Influence of vitamin D receptor polymorphism rs2228570 on pathological scarring

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Abstract. The physiological process of scarring is a common denominator of interest in a plethora of medical specialties. The molecular basis whereby this process results in pathological scarring for some individuals is poorly understood at present, with clues pointing towards individual predisposition for pathological scarring. Vitamin D and its subsequent pathway plays a key role in skin metabolism and homeostasis, with alterations in the level of vitamin D receptor (VDR) seen within pathological scars. The present study investigated the role of the rs2228570 polymorphism of *VDR* with regards to scar formation and evolution in a group of 71 female patients recovering from Caesarian section. Blood samples were taken at the time of surgery, and the follow-up was collected remotely at 3 and 6 months after surgery. The rs2228570 polymorphism was investigated using an RFLP-PCR protocol. The results demonstrated that the CC genotype, in combination with the Patient Observer Scar Assessment Scale (POSAS) and SCAR scores are associated with pathological scarring, with more studies being necessary to draw a firm conclusion.

Introduction

Scarring is a complex physiological process, whereby minimal deviation from the balance of synthesis and decomposition may lead to a resulting pathological scar. Far from being an esthetic issue, pathological scars are associated with symptoms which affect the patients' quality of life, including pain, loss of sensitivity and movement restriction (1).

Vitamin D has a plethora of roles within physiological skin function, including cell proliferation, differentiation, inflammation and fibrosis. Emerging research shows that vitamin D receptor (VDR) levels are observed at lower levels within pathological scars (2), as well as vitamin D supplementation being proposed as an early preventative option for keloid scars (3).

The rs2228570 polymorphism of the *VDR* is considered a benign polymorphic variant, with impact on drug response according to Clinvar (4). The polymorphism has been correlated with decreased levels of serum 25-hydroxyvitamin D, as well as a risk factor in combination with environmental stimuli for cutaneous malignant melanoma (4). Meta-analyses have been carried out to investigate the association between the *VDR* polymorphism and connective tissue disorders such as degenerative disc disease and periodontal disease (5-8).

The present study aimed to investigate the rs2228570 *VDR* polymorphism regarding the development and evolution of pathological scars in a group of female patients.

Patients and methods

The study group consisted of female patients having undergone Caesarian section at the 1st Gynaecology Clinic in Cluj-Napoca, Romania. A total of 84 patients were recruited for the study and followed up for 6 months, with initial in-person and 3 and 6-month phone check-ups. Among these 84 patients, 71 finished the follow-up and were included in the present study. After the 6-month check-up, the patients were grouped according to scar type as follows: 53 patients with physiological scars, 13 patients with hypertrophic scars and 5 patients with atrophic scars.

The inclusion criteria for the study were: patients over 18 years of age, willing and able to offer informed consent for the procedure and follow-up after undergoing planned Caesarian section with no pre-operative or post-operative complications.

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Abbreviations: VDR, vitamin D receptor; SNP, single nucleotide polymorphism; POSAS, Patient Observer Scar Assessment Scale; RFLP-PCR, restriction frag time polymerase chain reaction

Key words: VDR, SNP, RFLP-PCR, hypertrophic scar, atrophic scar

Table I. Characteristics of the study group (n=71) regarding the clinical and demographic data.

Parameters	Normal scarring (n=53)	Hypertrophic scarring (n=13)	Atrophic scarring (n=5)
Age mean	31.03±5.31	30.76±4.47	30.66±4.5
Weight (kg)	80.33±14.55	73.15±14.5	84.5±21.77
Height (m)	1.63±0.06	1.63±0.08	1.60±0.03
Preconceptional weight (kg)	65.07±14.69	59±12.28	72.2±24.83
Weight gain (kg)	14.26±5.37	14.15±5.77	13.2±4.43
Smoking			
Yes	10 (14.08)	1 (1.4)	1 (1.4)
No	43 (60.56)	12 (16.9)	4 (5.63)
P-value	0.002	>0.005	>0.005
Personal history			
Yes	10 (14.08)	4 (5.63)	1 (1.4)
No	43 (60.56)	9 (12.67)	4 (5.63)
P-value	0.002	0.004	>0.005
Family history			
Yes	3 (4.22)	1 (1.4)	0
No	50 (70.42)	12 (16.9)	5 (12.19)
P-value	0.002	>0.005	N/A
Fitzpatrick phototype			
1	6 (8.45)	0	1 (1.4)
2	14 (19.71)	2 (2.81)	1 (1.4)
3	19 (26.76)	10 (14.08)	0
4	11 (15.49)	1 (1.4)	2 (2.81)
5	3 (4.22)	0	1 (1.4)
POSAS			
3 months	18.88±7.16	21.61±4.87	12.2±5.4
6 months	16.74±6.67	7.53±2.25	7.4 ± 2.7
P-value	>0.005	0.001	0.004
SCAR			
3 months	5.71±2.43	7.53±2.25	7.4±2.7
6 months	4.45±2.7	8.69±1.1	8.2±1.93
P-value	>0.005	>0.005	>0.005
Treatment			
Yes	8 (11.26)	4 (5.63)	0
No	45 (63.38)	9 (12.67)	5 (12.19)
Lactation (months)	4.05±2.48	5.19±1.46	3.3±3.07

