Original Article

Identification of Genotypes and Allelic Frequencies of Vitamin D Receptor Gene Polymorphism (TaqI) in Egyptian Melasma Patients

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Abstract

Background: Melasma is a characteristic pattern of facial hyperpigmentation, occurring primarily on the forehead, cheeks, and chin, in a mask-like distribution. The pathogenesis of melasma is not fully understood. Vitamin D plays a role in skin pigmentation. It exerts its effect through vitamin D receptor (VDR), which is expressed in variable cells including normal melanocytes. **Aim and Objective:** The aim of the current work was to investigate if VDR gene polymorphism (TaqI) confers susceptibility to melasma in Egyptian patients. **Materials and Methods:** A total of 45 female patients with melasma were recruited and 50 healthy subjects that were matched on age, sex, body mass index, and skin phototype, were included as a control group.TaqI polymorphism was investigated using restriction fragment length polymorphism polymerase chain reaction (RFLP PCR). **Results:** Presence of (t) allele and (tt) genotype was significantly associated with melasma cases compared with control group ($P < 0.001$ for both). No significant association was found between (tt) genotype or (t) allele and clinical data of the studied cases. **Conclusion:** TaqI polymorphism is associated with melasma. Further, large-scale studies are recommended to underscore and validate the current findings. It is also necessary for future studies to extend the research to other populations and ethnicities. Investigating other VDR gene polymorphisms in melasma is also warranted. Since melasma is a multifactorial disease, gene–gene and gene–environment interactions should be considered in future genetic-epidemiologic researches to apply more comprehensive insight into the role of VDR gene in its pathogenesis.

Keywords: *Melasma, polymorphism, TaqI, vitamin D receptor gene*

Introduction

Skin pigmentation is determined by genetic, environmental, and endocrine factors, which influence both melanin synthesis in melanocytes and the distribution of melanin throughout the epidermis.[1]

Melasma is a common form of skin hyperpigmentation. It appears as symmetrical and irregular light-brown to grey-brown macules and patches on sun-exposed areas of the skin (i.e., forehead, temples, and cheeks).[2] These patches often develop gradually over time.[3]

Melasma can affect any one; it is particularly common in women and only 10% of those affected are men.[4]

The pathogenesis of melasma is not fully understood, but ultraviolet light exposure, pregnancy, estrogen ingestion, and positive family history are well recognized associations.[5] Vitamin D is produced in the skin after exposure to ultraviolet radiation or may be obtained from diet and supplements. Vitamin D is hydroxylated in the liver to 25-hydroxyvitamin D (25-OHD), which is the major circulating vitamin D metabolite. 25-OHD, in turn, is hydroxylated in the kidney to1,25-dihydroxyvitamin D [1,25(OH)2D], an endocrine hormone that functions to control serum levels of calcium and phosphorus. 1,25(OH)2D is also produced by non-renal tissues that possess 1 α hydroxylase, where it functions locally to control cellular growth and differentiation.[6] It also causes increase in tyrosinase activity and stimulates melanogenesis.[7]

Vitamin D exerts its effect via a nuclear hormone receptor called the vitamin D receptor (VDR), a member of the superfamily of nuclear receptors. VDR acts as a transcription factor and forms homodimers and heterodimers that are active in the transcription and transrepression of approximately

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900 genes in a ligand-independent and dependent manner.[8] These genes directly and indirectly influence cell cycling, proliferation, differentiation, and apoptosis.[9]

VDRs are present not only in cells typically involved in calcium and bone metabolism but also in other cell types, such as keratinocytes, melanocytes, fibroblasts, and immune cells of the skin.[10] It may also influence androgen and estrogen activation.[11]

The gene encoding for VDR is located on chromosome 12q[12] contains 11 exons[13] and spans approximately 75 kilobases of genomic DNA.[14]

A number of polymorphisms have been reported in VDR gene and include Bsm1 (alleles Bb), Apa1 (Aa), TaqI (Tt), and a polyadenyl microsatellite length polymorphism in the terminal untranslated region.[15-18]

TaqI polymorphism of VDR gene is a T/C nucleotide substitution (ATT to ATC) leading to a synonymous change at codon 352 (isoleucine) in exon IX.[19]

Based on the above-mentioned facts, namely, the role of ultraviolet light in vitamin D synthesis, the role of vitamin D in cutaneous pigmentation that is carried out through VDR, the expression of VDR in melanocytes, and its suggested role in estrogen activation; we aimed to investigate the association between VDR gene polymorphism TaqI and melasma in a sample of Egyptian patients searching for its role in disease occurrence and increased disease risk.

