Role of *hTERT* rs2736100 in pathological scarring

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Abstract. Hypertrophic and atrophic scars are the effect of a dysregulated wound-healing process in genetically predisposed individuals. The genetic predisposition has acquired significant attention due to the diverse phenotype of pathological scarring in individuals with a positive personal and family history. Recent studies have identified telomere shortening and decreased hTERT activity in pathological scarring, proposing the rs2736100 variant of human telomerase reverse transcriptase (*hTERT*) gene as a valuable variant gene candidate. We examined the scarring process in 71 female patients who had undergone Caesarean section and developed hypertrophic and atrophic scars with the objective to investigate the role of single nucleotide polymorphism (SNP) rs2736100 in pathological scarring. Genotyping was performed using RT-PCR and follow-up included the Patient Observer Scar Assessment Scale (POSAS) and SCAR scales. Comparative analysis for mean POSAS value between the check-ups at 3 and 6 months revealed a statistical decreased difference of 1.71 points [95% confidence interval (CI), 0.4-2.89; P=0.01], while SCAR highlighted a decreased difference of 0.670 (95% CI, -0.04-1.38; P=0.055). The C variant allele revealed a borderline statistical value for the risk of developing pathological scarring (OR=1.44; 95% CI, 0.876-1.332: P=0.066). In our study a pre-conceptional body mass index (BMI) >25 kg/m^2 was statistically associated with pathological scarring. The Fitzpatrick type 4 phototype displayed an increased frequency for the heterozygous genotype in the current study, and it was demonstrated that dark skin tone was associated with abnormal scar formation. Our study investigated the role of *hTERT* gene variant rs2736100 in hypertrophic and atrophic scarring in a Caucasian population group. We report a borderline statistically significant value for the variant C allele of *hTERT* SNP for the risk of developing pathological scarring in female patients that had undergone Caesarean section.

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Abbreviations: SNP, single nucleotide polymorphism; POSAS, Patient Observer Scar Assessment Scale; RT-PCR, real-time polymerase chain reaction

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Introduction

The background of pathological scarring relies on alterations in physiological tissue repairing mechanisms after surgical incision, inflammation, burn, or due to trauma. Pathological scarring brings forth a negative impact on life quality (1,2). Preventive initiatives and therapeutic measures lack significant efficacy, and the reoccurrence rate is variably elevated. The study of the risk factors may offer valuable insights for efficient prophylactic measures and specific treatment development for at individuals at risk (3).

Hypertrophic scarring includes increased deposition of extracellular matrix and invasive fibroblast growth (4), while an atrophic scar appears as topographical depression as a result of inadequate compensation for tissue loss during the physiological wound-healing process (5).

Genetic predisposition has attracted significant attention due to the diverse phenotype of pathological scarring in individuals with a positive personal and family history. Several studies (6-8) have investigated the genes responsible for local coagulation, inflammation, tissue formation (reepithelization, angiogenesis and matrix protein production), and tissue remodeling; with variable grades of achievements and suggesting a wider approach (9).

The human telomerase reverse transcriptase (*hTERT*) gene maintains the telomeres at a steady length, prolonging the cell lifespan by synthetizing the telomeric sequence. Enzyme activity is increased in cancer and germ cells compared to somatic cells (10). Recent studies have identified telomere shortening and decreased *hTERT* activity in pathological scarring. The rs2736100 SNP is responsible for a dysregulated *hTERT* expression and activity being associated with excessive

fibrosis and fibroblast proliferation in multiple cancers (11). The role of this particular SNP has not been investigated with regards to scarring; however, due to the gene involvement in the scarring process, the present SNP is a valid candidate for research of this association (12). Thus, the objective of this study was to investigate the potential association of rs2736100 variant of the *hTERT* gene in relationship with atrophic and hypertrophic scarring in a Caucasian population group.

Patients and methods

Study group. The study group was comprised of 71 female patients having undergone Caesarean section at the 1st Gynaecology Clinic in Cluj-Napoca, Romania. A total of 85 patients were initially included in the study, with one patient withdrawing verbally from the study and an additional group of 14 patients lost through follow-up. The inclusion criteria consisted of individuals of age more than 18 years, willingness to participate and consecutive written informed consent of the female patients after undergoing planned Caesarean section without any pre-/post-operative complications, as well as compliance to the necessary follow-ups. The main exclusion criteria included the presence of overlapped incisions from previous surgeries or trauma and a reluctance or lack of cooperation for follow-up. The initial check-up was carried out in-person and at 3 and 6 month the check-ups were carried out via phone. Data regarding skin characteristics [Fitzpatrick phototype (13)] as well as general information regarding age, height, weight, consumption of tobacco, personal and family history of atypical scars and weight gain during pregnancy were collected. The clinical investigation relied on Patient Observer Scar Assessment Scale (POSAS) and SCAR scales (14) for objectifying the scars, both by professionals and patients (14). Six months following surgery, the 71 individuals were grouped according to the scar type as follows: physiological scar group (53 patients), hypertrophic scar group (13 patients) and atrophic scar group (5 patients).

