Involvement of COL5A2 and TGF-β1 in pathological scarring

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Abstract. Dysregulation in the cutaneous wound-healing process is a consequence of alterations in the efficiency and activity of the various components involved in the healing process. This dysregulation may result in various clinical appearances of a lesion, such as skin ulcers, keloids, hypertrophic and atrophic scars. The collagen type V alpha 2 (*COL5A2*) gene provides a template for a component of type V collagen, found primarily within the skin basement membrane. Transforming growth factor (TGF)-β is involved in inflammation, angiogenesis, proliferation of fibroblasts, collagen synthesis and extracellular matrix remodeling. Hypertrophic scar fibroblasts possess a disrupted expression pattern of the TGF-β signaling compared to normal healing, while an increased TGF-β signaling reduces the epidermal proliferation rate, triggering atrophic scarring. In the present study, 71 female patients who had undergone planned Caesarean section, without postoperative complications, were examined. These patients were clinically and molecularly evaluated after developing scars in order to determine the role of TGF-β1 (rs201700967 and rs200230083) and COL5A2 (rs369072636) in pathological scarring. Clinical scar evaluation was carried out using SCAR and POSAS scales and genotyping was performed by RT-PCR. No statistical differences were found between the subgroups regarding the genotype and the pathological scarring, since all the patients included were wild-type allele carriers. Further investigations and a more representative study group may highlight the involvement of COL5A2 and TGF-β1 single nucleotide variants in pathological scarring.

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Abbreviations: SNP, single-nucleotide polymorphism; POSAS, patient observer scar assessment scale; RT-PCR, reverse transcription-polymerase chain reaction; TGF-β1, transforming growth factor-β1

Key words: COL5A2, TGF-β1, SNP, hypertrophic scar, atrophic scar

Introduction

Wound healing is a physiological process, which aims to restore cutaneous function and its integrity in the aftermath of an injury. It is a highly gene-driven dynamic process of interactions between multiple cell types, extracellular matrix and signaling molecules. Inflammation, proliferation, re-epithelization and tissue remodeling are the four primary programmed phases (1). Dysregulation in the cutaneous wound-healing process leads to insufficient or excessive healing activities resulting in skin ulcers, keloids, as well as hypertrophic and atrophic scarring (2). Genetic predisposition is responsible for the variable phenotype encountered in pathological scarring, either as an individual lesion or part of a connective-tissue disorder (3).

Collagens are a protein family responsible for strength and support in many tissues, including the skin (4). The collagen type V alpha 2 (*COL5A2*) gene provides a template for a component of type V collagen, found primarily within the skin basement membrane (5). Atrophic scars are frequently present in Ehlers-Danlos Syndrome due to heterozygosity for COL5A1 null alleles or for missense mutations in COL5A2 (6). Hypertrophic scars share a similar clinical appearance with keloid scars, the main difference being the proliferation of the scar tissue beyond the original borders of the lesion, in the case of keloid scars. However, the hardened cord-like tracts of abnormal collagen are seen in hypertrophic and keloid scars alike. Even though the histology of hypertrophic scars consists primarily of type III collagen, small amounts of type V collagen are present, suggesting COL5A2 involvement in the development of pathological scarring (7).

Transforming growth factor (TGF)-β belongs to the family of growth factors involved in the wound-healing process. The three isoforms of TGF-β (TGF-β1, -β2, -β3) participate in inflammation (8), angiogenesis, proliferation of fibroblasts, collagen synthesis and extracellular matrix remodeling. Hypertrophic scar fibroblasts possess an altered phenotype and a disrupted expression pattern of TGF-β signaling when compared to normal healing. In addition, the TGF-β plasma level is considered a predictive marker for hypertrophic

scarring in children (9). Increased TGF-β signaling reduces the epidermal proliferation rate and it is considered responsible for triggering the atrophic scarring onset and evolution among other key players in pathological scarring (10). Since genetic predisposition plays a pivotal role in the etiopathogenesis of scarring, mutations in the genes responsible for inflammation, proliferation and maturation including TGF-β require further investigation (1).

The aim of the present study was to investigate the candidate variant genes of *TGF-β1* (rs201700967 and rs200230083) and *COL5A2* (rs369072636) as possible genetic predictive indicators in pathological scarring for the Caucasian population.