Patients and Methods

Studied population

This case-control study was carried out on 45 female patients with melasma, and 50 healthy subjects matched on age, sex, skin phototype, and body mass index (BMI), who have no present, past, or family history of melasma, as a control group. Selected control subjects had no blood relationship with the studied patients.

Patients were selected from the dermatology outpatient clinic during the period between January 2015 and December 2015. Written consent forms approved by the local ethical research committee were obtained from studied cases and control subjects before the study initiation.

All studied patients were subjected to complete history taking, general, and dermatological examination. Clinical data describing patients' age, disease duration, skin phototype, and family history of melasma were collected. Triggering factors (pregnancy, sun exposure, and estrogen-containing contraceptives' use) were all documented. Cases were clinically assessed by melasma area and severity index (MASI) score.[20]

MASI scoring was categorized as follows: A score of <5.5 defining mild disease, a score of ≥5.5 and <8.7 defining moderate disease, a score of ≥8.7 and <13.1 defining severe disease and a score of ≥13.1 defining very severe disease. All cases and control subjects had type IV Fitzpatrick skin type and BMI <30 kg/m^2.

Exclusion criteria

Any case or control subject who had received any systemic drugs or blood transfusion in the previous 6 months was excluded. Subjects with recent (within 6 months) or present history of systemic or autoimmune diseases, orthopedic disorders, diabetes, systemic infections, and/or any cutaneous disease or pigmentary disorder other than melasma were also excluded.

DNA extraction and amplification

Every case and control subject underwent detection of VDR gene polymorphism TaqI by restriction fragment length polymorphism polymerase chain reaction (RFLP PCR).

DNA was extracted from whole blood using Thermo Scientific Gene JET Genomic DNA purification kit, (Thermo Scientific, Lithuania). DNA was eluted and stored at −20°Cfor PCR procedure.

PCR for the VDR TaqI gene was carried out to a total volume of 25 µl; containing 10 µl genomic DNA; 1 µl of each primer, 12.5 µl of mastermix (Genecraft; Germany); (Stratagene, USA), and 1.5 µl of distilled water.[21]

VDR Taq1 gene was analyzed using the following designed primers (Midland, Texas):

Forward: 5′-CAGAGCATGGACAGGGAGCAAG-3′

Reverse: 5′-CGGCAGCGGATGTACGTCTGCAG-3′

PCR amplification for the VDR TaqI gene was performed separately using Applied Bio systems 2720 thermal cycler (Applied Bio, Singapore).

PCR condition consisted of: one cycle of amplification at 94º C for 3 minutes followed by 30 cycles at 94º C for 30 sec, One cycle at 60º C for 30 sec, one cycle at 72º C for 30 sec, and one final cycle of extension at 72º C for 5 min. The amplification products were separated by electrophoresis through 3% agarose gel stained with ethidium bromide with positive band at 345 bp.

VDR Taq1 gene polymorphism using RFLP technique

We took 15 µl of the PCR products for the VDR TaqI and mixed it with 1 µl (1 unit) of Fast Digest Taq1 restriction enzyme (provided by Fermentas) with 6.5 µl nuclease-free water and 2.5 µl of 10× Fast Digest Buffer.

The mixture was well mixed and incubated at 65°C for 30 min then 10 µl of the products were loaded into 3% agarose gel containing ethidium bromide for electrophoresis. The uncut fragment was 345 bp and digestion products were 260 bp and 85 bp [Figure 1a and b].[21]

Statistical analysis

Data were collected, tabulated and statistically analyzed using a personal computer with "(SPSS) version 11" program. Fisher's exact test was used for comparison of qualitative variables in 2 × 2 tables when expected cell count of more than 25% of cases was less than 5. Chi-square test (χ^2) was used to study the association between two qualitative normally distributed variables. Mann–Whitney U test was used for comparison between two groups not normally distributed having quantitative variables. Odds ratio was used to describe the probability that people who are exposed to a certain factor will have a disease compared to people who are not exposed to the factor. Differences were considered statistically significant with $P < 0.05$.

