR: Frequency of fokI and taqI polymorphism of vitamin D receptor gene in Indian population and its association with 25-hydroxyvitamin D levels. *Indian J Hum Genet* 2009;15:108-113.