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High prevalence of circulating autoantibodies against thyroid hormones in vitiligo and correlation with clinical and historical parameters of patients

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Summary

Background Autoantibodies against thyroid hormones (THAbs) directed towards triiodothyronine (T3-Ab) and/or thyroxine (T4-Ab) are very rare in the general population. They are increased in some nonthyroidal autoimmune diseases, where they seem to predict autoimmune thyroid disorders (ATDs). So far, their presence in patients with vitiligo has not been evaluated, but it might have a possible predictive role.

Objectives To assess the prevalence of THAbs in a group of vitiligo patients and to correlate their presence with clinical and historical parameters.

Methods In total 79 patients with nonsegmental vitiligo and 100 controls were examined. Clinical characteristics of vitiligo and family and personal medical history were evaluated. Antinuclear autoantibodies, thyroid hormones and thyroid autoantibodies were measured. IgM T3-Ab, IgG T3-Ab, IgM T4-Ab and IgG T4-Ab were assayed by a radioimmunoprecipitation technique. Fisher's test, Student's t-test and χ^2-test were used for statistical analysis.

Results Overall 77 of 79 patients (97%) had at least one type of THAb (11 T3-Ab, 10 T4-Ab, 56 both). In the control group, only one person (1%) had THAbs. In patients with vitiligo, T3-Abs were significantly associated with leucotrichia (IgM+IgG, $P = 0{\cdot}033$; IgG, $P = 0{\cdot}039$; IgM, $P = 0{\cdot}005$) and thyroglobulin autoantibodies (IgM+IgG, $P = 0{\cdot}031$; IgG, $P = 0{\cdot}058$), while the absence of T3-Ab was related to personal history of cancer (IgM+IgG, $P = 0{\cdot}021$; IgG, $P = 0{\cdot}039$). T4-Abs were significantly associated with vitiligo activity (IgM+IgG, $P < 0{\cdot}001$; IgM, $P = 0{\cdot}037$) and duration (IgG, $P = 0{\cdot}013$).

Conclusions The surprisingly high prevalence of THAb in patients with vitiligo and their associations suggest a possible pathogenetic role in the disease and stress the tight link between vitiligo and ATDs. Further evaluation in a larger group of patients and an adequate follow-up are needed to define their potential predictive role.

What's already known about this topic?

- Vitiligo is strongly associated with autoimmune thyroid disorders.
- Autoantibodies against thyroid hormones (THAbs) have a very low prevalence in the general population and are increased in thyroid and extrathyroid autoimmune diseases.

What does this study add?

- For the first time, THAbs against triiodothyronine (T3-Ab) and thyroxine (T4-Ab) have been detected in a group of patients with vitiligo.
- T3-Ab and T4-Ab were associated with some clinical and historical parameters of vitiligo.

Vitiligo affects 0·5–1% of the worldwide population,[1,2] and is characterized by the appearance of achromic macules,[2] due to the disappearance of functional melanocytes from the epidermis. Many theories have been proposed to explain vitiligo pathogenesis, but none unravels the complexity of this disease.[3] Therefore, it is likely that theories are complementary.

The largely accepted autoimmune theory is sustained by strong evidence,[4–7] including the association with organ- and nonorgan-specific antibodies and autoimmune diseases.[7] In particular, vitiligo is strongly associated with autoimmune thyroid disorders (ATDs),[8] a group of diseases defined by an excess of serum thyroid autoantibodies [thyroid peroxidase antibodies (TPOAbs), thyroglobulin antibodies (TgAbs) and thyroid-stimulating hormone receptor antibodies (TSH-R-Abs)], which can be associated with clinical manifestations.[9] Other less-known thyroid-related autoantibodies are directed towards one or both thyroid hormones (THAbs), namely triiodothyronine (T3) and/or thyroxine (T4). These autoantibodies, which seem to be a particular subset of TgAbs, are the less frequent class of thyroid-related antibodies in human serum[10–13] and are reported to be increased in some autoimmune disorders. In fact, while they show a frequency of 0·07% in the general population,[14,15] in patients with Hashimoto thyroiditis, Graves disease, primary Sjögren syndrome or rheumatoid arthritis, the prevalence of THAbs averages 21%, 32%, 50% or 26%, respectively.[16] The rate of the *de novo* appearance in serum of THAbs and their persistence (as opposed to transient appearance) seem to be greater in patients with Hashimoto thyroiditis than in those without it,[17,18] as a confirmation of the importance of the autoimmune background in maintaining such autoantibodies.

