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Report

Catalase but not vitamin D receptor gene polymorphisms are associated with nonsegmental vitiligo in Northwestern Mexicans

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Abstract

Background Vitiligo is an acquired pigmentation disorder characterized by melanocyte loss via autoimmune mechanisms triggered by oxidative stress. Gene polymorphisms in antioxidant enzymes and immunomodulators such as catalase (CAT) and vitamin D receptor (VDR), respectively, have been linked to vitiligo in European and Asian populations. Our aim was to evaluate the role of CAT and VDR gene polymorphisms as well as CAT and vitamin D in nonsegmental vitiligo in Northwestern Mexicans.

Methods A total of 357 subjects, 173 nonsegmental vitiligo patients and 184 age-gender matched healthy controls, were genotyped by PCR-restriction fragment length polymorphism. CAT activity was determined in 39 patients and in 39 controls and vitamin D (VitD) levels in 35 individuals per group.

Results CAT 419 C/T gene polymorphism was not informative, -89 A/T was associated with risk ($P = 0.02$), and 389 C/T conferred protection against vitiligo along with AT haplotype ($P < 0.01$ in both cases). VDR *Bsm*I, *Apa*I, and *Taq*I gene polymorphisms were not associated with vitiligo, but *Bsm*I was more prevalent in patients with Koebner phenomenon ($P = 0.02$). Serum CAT activity and VitD levels were lower in patients than in controls, but they showed no association with any vitiligo clinical characteristics neither with their gene polymorphisms.

Conclusions Our results suggest a role for CAT gene polymorphisms in vitiligo susceptibility in the Mexican population and a lack of association with VDR gene polymorphisms.

Introduction

Vitiligo is an acquired pigmentation disorder characterized by the progressive loss of epidermal melanocytes resulting in the appearance of hypo or achromic patches.[1] It may affect any individual regardless of ethnicity, gender, or age, presenting a global prevalence that ranges from 0.2% to 1%.[2]

There are two main clinical types of vitiligo, segmental and nonsegmental, the latter being the most common and thus the most studied.[1] The pathophysiology of nonsegmental vitiligo, hereafter called vitiligo, is complex and has been recently related to a combination of intrinsic defects of the melanocyte turning it highly sensitive to oxidative damage, along with defects in immunoregulatory pathways that promote elicitation of autoimmune responses.[3]

Among the intrinsic defects, impairment of antioxidant enzymes has been widely studied in vitiligo. Catalase (CAT) has been a demanded target of investigation since the report of

its decreased activity and increased levels of its substrate, hydrogen peroxide (H_2O_2), in vitiligo lesions.[4] The latter effect has been further corroborated and often attributed to inhibition of CAT by high H_2O_2 levels.[5–7] However, differential expression/activity of the enzyme can also be attributed to allelic variants in the CAT gene such as single nucleotide polymorphisms (SNPs) located at its promoter or exonic regions.[6] In this context, a promoter SNP located in the -89 position, termed -89 A/T, has been associated with increased risk of developing vitiligo in Chinese and Indian populations.[6,7] A similar result was reported for exon 9 codon 389 SNP, 389 C/T, in Caucasian North American and English populations.[8,9]

Regarding immunomodulatory molecules, vitamin D (VitD) is a relevant target considering that some autoimmune diseases are associated with reduced VitD levels, including vitiligo.[10,11] VitD is a secosteroid hormone that exerts its biological effects through the nuclear vitamin D receptor (VDR) on target cells. VitD plays a main role in calcium and phosphate homeostasis but is also important in immune regulation as most immune cells express VDR.[10] Its main immune roles are potentiation of innate response and regulation of adaptive response through inhibition of dendritic cell maturation and T helper (Th) 1 and 17 cells function as well as activation of T regulatory cells (Treg).[10]

It is important to mention that any alterations in the structure of VDR could modify the efficiency of VitD functions. Among the potential modifications in VDR, SNPs in regulatory regions of its gene have shown to be involved in different pathologies, particularly the SNPs near 3′ untranslated region, to say *Bsm*I, *Apa*I, and *Taq*I, have been linked to vitiligo susceptibility in Chinese, Turkish, and Egyptian populations.[11–14]

Considering the aforementioned findings regarding CAT, VitD, and their related genes on other populations, we hypothesize that they may be involved in vitiligo pathogenesis and/or its clinical characteristics in a Northwestern Mexican population.

