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LETTER TO THE EDITOR

TSH levels, overweight, BMI, and skin expression levels of DCT and CCBL2 genes are related to vitiligo treatment response with narrow band UVB phototherapy

Vitiligo is a chronic skin disease characterized by a lack of pigmentation in the skin of affected patients (Boniface, Seneschal, Picardo, & Taieb, 2017). The study of the functional status of the skin could be helpful in predicting the response to phototherapy treatment. To date, no studies about clinical, biochemical, and gene expression profiles related to response to treatment are available, particularly with narrowband ultraviolet B light phototherapy (nb-UVB), the first choice treatment in vitiligo (Majid, 2010). Therefore, and to identify clinical, biochemical, and skin gene expression patterns with prognostic utility, we conducted a study with 45 subjects with vitiligo vulgaris (VV): 23 patients with active VV (AVV, 10 male and 13 female) and 22 patients with stable VV (SVV, 14 male and 8 female). Two skin biopsies (from vitiligo lesions and pigmented areas) of 4 mm in diameter were obtained before the administration of 48 nb-UVB phototherapy sessions, performed two times a week for a period of 6 months with an initial dose of 150 mJ/cm^2, and increments of 50 mJ/cm^2 every third session until the minimum dose of asymptomatic erythema was achieved. Each biopsy was used to isolate total RNA and to perform expression analysis with TruSeq Targeted RNA Expression analysis (RNA-Seq, Illumina, Inc., San Diego, CA) (Ocampo-Candiani et al., 2018), targeting 29 genes involved in skin pigmentation, apoptosis, cell survival, and signal transduction mechanisms.

The statistical analysis of clinical, biochemical parameters, response to treatment (defined as the degree of repigmentation achieved after phototherapy, and classified in three groups: *Low* [less than 10%]; *Middle* [10.1%

TABLE 1 Family history and early onset of vitiligo

Family history of vitiligo	≤30 years	>30 years	O.R.	C.I.	X^2	cP
Yes	18	2	5.78	1.00–62.29	5	.05
No	15	10	0.17	0.02–0.10	5	.05

Abbreviation: cP, corrected *p* value.

TABLE 2 Clinical and biochemical parameters observed for each nb-UVB treatment response group (low, middle, and high)

	Treatment response (mean ± SD)		
Clinical and biochemical parameters	Low *n* = 15	Middle *n* = 15	High *n* = 15
Age (years)	39.933 ± 15.462	39.267 ± 11.949	45.533 ± 15.528
Weight (kg)	90.433 ± 16.417[a]	73.587 ± 20.780	77.867 ± 19.511
BMI	31.067 ± 4.913[b]	27.653 ± 7.120	27.827 ± 5.211
Size (m)	1.705 ± 0.089	1.630 ± 0.084	1.667 ± 0.117
Hb (g/dL)	15.313 ± 1.373	14.233 ± 1.694	14.460 ± 1.594
Leukocyte (thou/mm^3)	6.565 ± 1.845	6.263 ± 1.256	6.652 ± 1.937
PLT (K/mcL).	239.000 ± 68.995	210.200 ± 39.223	229.333 ± 44.158
Glucose (mg/dL)	96.067 ± 6.112	97.000 ± 6.141	93.800 ± 11.378
Bun (mg/dL)	10.867 ± 3.378	9.867 ± 2.615	11.267 ± 3.595
Creatinine (mg/dL)	0.807 ± 0.175	0.700 ± 0.169	0.767 ± 0.168
T4 (mcg/dL)	7.167 ± 2.017	7.503 ± 1.260	7.703 ± 1.641
T3 (ng/dL)	135.145 ± 41.944	127.216 ± 20.863	120.778 ± 23.762
TSH (mIU/L)	2.277 ± 0.688[b]	2.034 ± 0.630	1.645 ± 0.767

Abbreviations: BMI, body mass index; Hb, hemoglobin concentration; PLT, platelet; TSH, thyroid-stimulating hormone.

[a]Low versus middle.

[b]Low versus high.

$p \leq .05$.

TABLE 3 Differences in gene expression profiles observed for treatment response groups