Results

Clinical characteristics of studied groups

Age of selected cases ranged from 21 to 53 years with a mean ± SD of 35.9 ± 7.65 years. The disease course was progressive in 33 cases (73.3%), stationary in 9 cases (20%), and regressive in three cases (6.7%). Disease duration varied in the studied cases from 1 to 108 months with 32.1 ± 35.1 months as a mean ± SD value. Family history of melasma was positive in 12 cases (26.7%).

MASI score varied between mild (MASI score <5.5) in three cases (6.7%), moderate (MASI score ≥5.5 and <8.7) in four cases (8.9%), severe (MASI score >8.7 and <13.1) in 14 cases (31.1%), and very severe (MASI score ≥13.1) in 24 cases (53.3%).Mean ± SD value of MASI in all cases was 24.7 ± 5.7.

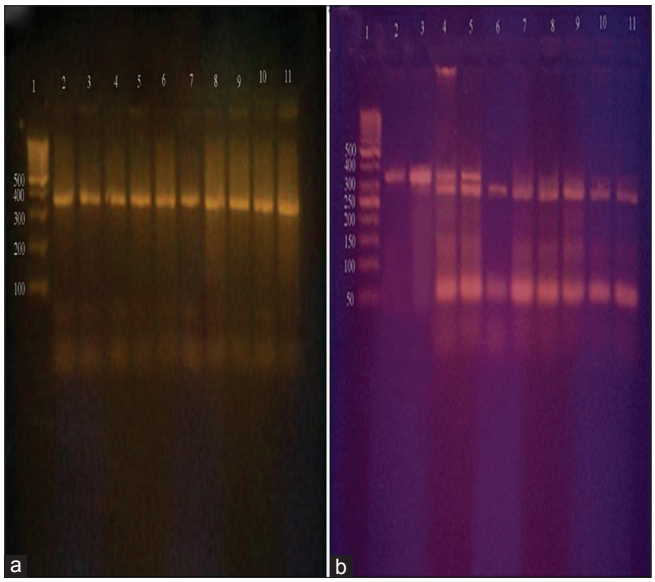

Figure 1: (a) VDR Taq1 gene, lanes from 2-11 show the length of the PCR amplicon which is 345 bp. Ladder 100 bp was used. (b) For VDR Taq1 gene polymorphism, the uncut fragment was 345 bp and digestion products were 260 and 85 bp. Ladder 50 bp was used. *Lanes 2 and 3 indicate TT genotype (345 bp). *Lane 4 and 5 indicates Tt genotype (345 bp, 260 bp and 85 bp). *Lanes 6,7,8,9,10, and 11 indicate tt genotype (260 bp and 85 bp)

Precipitating factors were pregnancy in 23 cases (51.1%), sun exposure in 15 cases (33.3%), and estrogen-containing contraceptives in 7 cases (15.6%).

Regarding control subjects, their ages ranged from 20–53 years with mean ± SD value of 34.8 ± 7.65 years.

Prevalence of alleles and genotypes in cases and controls

(TT) genotype was more prevalent among the control group than melasma patients (52% vs 22.2%). (tt) genotype was significantly associated with melasma patients ($P < 0.001$). (tt) genotype increases the risk of occurrence of melasma by 5.44 folds. (Tt) genotype was present in 26.7% of cases and 26% of controls. Regarding (t) allele, it was significantly associated with patients' group (64% vs 35%). It increases risk of occurrence of melasma by 3.37 folds [Table 1 and Figures 2, 3].

Relationship between genotypes and alleles and clinical data of cases

No significant association was found between genotypes or alleles and clinical data of the studied cases {patient age,

Table 1: Comparison between cases and control subjects regarding Taq I alleles and genotypes

TaqI Genotypes	Cases		Control		(CI 95%)	OR
	No.	%	No.	%		
tt	23	51.1	11	22	1.95-15.1	5.44
TT	10	22.2	26	52	–	–
Tt	12	26.7	13	26	0.82-7.01	2.40
TaqI Alleles	**Cases**		**Control**		**(CI 95%)**	**OR**
	No.	%	No.	%		
T	32	36	65	65	1.85-6.11	3.37
t	58	64	35	35		