Material and methods

Subjects

The present case-control study was approved by the Biomedical Research Ethics Committee of *Hospital General de Culiacán*, Sinaloa, México, following its ethical standards and those of the Helsinki Declaration of 1975, as revised in 1983. We enrolled a total of 357 individuals of Northwestern Mexican ancestry up to grandparents, that was divided into two groups, vitiligo and healthy controls (HCs). Written informed consent was collected from all participants.

The vitiligo group consisted of 173 nonsegmental vitiligo patients (81 males and 92 females) referred to General Hospital of Culiacan, Sinaloa, Mexico. Clinical diagnosis of vitiligo was determined by dermatologists based on the latest criteria of classification (Bordeaux, France, 2011).[1] Furthermore, demographical data and clinical characteristics of patients were assessed via questionnaire. Main aspects taken into account were gender, family history of vitiligo, age of onset, type of vitiligo according to lesion distribution, presence of other autoimmune diseases, presence of Koebner phenomenon, and disease activity. For the latter, the disease was considered stable if no lesions had appeared/extended in a period ≥1 year. The aforementioned clinical and demographic data of vitiligo patients is shown in Table 1.

On the other hand, the HCs group were 184 gender-age matched individuals free of vitiligo or other autoimmune diseases and without a family background of vitiligo.

Genetic analyses

DNA was isolated from peripheral blood with EDTA anticoagulant. All individuals included in the study (357, 173 patients and 184 HCs) were genotyped for CAT -89 A/T (rs7943316), 389 C/T (rs769217), and 419 C/T (rs11032709), and VDR *Bsm*I (rs1544410), *Apa*I (rs7975232), and *Taq*I (rs731236) gene polymorphisms by a PCR-restriction fragment length polymorphism method described elsewhere.[6,12] Primers and restriction enzymes used are listed in Table 2. Genotypes were visualized in 6% polyacrylamide gels and were confirmed randomly by an independent observer in 10% of the samples, obtaining the same results.

Serum analyses

Serum samples were separated from coagulated blood and stored at −70 °C until use. CAT activity was assessed by measuring the methanol conversion into formaldehyde using purpald as the colorimetric agent, while VitD levels were determined by competitive ELISA. Assays for CAT were done in 39 patients and 39 controls and for VitD in 35 individuals per group, all taken from the 357 total sample. They were free of pharmacological treatment, with an age of 18–45 years old and were matched by age, gender, and season of sampling. Samples were analyzed at the same time using the CAT assay kit and Vitamin D ELISA kit, both from Cayman Chemical (MN, USA) according to the manufacturer's instructions.

Table 1 Clinical and demographic data of vitiligo patients

Characteristic	Vitiligo patients*n* = 173 (%)
Male/female	81 (46.8)/92 (53.2)
Sporadic/familiar vitiligo[a]	74 (42.1)/99 (56.7)
Early onset/late onset	74 (42.1)/99 (57.9)
Localized[b]/disseminated[c]	24 (13.9)/149 (86.1)
Active/stable	99 (57.3)/74 (42.8)
With/without autoimmune disease	17 (9.8)/156 (90.2)
With/without Koebner phenomenon	35 (20.2)/138 (79.8)

[a]Reported 1st, 2nd, and/or 3rd degree affected relatives.
[b]Eight bilateral focal and 16 acrofacial cases.
[c]Fourteen universal and 135 generalized cases.

Table 2 Summary of conditions for the genetic analyses

SNP	Primer sequence (5′-3′)	Tm (°C)	PCR product size (bp)	Restriction enzyme	Cut allele size (bp)[a]	Ref
CAT gene						
-89 A/T	F-AATCAGAAGGCAGTCCTCCC R-TCGGGGAGCACAGAGTGTAC	66	237	*Hin*fI	A:162, 75	
389 C/T	F-GCCGCCTTTTTGCCTATCCT R-TCCCGCCCATCTGCTCCAC	69.5	202	*Bst*XI	T: 108, 94	6
419 C/T	F-CCTAAGTGCATCTGGGTGGT R-TACATCAGACAGTTGGGGCA	60.5	264	*Bst*NI	C:174, 90	
VDR gene						
*Bsm*I (G/A)	F-ACCTGGCCATTGTCTCTCAC R-CTAACCAGCGGAAGAGGTCA	60	600	*Bsm*I	G: 420, 180	12
*Apa*I (C/A); *Taq*I (C/T)	F-CAGAGCATGGACAGGGAGCAA R-CACTTCGAGCACAAGGGGC GTTAGC	68.5	490	*Apa*I; *Taq*I	C: 280, 210; T: 290, 200	