		Response to treatment			
Pathway	**Gene**	**≤10%**	**10.1% to 29.9%**	**≥30%**	***p* value**
Pigment	DCT unaffected	2.760 ± 0.218	3.148 ± 0.069	2.989 ± 0.081	
	DCT vitiligo	0.149 ± 0.116	1.777 ± 0.286	2.817 ± 0.232	.01 (A); .051 (B)*
	MC1R unaffected	0.929 ± 0.190	1.510 ± 0.214	1.005 ± 0.214	
	MC1R vitiligo	0.866 ± 0.228	1.443 ± 0.166	1.498 ± 0.168	
	MC4R unaffected	0.220 ± 0.156	0.106 ± 0.073	0.012 ± 0.012	
	MC4R vitiligo	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	
	POMC unaffected	0.441 ± 0.146	0.476 ± 0.169	0.739 ± 0.197	
	POMC vitiligo	0.638 ± 0.215	0.496 ± 0.174	0.535 ± 0.178	
	TYRP1 unaffected	3.508 ± 0.105	3.679 ± 0.079	3.442 ± 0.073	
	TYRP1 vitiligo	0.765 ± 0.189	1.496 ± 0.268	1.381 ± 0.218	.071 (A)**
	MLANA unaffected	2.804 ± 0.087	2.992 ± 0.086	2.583 ± 0.180	
	MLANA vitiligo	0.264 ± 0.126	0.475 ± 0.237	0.689 ± 0.194	
	PHACTR2 unaffected	2.771 ± 0.143	3.027 ± 0.107	2.880 ± 0.093	
	PHACTR2 vitiligo	2.983 ± 0.070	3.021 ± 0.067	2.890 ± 0.070	
Apoptosis	BAX unaffected	0.323 ± 0.123	0.842 ± 0.210	0.487 ± 0.177	
	BAX vitiligo	0.755 ± 0.221	0.670 ± 0.221	0.446 ± 0.143	
	BCL2 unaffected	1.707 ± 0.211	1.877 ± 0.166	1.776 ± 0.194	
	BCL2 vitiligo	1.506 ± 0.246	1.839 ± 0.204	1.683 ± 0.194	
	BCL3 unaffected	2.112 ± 0.204	2.377 ± 0.122	2.124 ± 0.225	
	BCL3 vitiligo	2.373 ± 0.131	2.242 ± 0.185	2.188 ± 0.165	
	CASP3 unaffected	2.214 ± 0.189	2.664 ± 0.100	2.206 ± 0.212	
	CASP3 vitiligo	2.504 ± 0.074	2.612 ± 0.084	2.448 ± 0.075	
	CASP7 unaffected	1.270 ± 0.207	1.405 ± 0.196	1.402 ± 0.206	
	CASP7 vitiligo	1.847 ± 0.160	1.460 ± 0.234	1.391 ± 0.173	
	CASP8 unaffected	2.405 ± 0.172	2.603 ± 0.093	2.048 ± 0.250	
	CASP8 vitiligo	2.447 ± 0.187	2.494 ± 0.173	2.411 ± 0.079	
	CASP10 unaffected	1.902 ± 0.157	2.141 ± 0.175	1.803 ± 0.201	
	CASP10 vitiligo	1.854 ± 0.227	1.881 ± 0.204	2.026 ± 0.157	
	CFLAR unaffected	2.749 ± 0.075	2.515 ± 0.100	2.507 ± 0.201	
	CFLAR vitiligo	2.630 ± 0.166	2.734 ± 0.082	2.655 ± 0.066	
	FASLG unaffected	0.136 ± 0.093	0.264 ± 0.138	0.093 ± 0.075	
	FASLG vitiligo	0.000 ± 0.000	0.065 ± 0.064	0.265 ± 0.150	
	TNF unaffected	1.785 ± 0.171	1.803 ± 0.178	1.506 ± 0.235	
	TNF vitiligo	1.608 ± 0.215	1.827 ± 0.205	1.522 ± 0.248	
	TNFRSF1A unaffected	3.073 ± 0.076	3.269 ± 0.080	3.133 ± 0.081	
	TNFRSF1A vitiligo	3.208 ± 0.070	3.203 ± 0.061	3.128 ± 0.062	
Oxidative stress	GGT1 unaffected	0.294 ± 0.118	0.497 ± 0.164	0.468 ± 0.179	
	GGT1 vitiligo	0.424 ± 0.180	0.233 ± 0.120	0.453 ± 0.156	
	CCBL2 unaffected	3.325 ± 0.040	3.466 ± 0.056	3.275 ± 0.060	.036 (C)*
	CCBL2 vitiligo	3.380 ± 0.035	3.303 ± 0.063	3.258 ± 0.052	
	GPD1 unaffected	2.451 ± 0.212	2.513 ± 0.201	2.907 ± 0.182	
	GPD1 vitiligo	2.326 ± 0.254	2.678 ± 0.152	3.001 ± 0.122	.075 (B)**
	TXN unaffected	4.269 ± 0.054	4.121 ± 0.071	4.218 ± 0.056	
	TXN vitiligo	4.312 ± 0.036	4.300 ± 0.028	4.319 ± 0.036	

(Continues)

TABLE 3 (Continued)