Data are presented as mean ± SD for continuous variables and as percentages for categorical variables. POSAS, Patient Observer Scar Assessment Scale; N/A, not applicable (due to sample size, the value cannot be computed).

Patients who were unwilling or unable to maintain contact for follow-up were excluded, alongside patients whose incisions overlapped previous surgeries or trauma.

After inclusion, a peripheral venous blood sample was collected in a K3EDTA vacutainer and stored at 4˚C until processing. Genomic DNA was extracted using the Wizard genomic DNA Purification Kit, produced by Promega Corp. After rehydration, the amount of DNA obtained through extraction, as well as purity was evaluated using spectrophotometrical analysis. The DNA samples were stored at -20˚C pending genotyping.

Patient data including demographic data, a full medical and pregnancy history, Fitzpatrick phototype as well as SCAR and Patient Observer Scar Assessment Scale (POSAS) scales (9) were completed for the first visit. During follow-ups, the SCAR and POSAS scores were filled out via phone interview, and photographs of the scars were sent by the patients.

The genotyping was carried out using an RFLP-PCR protocol. The amplification was carried out using Thermo Scientific™ PCR MasterMix (Thermo Fisher Scientific, Inc.), specific primers and the DNA extracted from peripheral venous blood samples.

Table II. Allele frequency and genotype distribution of the *VDR* variant gene.

Allele frequency and genotype distribution of the *VDR* variant gene	Normal scarring n (%)	Hypertrophic scarring n (%)	Atrophic scarring n (%)	OR	P-value
T allele	33	9	0	N/A	N/A
C allele	73	17	5	2.43	0.064
TT	5	0	0	N/A	N/A
CT	23	9	0	N/A	N/A
CC	25	4	5	2.37	0.088
CC + CT	48	13	5	2.41	0.077

OR, odds ratio; N/A, not applicable (due to sample size, the value cannot be computed); VDR, vitamin D receptor.

The primers used were: 5'-AGCTGGCCCTGGCACTGACTCTGCTCT-3' and 3'-ATGGAAACACCTTGCTTCTTCTCCCTC-5'. The cycling conditions were as follows: initial denaturation for 3 min at 94.5˚C, 35 cycles of 1 min at 94.5˚C, 1 min at 61˚C, 2 min at 72˚C and a final elongation of 10 min at 72˚C. The amplified samples were digested using Thermo Scientific™ FastDigest FokI, (Thermo Fisher Scientific, Inc.) incubated for 15 min at 37˚C. After digestion, the samples were migrated through 2% agarose gel (MetaPhor Agarose) dyed using RedSafe Nucleic Acid Staining Solution (MetaPhor Agarose), leading to the formation of 3 possible bands: a 265-bp band, representing the PCR product of the *VDR* gene (either undigested or major allele), a 196-bp band and a 69-bp band, resulting from the presence of the restriction site.

Statistical analysis. The investigation was conducted using SPSS for MacBook software (SPSS, Inc.). The Chi-square test was used to measure the Hardy-Weinberg equilibrium. The categorial variables are presented as percentages whereas the continuous valuables are expressed as mean ± SD. The Chi-square test was used to compare the clinical and demographic data. The dispersion parameters were calculated using the Kolmogorov-Smirnov test. Mann-Whitney-Wilcoxon test and Student's t-test were used to make comparisons between subgroups and to make correlations within continuous variables. The outcome differences appreciated by the SCAR and POSAS were compared using the Student's t-test.

Multinomial regression was considered to predict the possible outcomes and to determine the susceptibility of the *VDR* polymorphism between the subgroups. Allelic frequencies and genotype distribution were examined among the study group using Fisher's exact test [OR with 95% confidence intervals (CI)]. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Demographic study. The characteristics of the study group (n=71) regarding the clinical and demographic data are illustrated in Table I. There was no reported differences in age, weight, height, lactation duration and preconceptional weight within our study group. No differences were present in the personal and family history, smoking habit and the clinical evaluation scores POSAS and SCAR.