CAT, catalase; VDR, vitamin D receptor; SNP, single nucleotide polymorphism; Tm, primer annealing temperature; bp, base pairs; Ref, reference.
[a]Uncut allele size = PCR product size.

Statistical analyses

Genetic association test was performed using conditional logistic regression adjusted for age and gender, generating adjusted odds ratios (OR) and 95% confidence intervals for case-control comparisons. Associations of clinical variables with polymorphisms were evaluated by logistic regression. Differences in CAT activity and VitD levels were analyzed by Wilcoxon signed-rank test in case-control comparisons and by Mann-Whitney U in the rest. Statistical tests were run on SPSS Statistics v20 (IBM, Armonk, NY, USA), except for Hardy-Weinberg equilibrium and haplotype analysis which were done with deFinetti software (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) and SNPStats software (http://bioinfo.iconcologia.net/SNPstats), respectively.[15] Graphics were performed with GraphPad Prism v7 (La Jolla, CA, USA). Values of $P < 0.05$ were considered significant.

Results

Allele and genotype frequency comparison

Genotype frequencies were in agreement with Hardy-Weinberg equilibrium in both case and control groups, except for CAT 419 C/T gene polymorphism in controls ($P = 0.02$). As shown in Table 3, CAT -89 TT genotype and T alelle were more prevalent in the vitiligo group, conferring risk of developing vitiligo ($P = 0.015$, $P = 0.008$), while 389 TT genotype and T allele were more frequent in HCs group and were associated with protection against vitiligo ($P = 0.005$, $P = 0.007$). CAT 419 C/T polymorphism showed a low frequency in both groups being uninformative for the association analysis. No differences in the frequency of the VDR gene polymorphisms were observed between groups.

On the other hand, we found no association of CAT gene polymorphisms with the clinical characteristics of vitiligo (onset, clinical type, disease activity, presence of autoimmune disease, Koebner phenomenon, gender, and/or family history) (Tables S1–S3) but VDR *Bsm*I polymorphism appears to be related to the presence of Koebner phenomenon (Table 4).

Haplotype frequency comparison

For haplotype analysis, we excluded CAT 419 C/T polymorphism considering its low frequency. Linkage disequilibrium was observed for CAT ($D' = 0.99$, $r^2 = 0.39$) and VDR gene polymorphisms (BsmI-ApaI D'=0.93, $r^2 = 0.46$; BsmI-TaqI $D' = 0.97$ $r^2 = 0.79$; ApaI-TaqI $D' = 0.99$ $r^2 = 0.43$). As shown in Table 5, *CAT* haplotypes are associated with protection: AT against vitiligo *per se* and AC against active vitiligo ($P = 0.006$ and 0.039, respectively). No association of VDR haplotypes with vitiligo or its clinical characteristics were found (data not shown).

CAT activity and VitD levels comparison

We found a decrease in both serum CAT activity and VitD levels in the vitiligo group compared to HCs group ($P = 0.002$ and 0.017, respectively) (Fig. 1). However, CAT activity and VitD levels were not associated with any of the clinical characteristics of vitiligo or gene polymorphisms studied (Tables S4 and S5).

Discussion

In the present study, we evaluated the role of CAT and VDR gene polymorphisms, as well as serum CAT activity and VitD levels in vitiligo in a Mexican population considering their reported effects on other populations. Our results show that CAT -89 TT genotype and T allele are associated with an increased risk of developing vitiligo ($P = 0.015$, OR = 2.265 and $P = 0.008$, OR = 1.618), corroborating the findings in Chinese, Indian, and Italian populations and the suggestion that CAT -89 T allele contributes to the risk of developing vitiligo because of its position in the promoter of CAT gene relating to a decrease in its transcription rate.[6,7,16] However, this SNP was not found associated with vitiligo in Turkish and Korean populations, which might be attributed to ethnicity considering the interpopulation variation in -89 A/T polymorphism.[6,7,17,18]