Genotyping investigation. Peripheral venous blood samples were collected in K3EDTA vacutainers, followed by storage at 4˚C until processing. The Wizard Genomic DNA Purification Kit (Promega Corp.) was used to extract genomic DNA. This was stored at -20˚C after rehydration, pending genotyping. The mentioned SNP was genotyped using TaqMan assay (Thermo Fisher Scientific, Inc.) and the 7500 Fast Dx Real-Time Polymerase Chain Reaction (PCR) system (Applied Biosystems; Thermo Fisher Scientific, Inc.). BioRad CFX96 Real-Time PCR Detection System (BioRad Laboratories, Inc.) software was used to interpret the results.

Statistical analysis. Mean ± standard deviation or absolute and relative frequencies (%) were used in the descriptive statistics for clinical and genetic variables. SPSS for MacBook (SPSS Inc.) was the software used for conducting the statistical investigation. The Chi-square test was used to measure the Hardy-Weinberg equilibrium. The Chi-square test was also 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 addressed to analyze the comparisons between subgroups and to correlate within continuous variables. The outcome differences appreciated by the SCAR and POSAS 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 interval (CI)]. A significant statistical difference was considered at a P-value <0.05.

Results

Clinical and demographic study. Demographic and clinical patient features according to the study subgroup are illustrated in Table I. No statistical significant difference was observed regarding the age, height, weight, pre-conceptional weight and duration of lactation. Statistically significant differences were reported for the Fitzpatrick phototypes, the POSAS and SCAR scores for the clinical evaluation and in the family and personal history.

The comparative analysis for POSAS and SCAR mean values between check-ups at 3 and 6 months are further elaborated in Table I.

Analysis of hTERT rs2736100. The molecular analysis results are presented in Table II, illustrating the allele frequency and genotype distribution of the *hTERT* variant gene: rs2736100. The association analysis of POSAS and SCAR with the *hTERT* genotypes did not reveal any strong association (POSAS: F (2, 70)=0.019; P=0.8; SCAR: F (2, 73)=0.010, P=0.88, respectively).

The analysis of genotype effect on BMI with an age-adjusted criteria showed a borderline statistical differences between the subgroups. A BMI >25 kg/m^2 was statistically associated with pathological scarring (χ^2=5.001, P=0.048). No strong association was exhibited between the excessive weight gain during pregnancy (>15 kg) and pathological scarring (χ^2=2.12, P=0.322). No suggestive association was revealed between the personal history and the pathological scarring regarding the genotype distribution.

Under the Chi-square test, the genotype distribution was statistically associated with the Fitzpatrick skin phototype (χ^2=15.9, P=0.04), having under Cramer's test (V=0.319) a moderate association profile.

Fisher's exact test was used under the dominant and recessive models to evaluate the association between the genotype distribution and the pathological scarring subgroups. The variant CC genotype failed to exhibit any association for the hypertrophic and atrophic scars both separately and combined. The variant C allele revealed a borderline statistical value for the risk of developing pathological scarring (OR=1.44; 95% CI 0.876-1.332; P=0.066).

Discussion

Hypertrophic and atrophic scars are the effect of a dysregulated wound-healing process in genetically predisposed individuals, having many possible alterations in inflammation, cell proliferation or tissue remodeling steps of the physiological process (15). Several genetic abnormalities have been the investigative focus in pathological scarring research, but the *hTERT* variant rs2736100 represents a

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

Parameters		Normal scarring (n=53)	Hypertrophic scarring (n=13)	Atrophic scarring (n=5)
Mean age		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, n (%)	Yes	10 (14.08)	1 (1.4)	1 (1.4)
	No	43 (60.56)	12 (16.9)	4 (5.63)
	P-value	**0.02**	>0.05	>0.05
Personal history, n (%)	Yes	10 (14.08)	4 (5.63)	1 (1.4)
	No	43 (60.56)	9 (12.67)	4 (5.63)
	P-value	**0.02**	**0.04**	>0.05
Family history, n (%)	Yes	3 (4.22)	1 (1.4)	0 (0)
	No	50 (70.42)	12 (16.9)	5 (12.19)
	P-value	**0.02**	>0.05	N/A
Fitzpatrick phototype, n (%)	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-value	>0.05	**0.01**	**0.04**
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	>0.05	>0.05	>0.05
Treatment, n (%)	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

Data are presented as mean ± SD for continuous variables and as frequencies (n, %) for categorical variables. POSAS, Patient Observer Scar Assessment Scale. All P-values indicating significant differences are indicated in bold print.