CI: Confidence Interval, OR: Odds Ratio

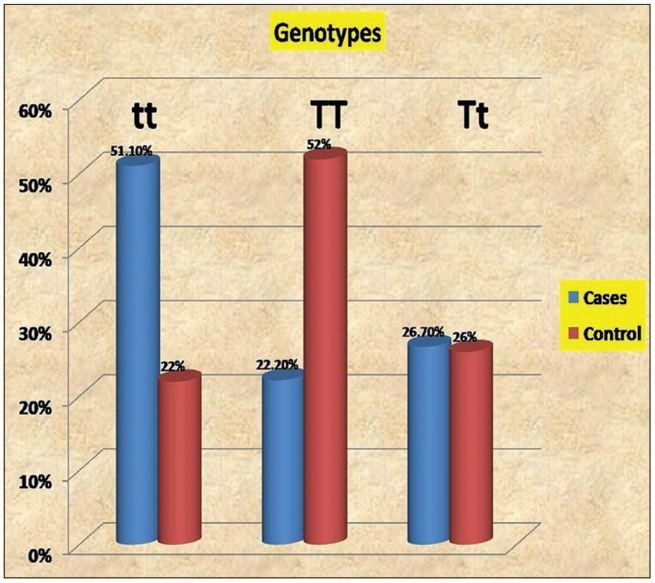

Figure 2: Distribution of TaqI genotypes in studied cases and controls

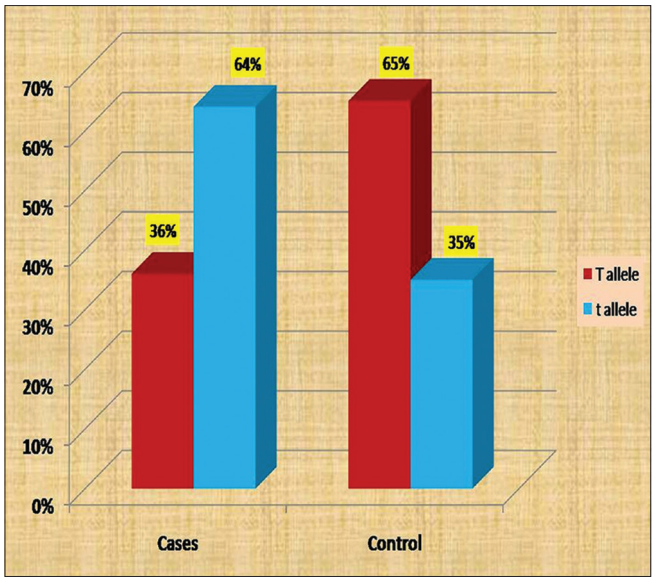

Figure 3: Distribution of TaqI alleles in studied cases and controls

disease duration, disease course, disease severity, precipitating factors or family history of melasma} [Tables 2 and 3].

Discussion

A polymorphism is a genetic variant that appears in at least 1% of the population. These genetic changes can occur in non-coding parts of the gene, so they would not be seen in the protein product. Changes in these regulatory parts of the gene would then affect the degree of expression of the gene, and thus the levels, functions, or stability of the protein.[22]

The association of a certain polymorphism with a phenotype or disease does not necessarily mean that the polymorphism is causing it.[23] To the best of our knowledge, TaqI polymorphism of VDR gene was not investigated before in melasma. It was studied in vitiligo and cutaneous malignant melanoma with contradictory results.[24-27]

Vitamin D was reported to have stimulatory effects on melanocyte proliferation.[8] In addition, it increases the number of DOPA-positive melanocytes and had a synergistic effect with a low dose of UVB-light.[9] It reduces the apoptotic activity induced by UVB in keratinocytes and melanocytes by the production of interleukin-6 and sphingosine-1-phosphate, thus preventing the loss of pigment in the skin.[10,11]

Sunlight induces production of vitamin D whose metabolite has anti-proliferative and prodifferentiated effects on melanocytes mediated through VDR. 1,25(OH)2D appears to be able to inhibit the growth of both normal and malignant melanocytes.[28]

However, there is no previous report stating that sunlight has an effect on VDR expression or VDR polymorphism. This issue may be considered in future studies.