Regarding CAT 389 C/T polymorphism, we found that TT genotype and T allele are associated with protection against

Table 3 Allelic and genotypic frequencies of CAT and VDR gene polymorphisms

CAT gene	Vitiligo[a] *n* = 173 (%)	HCs[a] *n* = 184 (%)	*P*[b]	OR (95% CI)[b]
-89 A/T				
AA	35 (20.2)	53 (28.8)	–	1.0 (reference)
AT	90 (52)	96 (52.2)	0.151	1.497 (0.864–2.595)
TT	48 (27.8)	35 (19)	0.015	2.265 (1.172–4.376)
T	0.537	0.451	0.008	1.618 (1.134–2.309)
389 C/T				
CC	96 (55.5)	85 (46.2)	–	1.0 (reference)
CT	70 (40.5)	76 (41.3)	0.232	0.766 (0.495–1.186)
TT	7 (4)	23 (12.5)	0.005	0.284 (0.117–0.689)
T	0.243	0.331	0.007	0.616 (0.433–0.875)
419 C/T				
CC	169 (97.7)	180 (97.8)	–	1.0 (reference)
CT	4 (2.3)	3 (1.6)	0.614	1.475 (0.326–6.663)
TT	0 (0)	1 (0.6)	0.978	– (–)
T	0.012	0.014	0.906	1.088 (0.270–4.389)
VDR gene[c]				
*Bsm*I				
GG (bb)	90 (52)	99 (53.8)	–	1.0 (reference)
GA (Bb)	69 (39.9)	66 (35.9)	0.574	1.139 (0.724–1.791)
AA (BB)	14 (8.1)	19 (10.3)	0.714	0.868 (0.405–1.857)
A (B)	0.280	0.283	0.975	0.994 (0.686–1.441)
*Apa*I				
CC (aa)	56 (32.4)	63 (34.2)	–	1.0 (reference)
CA (Aa)	83 (48)	91 (49.5)	0.875	1.039 (0.649–1.671)
AA (AA)	34 (19.6)	30 (16.3)	0.391	1.327 (0.695–2.536)
A (A)	0.436	0.410	0.363	1.175 (0.830–1.662)
*Taq*I				
TT (TT)	97 (56.1)	102 (55.4)	–	1.0 (reference)
TC (Tt)	69 (39.9)	71 (38.6)	0.981	1.005 (0.649–1.557)
CC (tt)	7 (4)	11 (6)	0.469	0.678 (0.237–1.942)
C (t)	0.240	0.253	0.811	0.952 (0.636–1.424)

CAT, catalase; VDR, vitamin D receptor; HCs, healthy controls; OR, odds ratio; CI, confidence interval.
[a]In agreement with Hardy Weinberg equilibrium except in CAT 419 C/T for HCs group ($P = 0.02$).
[b]Adjusted values for age and gender.
[c]BAT nomenclature is shown in parenthesis.

vitiligo ($P = 0.005$, OR = 0.284 and $P = 0.007$, OR = 0.616) which contrasts with previous studies.[16,19,20] According to the meta-analysis of He *et al.*,[19] C/T polymorphism is not associated with vitiligo, but they mention the importance of studying Latin and Hispanic populations as they might reveal a different pattern of genetic association, which can be seen in the present study. Considering CAT 389 C/T SNP is a silent substitution, its observed effect could be because of linkage with other polymorphisms that alter gene expression.[9]

For CAT 419 C/T polymorphism, our results were consistent with previous reports in its overall low frequency and lack of association with vitiligo.[6,8,9] Similarly, we did not find an association of VDR gene polymorphisms with vitiligo which contrasts with previous findings that have reported a protective effect of *Apa*I AA genotype.[11,14] This discrepancy could be explained by the genetic heterogeneity of the reported populations further highlighting the importance of studying Latin/Hispanic populations.

Table 4 Allelic and genotypic frequency of VDR *Bsm*I polymorphism in vitiligo patients according to presence of KP

VDR *Bsm*I[a]	Presence of KP *n* = 34 (%)	Absence of KP *n* = 139 (%)	*P*	OR (95% CI)
GG (bb)	13 (38.2)	77 (55.4)	–	1.0 (reference)
GA (Bb)	21 (61.8)	48 (34.5)	0.017	2.591 (1.188–5.653)
AA (BB)	0 (0)	14 (10.1)	0.999	– (–)
A (B)	0.309	0.273	0.520	1.209 (0.678–2.157)

VDR, vitamin D receptor; KP, Koebner phenomenon; OR, odds ratio.
[a]BAT nomenclature is shown in parenthesis.