Pathway	Gene	Response to treatment			
		≤10%	10.1% to 29.9%	≥30%	*p* value
Cell survival	CAPN3 unaffected	2.766 ± 0.165	3.047 ± 0.064	2.865 ± 0.072	
	CAPN3 vitiligo	2.303 ± 0.132	2.351 ± 0.153	2.223 ± 0.179	
	MITF unaffected	3.100 ± 0.079	3.128 ± 0.057	2.815 ± 0.208	
	MITF vitiligo	2.882 ± 0.061	2.866 ± 0.054	2.839 ± 0.078	
	CDC5L unaffected	3.360 ± 0.030	3.434 ± 0.061	3.338 ± 0.057	
	CDC5 L vitiligo	3.347 ± 0.055	3.423 ± 0.026	3.349 ± 0.049	
	MAPK1 unaffected	3.264 ± 0.064	3.368 ± 0.064	3.207 ± 0.080	
	MAPK1 vitiligo	3.317 ± 0.058	3.336 ± 0.063	3.193 ± 0.054	
Signal transduction	CSNK1G3 unaffected	2.515 ± 0.078	2.239 ± 0.163	2.000 ± 0.227	
	CSNK1G3 vitiligo	2.512 ± 0.069	2.359 ± 0.139	2.351 ± 0.052	
	NFKB1 unaffected	2.524 ± 0.224	2.830 ± 0.056	2.698 ± 0.064	
	NFKB1 vitiligo	2.884 ± 0.068	2.921 ± 0.049	2.722 ± 0.157	
	WNT7A unaffected	1.246 ± 0.260	1.302 ± 0.239	0.644 ± 0.200	
	WNT7A vitiligo	0.803 ± 0.271	1.405 ± 0.255	1.291 ± 0.216	

Note: The endogenous expression controls included were TPT1 and GAPDH. The results are presented as the means ± Standard Error. (A) Low versus middle; (B) low versus high; (C) middle versus high.
Abbreviations: apoptotic protein subfamily BCL-2, BCL3; CAPN3, calpain 3; CCBL2, beta-lyase conjugated cysteine; CDC5L, cell division cycle 5; CSNK1G3, casein kinase 1 Gamma 3; DCT, dopachrome tautomerase; GGT1, gamma-glutamyltransferase 1; GPD1, glycerol-3-phosphate dehydrogenase 1; MAPK1, MAP kinase 1; MC1R and MC4R, melanocortin 1 and 4 receptors; MITF, microphthalmia-associated transcription factor; MLANA, melan-A; NFKB1, nuclear factor Kappa B Subunit 1; PHACTR2, phosphatase and actin regulator 2; POMC, proopiomelanorcotin; proapoptotic gene BAX, caspases (CASP) 3, 7, 8 and 10; the regulator of apoptosis CFLAR, and the ligand gene that stimulates apoptosis FASLG; TNF and TNFRSF1A, tumor necrosis factor alpha receptor and its ligand; TXN, thioredoxin; TYRP1, tyrosinase 1; WNT7A, Wnt 7A member 7A.
*$p \leq .05$; **$p > .05$; $p < .1$.

and 29.9%]; and *High* [greater than 30%]), and gene expression profiles were performed to identify markers confirming the response to treatment.

Twenty patients (44.4%) presented a hereditary family history of vitiligo, 18 of which developed the disease before the age of 30. Therefore, hereditary history was established as an early age risk factor (cP = .05) (Table 1). Although 36 subjects (80%) presented some alteration in Antinuclear Antibody analysis, no association was found with the type of vitiligo or response to treatment.

After the evaluation, 42 (93.33%) subjects showed some degree of response to treatment: 7 subjects with AVV and 8 with SVV showed a low response, 7 subjects with AVV and 8 with SVV had a medium response to treatment, and 9 subjects with AVV and 6 with SVV showed a high response. The three subjects who did not present repigmentation after the treatment had AVV (6.67%). Concerning biochemical data, the ANOVA analysis showed high thyroid-stimulating hormone (TSH) levels in the low response group when compared to the other groups ($p \leq .05$). Further, this low response group also presented the highest weight and BMI ($p \leq .05$) (Table 2). The association between thyroid problems and the development of vitiligo is well known, especially in people with hypothyroidism (Gupta & Ammini, 2012; Kasumagic-Halilovic, Prohic, Begovic, & Ovcina-Kurtovic, 2011). On the other hand, there is growing evidence of an association between overweight and autoimmune-associated pathologies (Versini, Jeandel, Rosenthal, & Shoenfeld, 2014), which in turn have been associated with vitiligo (Spritz, 2006, 2007).

Moreover, the expression analysis identified significant differences in the expression of the DCT and CCBL2 genes and treatment response with predictive accuracy ($p \leq .05$) (Table 3). The expression profiles in the low response group were lower for DCT gene; and as this gene participates directly in the regulation of melanin levels in the skin (Salinas-Santander et al., 2018), it could be assumed that the repigmentation process of the skin is also affected. On the other hand, the CCBL2 gene, which participates in the oxidative stress response, shows a different expression pattern in unaffected skin when compared to the medium and high treatment response groups ($p \leq .05$) (Table 3). This result may imply the existence of a compensating effect against the stress in the skin of patients experiencing an intermediate response, which could have been induced by the same nb-UVB treatment, as previously published (Karsli, Akcali, Ozgoztasi, Kirtak, & Inaloz, 2014).

In conclusion, we found a group of markers with potential predictive utility when concerning the response to nb-UVB phototherapy.

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CONFLICT OF INTEREST

We the authors, declare that we have not conflict of interest.

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The University Hospital-UANL Institutional Review Board approved and registered the study under the code DE13-001.

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