Table II. Allele frequency and gentotype distribution of the hTERT variant gene: rs2736100.

Allele frequency and genotype distribution of the *hTERT* variant gene	Normal scarring (n=53)	Hypertrophic scarring (n=13)	Atrophic scarring (n=5)
A allele (wild-type)	52	9	7
C allele (variant type)	54	17	3
AA	15	2	2
AC	22	5	3
CC	16	6	0
AC + CC	28	11	3

novel research target for investigating the genetic influence of scarring.

In our study, 74.64% of the female patients developed normal scarring tissue, while 18.3 and 7.04% were found with hypertrophic and atrophic scars, respectively. The pathological scarring distribution respects the reported scar prevalence after surgery in the Caucasian population (16).

De Felice *et al* demonstrated the impact of telomere shortening on keloids, being associated with increased oxidative stress due to the absent expression of *hTERT* in keloids.

No data have been acquired regarding hTERT and hypertrophic and atrophic scars in Caucasians (12). Other studies have investigated the implication of hTERT in cancer and fibroproliferative diseases, highlighting the causative role of hTERT (10,17).

The AC genotype was the most frequently encountered in our study and the comparative analysis of all genotypes did not reveal any statistical difference between normal and pathological scars. Another research interest was the velocity of wound healing based on the POSAS and SCAR score values which were decreased between the 3- and 6-months check-ups, but no statistical difference was observed other than the similar pattern of decrease for each genotype.

The type 4 Fitzpatrick phototype displayed an increased frequency for heterozygous genotype in the current study and it is demonstrated that higher grade phototypes are associated with abnormal scar formation (7). Our study did not reveal this association due to the lower number of individuals included in the study.

Obesity is considered a risk factor for keloid development. Our data revealed similar results for the hypertrophic and atrophic scars. Literature suggests that an overweight status is a valid risk factor for hypertrophic scarring, while obesity is closely associated with keloid development through the systemic pro-inflammatory state which acts as a predisposing condition (18). In our study, a pre-conceptional BMI >25 kg/m^2 was statistically associated with pathological scarring, but it failed to exhibit a higher frequency of pathological scarring in the overweight individuals compared to normoponderal ones.

A positive personal history is a well-known risk factor for abnormal scarring tissue reoccurrence, particularly keloids, but hypertrophic and atrophic scarring lack this validation in other studies (19). No evidence was highlighted in the current study for the reoccurrence risk based on a positive personal history of abnormal scarring.

The implication of a smoking habit in pathological scarring is questionable. Some reports state a lower rate of hypertrophic scarring among smoking individuals (20), while others have highlighted the negative impact of smoking in surgical wound-healing (21). Our study did not investigate smoking as a risk factor due to the low smoking rate among the pregnant women investigated.

The limitations of the present study included a low patient enrolment rate with pathological scarring, an exclusive all female participants and no evidence to extrapolate the data for the male population, and a short follow up period. A better understanding of the risk factors for developing pathological scarring would improve treatment outcome since more selective preventative measures can be applied. Patients with documented predisposition can benefit from prophylactic post-surgical measures aimed at reducing scar tissue. Investigation on animal models with overexpressed or knockout of the *hTERT* gene can evaluate the wound-healing process and the risk of pathological scarring after surgery.

In conclusion, the present study investigated the role of the *hTERT* gene variant rs2736100 in hypertrophic and atrophic scarring in a female Caucasian population group. We reported a borderline statistical significance value for the variant C allele of the *hTERT* variant gene for the risk of developing pathological scarring in female patients having undergone Caesarean section.

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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, SRH, 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 the 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 (approval no. 299/26.7.2018) by the Ethics Committee of the 'Iuliu Hatieganu' 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.

Patient consent for publication

The consent form for this study has elaborated on the use of the data for publication for scientific purposes of the values recorded. The present study did not include any identifiable patient data.

Competing interests

The authors declare that they have no competing interests.