THAb positivity has been also reported in Waldenström macroglobulinaemia,[19] laryngeal cancer treated with cobalt-60 irradiation[11,20] and hepatocarcinoma.[21] In addition, studies in patients subjected to diagnostic fine-needle thyroid aspiration, and who were THAb negative at baseline, demonstrated that a thyroid lesion could elicit the appearance of THAbs *de novo*.[17] Their pathogenetic role is still unknown,[11] but they may predict an ATD,[15,18] as observed in patients with rheumatoid arthritis.[16] Indeed, in experimental models of ATD, THAbs are the earliest thyroid autoantibodies to be detected in serum.[17,22] Finally, depending on their serum concentration and binding affinity for thyroid hormone(s), THAbs may affect the measurement of serum thyroid hormones, most frequently causing an overestimation of the real serum concentration.[11,23]

As THAbs have been found in autoimmune diseases, and as vitiligo is an autoimmune disorder frequently associated with ATD, we wished to evaluate THAbs in patients with vitiligo. If THAbs were indeed detected, we would be interested in exploring a possible correlation with vitiligo features, comorbidities and history.

Patients and methods

Patient selection

Seventy-nine white patients (26 male, 53 female) affected by nonsegmental vitiligo were examined at our specialized vitiligo outpatient service between 2010 and 2013. The study was carried out according to the principles of the Declaration of Helsinki and was approved by the local institutional review board; informed written consent was obtained from each patient.

Consecutive patients coming from all over Italy, aged > 18 years and showing nonsegmental vitiligo according to the Vitiligo European Task Force definition,[24] were included in the study. Exclusion criteria were the presence of segmental vitiligo, the use of systemic immunosuppressive or antithyroid drugs (methimazole, propylthiouracil), previous ablative doses of ^{131}I, preceding thyroidectomy, established thyroid disease, fine-needle thyroid biopsy in the previous year and lack of informed consent.

One hundred age- and sex-matched subjects with neither vitiligo nor thyroid disease who were observed between 2008 and 2010 were consecutively selected as healthy controls. Essentially, these subjects were either nonconsanguineous relatives of patients with a number of endocrine diseases or persons who turned out to have no endocrine disease at all. Thyroid tests [TSH, free T3 (FT3), free T4 (FT4), TgAb, TPOAb] of all of these control subjects were always within the respective reference ranges.

Clinical evaluation

Vitiligo evaluation was performed using a modified Vitiligo European Task Force form, as previously described.[25,26] According to the above-mentioned assessment, we evaluated the head and neck, trunk, upper extremities and lower

extremities for the extent of depigmentation and stage of disease (grade of depigmentation and possible leucotrichia, scored 0–4). A total score for the aforementioned parameters was also performed (0–100% for vitiligo area and 0–16 for staging). Sex, age, age at onset, duration of the disease, phototype, Koebner phenomenon, leucotrichia, emotional stress at onset, early hair greying (> 50% white hair before the age of 40 years), signs of inflammation/pruritus, activity of the disease (active, appearance of vitiligo lesions/enlargement of the existing macules during 6 months before our clinical evaluation; borderline, 6–18 months; stable, ≥ 18 months) and previous repigmentation were also investigated. A double examination of the whole body under natural light and Wood's lamp was performed by two research dermatologists.

Family and personal medical history

Family history of vitiligo, ATD, other autoimmune comorbidities (diabetes mellitus type 1, Addison disease, systemic lupus erythematosus, autoimmune atrophic gastritis, coeliac disease, alopecia areata, rheumatoid arthritis, Sjögren syndrome, primary biliary cirrhosis) and cutaneous or visceral cancers were investigated. Personal medical history involved the above-mentioned autoimmune comorbidities of vitiligo (excluding thyroiditis) and cutaneous or visceral cancers.

Serological evaluation

Serum TSH, FT4, FT3, TgAb, TPOAb, TSH-R-Ab and antinuclear antibodies (ANA) were assayed by different laboratories, in the patients' towns of residence. Overt hyperthyroidism was defined as TSH below the lower limit reported by each lab and FT3 and/or FT4 above the upper limit reported by each lab. Subclinical hyperthyroidism was defined as TSH below the lower limit and FT3 and FT4 in the normal range. Overt hypothyroidism was defined as TSH above the highest limit and FT3 and/or FT4 below the lowest limit. Subclinical hypothyroidism was defined as TSH above the upper limit and FT3 and FT4 in the normal range.[27]