With respect to SNPs effect on clinical characteristics of vitiligo, no association was found with CAT gene polymorphisms, as previously reported.[7,20] As for VDR gene polymorphisms, we observed that *Bsm*I heterozygous genotype (GA) was associated with the presence of Koebner phenomenon (see Table 4), a trait often related to active vitiligo.[1] In this sense, *Bsm*I polymorphism might be indirectly implicated in disease progression regardless of its lack of association with vitiligo *per se*, a finding that remarks the importance of analyzing VDR gene variants according to vitiligo clinical characteristics, an omission of previous association studies.[11,13,14,21]

Haplotype analysis showed an association of CAT -89/389 haplotypes with protection against vitiligo: AT haplotype against vitiligo *per se* and AC haplotype against active vitiligo (see

Table 5 Haplotype frequencies of CAT and VDR gene polymorphisms in vitiligo patients and HCs and according to disease activity

Haplotype[a]	Vitiligo	HCs	AV	SV
$CAT_{-89,389}$				
TC	0.538	0.493	0.576	0.486
AT	0.243[c]	0.332[c]	0.243	0.242
AC	0.220	0.217	0.182[d]	0.270[d]
$VDR_{BsmI,ApaI,TaqI}$[b]				
GCT (baT)	0.545	0.584	0.538	0.554
AAC (BAt)	0.231	0.253	0.196	0.277
GAT (bAT)	0.166	0.133	0.188	0.136

CAT, catalase; VDR, vitamin D receptor; HCs, healthy controls; AV, active vitiligo; SV, stable vitiligo.
[a]BAT nomenclature (VDR gene) is shown in parenthesis.
[b]Only major haplotypes are shown.
[c]$P = 0.006$, OR = 0.61 CI = 0.43–0.87.
[d]$P = 0.039$, OR = 0.55 CI = 0.32–0.97.

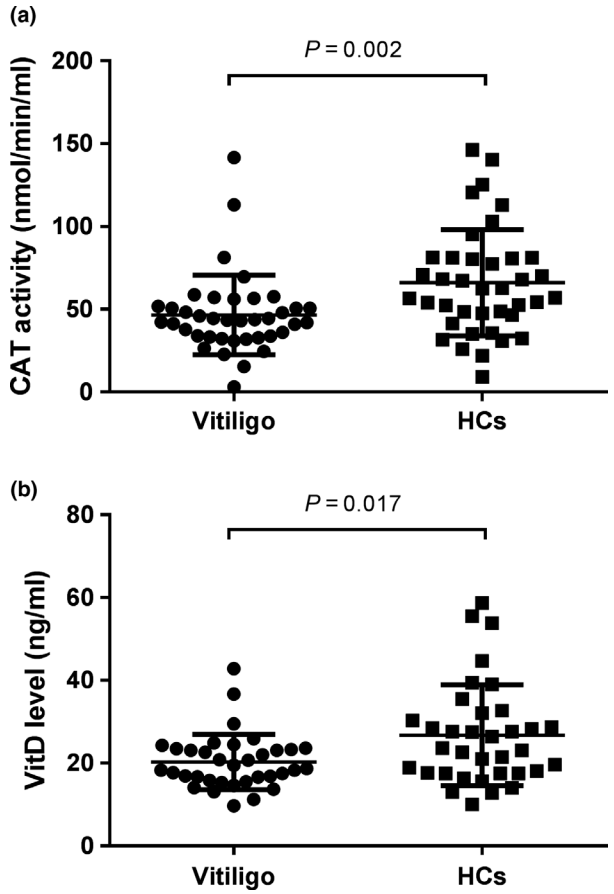

Figure 1 Serum measures of catalase and vitamin D in vitiligo patients and controls. (a) Catalase activity of 39 nonsegmental vitiligo patients versus 39 healthy controls. (b) Vitamin D levels of 35 nonsegmental vitiligo patients versus 35 healthy controls. Values are expressed as the mean ± standard deviation. Graphs were performed on GraphPad Prism v7

Table 5). The former has been previously reported in the Korean population, but the latter has not been described.[6,18] Considering the claim that any effect of 389 C/T alleles could be attributable to linkage with other CAT gene variants, we speculate that -89 A allele might be the one conferring protection particularly in the second case as it is more frequent among patients with stable vitiligo although not statistically significant (Table S2).