Table 2: Relationship between vitamin D receptor (VDR) genotypes and clinical data of the studied cases

Clinical data	Genotypes				*P*
	tt *n*=23		TT *n*=10		
Age/years					
X±SD	36.5±8.10		35±8.47		0.63
Range	21-53		21-49		NS
Duration of disease/months					
X±SD	31.2±38.3		39.1±32.4		0.28
Range	1-108		1-96		NS
	No.	%	No.	%	
Course					
Progressive	18	78.3	6	60	0.49
Regressive	2	8.7	1	10	NS
Stationary	3	13	3	30	
Precipitating factors					
Pregnancy	11	47.8	5	50	0.99
Sun exposure	7	30.4	3	30	NS
Estrogen-containing contraceptives	5	21.7	2	20	
Family history					
Positive	5	21.7	4	40	0.271
Negative	18	78.3	6	60	NS
MASI Score					
Mild	2	8.7	0	0	
Moderate	4	17.4	0	0	0.31
Severe	7	30.4	3	30	NS
Very severe	10	43.2	7	70	

NS: Non significant, MASI Score: Melasma Area and Severity Index Score, X±SD: Mean±Standard deviation

Genetic variations within VDR gene could lead to significant receptor dysfunction and could further affect the formation of biologically active 25(OH)D.[24] Therefore, these genetic variations may have potential role in pigmentary disorders.

Due to the lack of reports concerning the role of VDR allelic variants in melasma, we aimed to investigate VDR gene polymorphism TaqI in a sample of Egyptian cases.

This case-control study revealed that (tt) genotype was significantly associated with melasma and increases the risk of disease occurrence by 5.4 folds.

TaqI polymorphism is located near the 30 end of the gene. It does not alter the amino-acid sequence nor change the encoded VDR protein.[29] Thus, the significance of this variant is unclear.[30] However, it was postulated that VDR polymorphisms could be functional themselves[26] and affect the transcriptional activity of the VDR.[31]

It was also suggested that VDR polymorphisms may have important consequences for vitamin D metabolism, a major orchestrator of melanogenesis,[24] and display cell type-specific effects on mRNA stability and may regulate VDR gene transcription.[18]

Table 3: Relationship between VDR alleles and clinical data of the studied cases

Clinical data	Allele				*P*
	T *n*=32		t *n*=58		
Age/years					
X±SD	35.3±7.30		36.2±7.51		0.78
Range					NS
Duration of disease/months					
X±SD	33.1±32.2		30.2±36.1		0.42
Range					NS
	No.	%	No.	%	*P*
Course					
Progressive	20	62.5	30	51.7	
Regressive	1	3.2	7	12.1	0.65
Stationary	11	34.3	21	36.2	NS
Precipitating factors					
Pregnancy	17	53.1	20	34.4	0.84
Sun exposure	10	31.2	18	31.2	NS
Estrogen-containing contraceptives	5	15.7	20	34.4	
Family history					
Positive	12	37.5	18	31.1	0.45
Negative	20	62.5	40	68.9	NS
MASI Score					
Mild	4	12.6	10	17.2	
Moderate	0	0.0	9	15.6	0.33
Severe	9	28.1	17	29.3	NS
Very severe	19	59.3	22	37.9	

NS: Non significant, MASI Score: Melasma Area and Severity Index Score, X±SD: Mean±Standard deviation

In addition, data suggest that genetic variants of VDR gene could keep the balance between harmful and beneficial effects of ultraviolet light on the skin.[32] Therefore, the effect of vitamin D mediated by VDR is likely to induce or alter melasma risk as a result of differential effects of sunlight exposure on skin.

In the present study, (t) allele was significantly associated with melasma and increases the risk of the disease by 3.37 folds.

It has been reported that (T) allele of Taq1 polymorphism is associated with greater VDR-mediated transcription[30] but the significance of (t) allele is still unclear.

Conclusion

From the above findings we can conclude that TaqI polymorphism is associated with and increases the risk of melasma.However, the mechanism of such effects is still unknown and needs further research.

Genetic association studies and gene polymorphism studies are investigative research work to demonstrate the participation of certain genes in disease development. These associations cannot be used for diagnosis or prognosis. They may be used, in future, for clinical interference and prevention or for treatment.

Therefore, identification of individuals with Taq1 polymorphism may help to prevent the development of melasma by prophylactic avoidance of avoidable risk factors; strict sun protection, avoidance of all forms of hormonal treatment in form of contraceptive pills or replacement therapy.

Furthermore, larger prospective studies to underscore and validate current findings are needed.It is necessary also for future studies to extend the research to other populations and ethnicities. Investigating other VDR gene polymorphisms in melasma is also warranted. Since melasma is a multifactorial disease, gene–gene and gene–environment interactions, should be considered in future genetic-epidemiologic researches to apply more comprehensive insight into the role of VDR gene in its pathogenesis.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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