Patients and methods

Study group and sampling. The study group comprised 71 female individuals enrolled after undergoing a Caesarean section at the First Gynecology Clinic in Cluj-Napoca. The subjects were >18 years of age, with a mean age of 31,03 years. The age range of the participants was 18-41 years. Inclusion criteria were Caesarean section without any pre-/post-operative complications and follow-up compliance. Exclusion criteria were overlapped incisions from previous surgeries or trauma.

An in-person initial consultation was performed, and periodic check-ups at 3 and 6 months followed. The SCAR (11) and POSAS (https://www.posas.nl/) scales were applied to clinically investigate the scars; both by patients and professionals. The 3- and 6-month consultations were performed by phone, with the patients completing the questionnaires during the conversation, and sending photographs of the scar to the investigator for further evaluation. Pending follow-up, the study group was divided into: Physiological scar group (53 patients), hypertrophic scar group (13 patients) and atrophic scar group (5 patients) based on the final appearance of the scar at 6 months.

Simultaneously, venous blood samples were collected in K3EDTA vacutainers and stored at 4˚C until genotyping. Genomic DNA extraction was performed using a commercial kit according to the manufacturer's protocol (The Wizard Genomic DNA Purification Kit, Promega Corp.). Storage at -20˚C after rehydration followed until further processing.

The present study was approved by the Ethics Committee of the 'Iuliu Haţieganu' University of Medicine and Pharmacy, Cluj-Napoca. All the patients included were of legal age and capable of understanding the purpose and potential risks involved. Patient consent was obtained for the study.

Genotyping investigation. COL5A2 (rs369072636) and TGF-β1 (rs201700967 and rs200230083) genotyping were performed using TaqMan assay (Thermo Fisher Scientific, Inc.) and the 7500 Fast Dx Real-Time Polymerase Chain Reaction (PCR) system (Applied Biosystem; Thermo Fisher Scientific, Inc.), under the manufacturer's protocols. Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad) software was used to interpret the results.

Statistical analysis. SPSS for MacBook version 25 (SPSS. Inc.) was used for conducting the statistical investigation. Mean ± standard deviation or absolute and relative frequencies (%) were used in the descriptive statistics for clinical and genetic variables. The Hardy-Weinberg Equilibrium was measured using the Chi-square test. Clinical and demographical data were compared using the Chi-square test. The Kolmogorov-Smirnov test was applied to evaluate the dispersion parameters. The Mann-Whitney U test and the Student's t-test were addressed to compare between subgroups and to correlate within continuous variables. SCAR and POSAS differences in outcomes were compared using the Student's t-test. Allelic frequencies and genotype distribution were examined among the study group using Fisher's exact test [odds ratio [OR with 95% confidence intervals (CIs)]. A significant statistical difference was considered at $P<0.05$.

Results

Clinical and demographic study. For the age, height, weight, pre-conceptional weight and lactation duration variables no difference was observed between the subgroups. The demographic and clinical characteristics are presented in detail in Table I. Significant statistical differences were computed with regard to the clinical evaluation from POSAS, personal and family history. No differences were observed between the groups regarding the Fitzpatrick phototype and SCAR scales.

It was found that 74.64% of the patients had normal scarring tissue, while 18.3 and 7.04% developed hypertrophic and subsequently atrophic scars. The pathological scarring distribution verifies the reported scar prevalence after surgery in the Caucasian population (data not shown).

A decreased value of 1.71 points in difference was statistically significant between the 3- and 6-month check-ups for the POSAS comparative analysis (95% CI, 0.4-2.89; P=0.01). The SCAR comparative analysis did not reveal a statistical difference, 0.670 (95% CI, 0.04-1.38; P=0.055) (data not shown).

Analysis of COL5A2 (rs369072636). The *COL5A2* genotypes are GG wild-type homozygote, GA heterozygote and AA variant homozygote. In the present study, all 71 participants were genotyped, yielding a GG wild-type homozygote. The Hardy-Weinberg Equilibrium was not measured due to the lack of the variant genotype.

Analysis of β1-TGF (rs201700967 and rs200230083). The possible β1-TGF (rs201700967) genotypes are CC wild-type homozygote, CT heterozygote and TT variant homozygote. In the present study, all 71 participants were genotyped, yielding a CC wild-type homozygote. The Hardy-Weinberg Equilibrium was not measured due to lack of the variant genotype.