References

1. Gauglitz GG, Korting HC, Pavicic T, Ruzicka T and Jeschke MG: Hypertrophic scarring and keloids: Pathomechanisms and current and emerging treatment strategies. Mol Med 17: 113-125, 2011.
2. Ilie M, Caruntu C, Tampa M, Georgescu SR, Matei C, Negrei C, Ion RM, Constantin C, Neagu M and Boda D: Capsaicin: Physicochemical properties, cutaneous reactions and potential applications in painful and inflammatory conditions (Review). Exp Ther Med 18: 916-925, 2019.
3. Scrimali L, Lomeo G, Nolfo C, Pompili G, Tamburino S, Catalani A, Siragò P and Perrotta RE: Treatment of hypertrophic scars and keloids with a fractional CO2 laser: A personal experience. J Cosmet Laser Ther 12: 218-221, 2010.
4. Li-Tsang CW, Lau JC and Chan CC: Prevalence of hypertrophic scar formation and its characteristics among the Chinese population. Burns 31: 610-616, 2005.

5. Gozali MV, Zhou B and Luo D: Effective treatments of atrophic acne scars. J Clin Aesthet Dermatol 8: 33-40, 2015.
6. Shih B and Bayat A: Genetics of keloid scarring. Arch Dermatol Res 302: 319-339, 2010.
7. Glass DA: Current understanding of the genetic causes of keloid formation. J Investig Dermatology Symp Proc 18 (Suppl): S50-S53, 2017.
8. Huang C, Nie F, Qin Z, Li B and Zhao X: A snapshot of gene expression signatures generated using microarray datasets associated with excessive scarring. Am J Dermatopathol 35: 64-73, 2013.
9. Wu J, Ma B, Yi S, Wang Z, He W, Luo G, Chen X, Wang X, Chen A and Barisoni D: Gene expression of early hypertrophic scar tissue screened by means of cDNA microarrays. J Trauma 57: 1276-1286, 2004.
10. Shammas MA: Telomeres, lifestyle, cancer, and aging. Curr Opin Clin Nutr Metab Care 14: 28-34, 2011.
11. Zhu HY, Li C, Bai WD, Su LL, Liu JQ, Li Y, Shi JH, Cai WX, Bai XZ, Jia YH, *et al*: MicroRNA-21 regulates hTERT via PTEN in hypertrophic scar fibroblasts. PLoS One 9: e97114, 2014.
12. De Felice B, Wilson RR and Nacca M: Telomere shortening may be associated with human keloids. BMC Med Genet 10: 110, 2009.
13. Fitzpatrick TB: The validity and practicality of sun-reactive skin types I through VI. Arch Dermatol 124: 869-871, 1988.
14. Idriss N and Maibach HI: Scar assessment scales: A dermatologic overview. Ski Res Technol 15: 1-5, 2009.
15. Li G, Zhou R, Zhang Q, Jiang B, Wu Q and Wang C: Fibroproliferative effect of microRNA-21 in hypertrophic scar derived fibroblasts. Exp Cell Res 345: 93-99, 2016.
16. Kiprono SK, Chaula BM, Masenga JE, Muchunu JW, Mavura DR and Moehrle M: Epidemiology of keloids in normally pigmented Africans and African people with albinism: Population-based cross-sectional survey. Br J Dermatol 173: 852-854, 2015.
17. Leão R, Apolónio JD, Lee D, Figueiredo A, Tabori U and Castelo-Branco P: Mechanisms of human telomerase reverse transcriptase (hTERT) regulation: Clinical impacts in cancer. J Biomed Sci 25: 22, 2018.
18. Butzelaar L, Soykan EA, Galindo Garre F, Beelen RH, Ulrich MM, Niessen FB and Mink van der Molen AB: Going into surgery: Risk factors for hypertrophic scarring. Wound Repair Regen 23: 531-537, 2015.
19. Butzelaar L, Ulrich MMW, Mink van der Molen AB, Niessen FB and Beelen RHJ: Currently known risk factors for hypertrophic skin scarring: A review. J Plast Reconstr Aesthetic Surg 69: 163-169, 2016.
20. Deliaert AE, Van den Kerckhove E, Tuinder S, Noordzij SM, Dormaar TS and van der Hulst RR: Smoking and its effect on scar healing. Eur J Plast Surg 35: 421-424, 2012.
21. Poetschke J and Gauglitz GG: Aktuelle optionen zur behandlung pathologischer Narben. J Dtsch Dermatol Ges 14: 467-478, 2016.