On the other hand, VDR haplotypes were not associated with vitiligo or any of its clinical characteristics. In this context, VDR haplotypic frequencies in vitiligo have been reported only by Aydıngöz *et al.*,[13] showing a decrease in GAT haplotype in patients. Nevertheless, they did not analyze their implication on clinical characteristics of vitiligo, a putative interesting topic if we consider the contrasting results of VDR genetic association studies in different populations.

Concerning serum analyses, we found that both CAT activity and VitD levels were decreased in the patient group in accordance with the general consensus that vitiligo is associated with low activity/levels of CAT and VitD.[4–7,11,14,21] On the other hand, CAT activity was not associated with clinical characteristics of vitiligo contrasting with the association of decreased CAT activity with active and generalized vitiligo reported in Tunisian and Indian populations, respectively.[5,7] This difference might be related to confounding factors such as age, physical activity, seasonal changes, and exposure to chemicals, which are known to affect CAT activity.[22] Similarly, VitD levels were not related to clinical characteristics agreeing with previous studies and suggesting its lack of effect in the clinical outcome of vitiligo regardless of its role with the disease *per se*.[23,24] Moreover, CAT and VitD measures were not related with the analyzed SNPs in CAT and VDR genes, respectively, differing from the findings that associate -89 T allele to lower CAT activity and *Apa*I A allele to higher VitD levels.[6,7,11,14] This discrepancy could be attributed to ethnicity since CAT -89 T allele and *Apa*I A allele frequencies vary according to the population studied.[6,7,11,16] Nevertheless, these relationships cannot be yet generalized considering the few populations analyzed as well as CAT and VitD decrease might be a consequence, and not a cause, of the disease.

In conclusion, it appears that CAT, but not VDR, gene polymorphisms modulate the risk of developing vitiligo which is related to low CAT activity and VitD levels. As far as we know, this is the first association study of CAT and VDR genes with vitiligo in the Latin American population. Therefore, we believe this contribution will enrich the knowledge in the field of vitiligo genetic epidemiology and should encourage further studies in order to better assess the role of CAT and VitD in vitiligo susceptibility and/or pathogenesis.

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References

1 Ezzedine K, Lim HW, Suzuki T, *et al.* Revised classification/nomenclature of vitiligo and related issues: the Vitiligo Global Issues Consensus Conference. *Pigment Cell Melanoma Res* 2012; **25**: E1–E13.
2 Krüger C, Schallreuter KU. A review of the worldwide prevalence of vitiligo in children/adolescents and adults. *Int J Dermatol* 2012; **51**: 1206–1212.
3 Manga P, Elbuluk N, Orlow SJ. Recent advances in understanding vitiligo. *F1000Res* 2016; **5**(F1000 Faculty Rev): 2234.
4 Schallreuter KU, Wood JM, Berger J. Low catalase levels in the epidermis of patients with vitiligo. *J Invest Dermatol* 1991; **97**: 1081–1085.
5 Dammak I, Boudaya S, Ben Aldallah F, *et al.* Antioxidant enzymes and lipid peroxidation at the tissue level in patients with stable and active vitiligo. *Int J Dermatol* 2009; **48**: 476–480.