Analysis of the variant gene for VDR. The Hardy-Weinberg equilibrium was respected for the investigated gene variant. The allele frequency and genotype distribution are illustrated in Table II. Both the dominant and recessive models of Fisher's exact test for evaluating the risk for pathological scarring did not reveal any statistical differences for the variant gene carriers of *VDR* between the subgroups.

Analysis of the variant gene for VDR and clinical parameters. The Student's t-test was used to assess the clinical outcome indicated by the SCAR and POSAS in association with the variant gene of *VDR*. The POSAS at 6 months did not reveal any significant statistical difference compared to the POSAS at 3 months regarding the genotype distribution. In comparison, the SCAR at 3 months did reveal a significant statistical association between the CC genotype and the poor outcome (P=0.041), highlighting the CC genotype as a causative factor (OR=1.8, CI 0.204-6.876, P=0.048). SCAR at 6 months failed to reveal this causative interplay.

The multivariate regression conducted to appreciate the association between the POSAS, SCAR, scarring type and genotype did not reveal a significant association (R^2=0.523, beta: 0.81; 95% CI: 0.534-0.966). Furthermore, a high score for both SCAR and POSAS did not illustrate a significant association with the CT heterozygous genotype for developing hypertrophic scars.

Discussion

The results obtained in the present study do not support a strong association between the specific SNP investigated and the process of scarring, however the results may prompt further investigation.

As a secondary finding, the present study supports the use of complementary scales such as SCAR and POSAS, in order to screen for potential pathological scarring. This accomplishes a similar goal to genotyping for risk markers: early detection and therapeutic intervention, bypassing the traditional treatment algorithm, which involves waiting for the

scar to heal entirely, and then intervening where needed with invasive or non-invasive strategies.

As the outcome of scarring is heavily influenced by the mechanism of the lesion itself, postoperative care and populational factors, no comparison can be made regarding the frequency of the pathological scars revealed in the present study and other studies investigating scarring currently available in literature.

The strong point of the study lies in the selection of the study group: with patients in identical conditions, operated according to the internal protocol of the clinic. This selection excludes a series of intraoperative and postoperative factors which influence scarring, including incision and suture technique and materials, as well as postoperative care.

Given the weak correlations obtained in our study, a larger study group might have offered a more realistic image on the influence of rs2228570 on the process and outcome of scarring. Another issue that presented itself was that out of the initial 84 patients recruited, 13 were excluded before the finalization of the study. One patient retracted herself from the study, however another 12 patients could not be contacted. Synchronizing the study check-ups with the required post-pregnancy check-ups could be a solution to ensure a lower rate of drop-out.

A longer follow-up time would be a valid way to expand the results of this particular study, as scars may evolve past the 6-month marker used within the present methodology. However, this would require a much larger sample size, due to a predictably higher drop-out rate.

To the best of our knowledge, this is the first study investigating this particular polymorphism of *VDR* with regards to scarring. The rs2228570 polymorphism has been studied extensively in other connective tissue pathologies, such as lumbar disc degeneration, with mixed results; in some subsets of the population, the polymorphic variant is associated with the development of disc degeneration (5).

It seems to be the case that vitamin D, alongside a plethora of other factors and genes, are responsible for the individual predisposition to develop pathological scars. The research and clinical applications of genetic markers which predict the tendency towards pathological scarring would prove invaluable, allowing for a predictive, pro-active and personalized therapeutical approach for scarring.

In conclusion, no association was found between rs2226570 *VDR* polymorphism and scarring outcome. A potential correlation between pathological scarring and the CC genotype combined with SCAR score results was found at the 3-month mark, but this did not reach significance at the end of the study.

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Availability of data and materials

The individual genotyping results, as well as any other information pertaining to the study are available by reasonable request to the corresponding author.

Authors' contributions

RFI, AC, and IVP contributed substantially to the design of the study. CSA performed the analysis of the resulting data. SRH, IL, RET, and ICR were involved in the acquisition of data. All authors critically revised the manuscript, approved the final version and agree to be accountable for all aspects of the work.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the 'Iuliu Haţieganu' University of Medicine and Pharmacy, Cluj-Napoca, Romania. All patients included were of legal age and capable of understanding the purpose and potential risks involved. Consent was granted freely and without coercion. The consent form for this study elaborated on the use of the data for publication for scientific purposes of the values recorded. The present study does not include any identifiable patient data.

Patient consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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