Discussion

Abnormal collagen fibrillogenesis due to mutations in *COL5A2* gene is responsible for dermal fragility and altered wound-healing process. COL5A2 encodes the α2(V) chain of type V collagen. Atrophic scars present in Ehlers-Danlos Syndrome were associated with the heterozygosity for COL5A1 null alleles or for missense mutations in COL5A2. The investigated mutation was reported to reduce the quantity of normal type V collagen available for collagen fibrin synthesis (6). Our study did not encounter the homozygote or heterozygote genotypes due to the low frequency

Table I. Demographic and clinical patient features according to the study subgroup.[a]

Parameters	Normal scarring group (n=53)	Hypertrophic scarring group (n=13)	Atrophic scarring group (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
Preconception 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=0.01	Not determined	Not determined
Personal history			
Yes	10 (14.08)	4 (5.63)	1 (1.4)
No	43 (60.56)	9 (12.67)	4 (5.63)
	P=0.01	P=0.02	Not determined
Family history			
Yes	3 (4.22)	1 (1.4)	0 (0)
No	50 (70.42)	12 (16.9)	5 (12.19)
	P=0.02	Not determined	Not determined
Fitzpatrick phototype			
1	6 (8.45)	0 (0)	1 (1.4)
2	14 (19.71)	2 (2.81)	1 (1.4)
3	19 (26.76)	10 (14.08)	0 (0)
4	11 (15.49)	1 (1.4)	2 (2.81)
5	3 (4.22)	0 (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=0.1	P=0.01	P=0.01
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=0.2	P=0.055	P=0.6
Treatment			
Yes	8 (11.26)	4 (5.63)	0 (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

[a]Data are presented as mean ± SD for continuous variables and as frequencies for categorical variables. POSAS, patient observer scar assessment scale; SCAR, Scar Cosmesis Assessment and Rating; SD, standard deviation. P-values indicate statistical significance.

described in existing databases (https://www.ncbi.nlm.nih.gov/snp/rs369072636#frequency_tab). The frequency among the Caucasian population has not been reported yet in the literature and our second research objective was to provide insight regarding this.

Hypertrophic scars present proliferation of the dermal tissue due to excessive deposition of fibroblast-derived collagen and other extracellular matrix proteins and can be triggered by chronic inflammation, persistent fibrosis or infection (12). The *COL5A2* gene investigated plays a role in the dysregulated wound-healing process and a larger study group may validate a genetic predisposition in Caucasians.

TGF-β was indicated based at the plasma level to be a predictive factor in children after burn-induced hypertrophic scars. Elevated plasma levels of TGF-β were associated with no hypertrophic scar development compared to low levels (13). Other findings suggest that the high frequency of $CD4^+$/TGF-β-producing T cells was identified in hypertrophic tissue (14).

Manipulation of TGF-β in the prevention of hypertrophic and atrophic scarring by antibody neutralization revealed

in animal models an inhibitory effect on fibrosis, reducing the TGF-β signaling in both normal and pathological scarring (15). The TGF-β1 single nucleotide variants (rs201700967 and rs200230083) are both a C/T transition substitution for the coiled-coil domain containing 9.

TGF-β1 is a multipotent cytokine responsible for regulating cell growth and differentiation as well as extracellular matrix organization in dermal tissue, as well as stimulating the angiogenesis and vasodilatation in hypertrophic scars. Atrophic scars reveal an opposite phenotype compared to the hypertrophic ones, revealing a loss in function or an attenuated activity of the TGF-β1 (16).

Limitations consist of a non-representative study group due to a low rate of enrollment to contrast the reduced population frequencies of the investigated mutations. No data regarding these genetic variants were available in literature for the Caucasian population.

Due to lack of data regarding the minor allele frequency in the case or control population, a relevant sample size estimate cannot be calculated. Further advanced investigation of the role of COL5A2 and TGF-β1 in the etiopathogenesis of scarring is necessary for a better understanding of the possible predisposition indicator that they may hold. The signaling pathways of COL5A2 and TGF-β1 have common points of interactions and no data are available over their inhibiting or stimulating effects on their expression.

In conclusion, the present study focused on the investigation of *COL5A2* and *TGF-β1* gene variants in pathological scarring in the absence of a genetic disorder in a Caucasian population group. Further investigations and a more representative study group may highlight implication of COL5A2 and TGF-β1 single nucleotide variants in pathological scarring. However, no statistical differences were found between the subgroups regarding the genotype and the pathological scarring relationship, since we lack a representative study group.

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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 and interpretation 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 has been approved by the Ethics Committee of the 'Iuliu Haţieganu' University of Medicine and Pharmacy, Cluj-Napoca. All patients included were of legal age and capable of understanding the purpose and potential risks involved. Consent was granted freely and without coercion.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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