6 Liu L, Li C, Gao J, *et al*. Promoter variant in the catalase gene is associated with vitiligo in Chinese people. *J Invest Dermatol* 2010; **130**: 2647–2653.
7 Mansuri MS, Jadeja SD, Singh M, *et al*. The catalase gene promoter and 5′-untranslated region variants lead to altered gene expression and enzyme activity in vitiligo. *Br J Dermatol* 2017; **177**: 1590–1600.
8 Casp CB, She JX, McCormack WT. Genetic association of the catalase gene (CAT) with vitiligo susceptibility. *Pigment Cell Res* 2002; **15**: 62–66.
9 Gavalas NG, Akhtar S, Gawkrodger DJ, *et al*. Analysis of allelic variants in the catalase gene in patients with the skin depigmenting disorder vitiligo. *Biochem Biophys Res Commun* 2006; **345**: 1586–1591.
10 Hewison M. Vitamin D, immunity and human disease. *Clin Rev Bone Miner Metab* 2010; **8**: 32.
11 Li K, Shi Q, Yang L, *et al*. The association of vitamin D receptor gene polymorphisms and serum 25-hydroxyvitamin D levels with generalized vitiligo. *Br J Dermatol* 2012; **167**: 815–821.
12 Becerra-Loaiza DS, Sánchez-Zazueta JG, Ochoa-Ramírez LA, *et al*. Study on vitamin D receptor gene polymorphisms in patients with urinary tract infections conducted in Northwestern Mexico. *Rev Mex Urol* 2018; **78**: 419–424.
13 Aydıngöz IE, Bingül I, Doğru-Abbasoğlu S, *et al*. Analysis of vitamin D receptor gene polymorphisms in vitiligo. *Dermatology* 2012; **224**: 361–368.
14 Sobeih S, Mashaly HM, Gawdat H, *et al*. Evaluation of the correlation between serum levels of vitamin D and vitamin D receptor gene polymorphisms in an Egyptian population. *Int J Dermatol* 2016; **55**: 1329–1335.
15 Solé X, Guinó E, Valls J, *et al*. SNPStats: a web tool for the analysis of association studies. *Bioinformatics* 2006; **22**: 1928–1929.
16 Caputo V, Niceta M, Fiorella S, *et al*. Vitiligo susceptibility and catalase gene (CAT) polymorphisms in sicilian population. *G Ital Dermatol Venereol* 2018; **153**: 619–623.
17 Akbas H, Dertlioglu SB, Dilmec F, *et al*. No association between catalase (CAT) gene polymorphisms and susceptibility to vitiligo in a Turkish population. *Clin Ter* 2013; **164**: e173–e177.
18 Park HH, Ha E, Uhm YK, *et al*. Association study between catalase gene polymorphisms and the susceptibility to vitiligo in Korean population. *Exp Dermatol* 2006; **15**: 377–380.
19 He J, Li X, Li Y, *et al*. Lack of association between the 389C>T polymorphism (rs769217) in the catalase (CAT) gene and the risk of vitiligo: an update by meta-analysis. *Australas J Dermatol* 2015; **56**: 180–185.
20 Mehaney DA, Darwish HA, Hegazy RA, *et al*. Analysis of oxidative stress status, catalase and catechol-O-methyltransferase polymorphisms in Egyptian vitiligo patients. *PLoS One* 2014; **9**: e99286.
21 Zhang JZ, Wang M, Ding Y, *et al*. Vitamin D receptor gene polymorphism, serum 25-hydroxyvitamin D levels, and risk of vitiligo: a meta-analysis. *Medicine (Baltimore)* 2018; **97**: e11506.
22 Kodydková J, Vávrová L, Kocík M, *et al*. Human catalase, its polymorphisms, regulation and changes of its activity in different diseases. *Folia Biol (Praha)* 2014; **60**: 153–167.
23 Doss RW, El-Rifaie AA, Gohary YM, *et al*. Vitamin D receptor expression in vitiligo. *Indian J Dermatol* 2015; **60**: 544–548.
24 Saleh HM, Abdel Fattah NS, Hamza HT. Evaluation of serum 25-hydroxyvitamin D levels in vitiligo patients with and without autoimmune diseases. *Photodermatol Photommunol Photomed* 2013; **29**: 34–40.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Alelle and genotype frequencies of CAT and VDR gene polymorphisms in vitiligo patients according to gender and family history of vitiligo (FHV).
Table S2. Alelle and genotype frequencies of CAT and VDR gene polymorphisms in vitiligo patients according to disease activity, clinical type, and type of onset.
Table S3. Alelle and genotype frequencies of CAT and VDR gene polymorphisms in vitiligo patients according to presence of autoimmune comorbidities (AIC) and Koebner phenomenon (KP).
Table S4. Serum catalase (CAT) activity and vitamin D (VitD) levels according to clinical and demographic characteristics of vitiligo patients.
Table S5. Serum catalase (CAT) activity and vitamin D (VitD) levels according to CAT and VDR genotypes.