Patients underwent blood sampling. Collected blood samples were centrifuged at 1500 *g* for 10 min and the supernatant serum was aspirated. Four types of THAb were measured: IgM-T3-Ab, IgG-T3-Ab, IgM-T4-Ab and IgG-T4-Ab. THAbs of either class were measured by the radioimmunoprecipitation technique using antihuman IgM or antihuman IgG serum (Behringwerke, Marburg, Germany) and [^{125}I]T3 or [^{125}I]T4 (Johnson and Johnson, Milan, Italy). Serum samples (500 μL) were incubated with 0·5 μCi [^{125}I]T3 or [^{125}I]T4 for 60 min at 23 °C. Aliquots of this mixture (20 μL) were then incubated with 150 μL antihuman IgM or antihuman IgG, both prediluted 1 : 10 with saline containing bovine serum albumin (Sigma, St. Louis, MO, U.S.A.) at a final concentration of 0·5%. After 24-h incubation at 4 °C, tubes were centrifuged at 2000 *g* for 20 min, and the supernatant was aspirated. Sera were considered THAb positive when precipitated radioactivity (percentage bound over total) was > 2 SDs from the normal mean. These cut-off points were 3·9% (IgM-T3), 3·4% (IgM-T4), 3·6% (IgG-T3) and 3·9% (IgG-T4). For each of the four THAbs, all patient and control sera were assayed simultaneously, that is, in the same run. When levels were above normal (positive) or borderline, THAbs were assayed twice. This radioimmunoprecipitation technique had already been successfully used to evaluate serum THAb in thyroid and extrathyroid autoimmune disorders. This method is not commercially available and has been developed in the research laboratory of the Department of Clinical and Experimental Medicine, University of Messina, Messina, Italy.[13,16–18]

Statistical analysis

Six categories of patients were created, according to the positivity for the different classes/types of THAb. Statistical analysis was performed in order to evaluate the possible correlations between clinical parameters of vitiligo, personal and family history of disease and serological values as follows: (i) patients simultaneously positive for both IgM and IgG T3-Ab vs. patients negative for both IgM and IgG T3-Ab, (ii) patients simultaneously positive for both IgM and IgG T4-Ab vs. patients negative for both IgM and IgG T4-Ab, (iii) patients positive for IgM T3-Ab vs. patients negative for IgM T3-Ab, (iv) patients positive for IgG T3-Ab vs. patients negative for IgG T3-Ab, (v) patients positive for IgM T4-Ab vs. patients negative for IgM T4-Ab and (vi) patients positive for IgG T4-Ab vs. patients negative for IgG T4-Ab. The SPSS 15·0 for Windows program (IBM, Armonk, NY, U.S.A.) was utilized for the data collection and statistical analysis performed in the current study. Basic summary statistics, such as means with SDs and proportions were used to describe the patients' characteristics. The χ^2 and Fisher's tests were used for comparing categorical data, while Student's *t*-test was used for comparing continuous data. Results were considered significant for P values $< 0{\cdot}05$.

Results

The clinical features of the 79 patients are reported in Table 1. Twenty-five of the 79 patients (32%) had a family history of vitiligo, five (6%) had premature hair greying, 24 (30%) had ATD and four (5%) had other autoimmune comorbidities (alopecia areata, $n = 1$; Sjögren syndrome, $n = 1$; rheumatoid arthritis, $n = 2$). Family history of cancer was found in 12 patients (15%). In particular, two (3%) had a family history of melanoma, while 10 (13%) had a family history of visceral cancer (three cases of colorectal cancer, three lung cancer, two gastric cancer and two prostate cancer).

Personal history of autoimmune comorbidities of vitiligo (excluding thyroiditis) was found in 10 patients (13%), with diabetes mellitus type 1 ($n = 1$), autoimmune atrophic gastritis ($n = 5$), coeliac disease ($n = 3$) and alopecia areata ($n = 1$). Concerning cancer comorbidities, one patient (1%) each had melanoma, seminoma cancer or endometrial cancer.

Table 1 Patients' characteristics

Sex	26 (33) male; 53 (67) female
Age at observation (years)	38·45 ± 16·0; range 18–73
Vitiligo duration (years)	11·67 ± 11·85
Age of vitiligo onset (years)	26·84 ± 14·5
Extension of vitiligo macules (% of total body surface area)	7·5 ± 15·19
0·1–5%	45 (57)
5·1–10%	23 (29)
10·1–15%	6 (8)
15·1–25%	2 (3)
25·1–50%	1 (1)
> 50%	2 (3)
Staging of vitiligo macules	4·92 ± 2·34
Head/neck	1·1 ± 0·80
Upper limbs	1·60 ± 0·75
Trunk	1·39 ± 0·94
Lower limbs	1·14 ± 0·89
Phototype	
I	4 (5)
II	50 (63)
III	24 (30)
IV	0
V	1 (1)
Leucotrichia	21 (27)
Koebner phenomenon	40 (51)
Stress at onset	54 (68)
Activity of disease	
Stable	16 (20)
Borderline	20 (25)
Active	43 (54)
Previous repigmentation	50 (63)
Inflammation	22 (28)
Early hair greying	5 (6) personal; 2 (3) familial

Data are expressed as n (%), except continuous variables, reported as mean ± SD.

Serological evaluation

The thyroid-related hormonal features of the patients with vitiligo are reported in Table 2. TPOAbs, TgAbs and TSH-R-Abs were found to be increased (and therefore positive) in 15 (19%), 20 (25%) and four (5%) patients, respectively, while ANAs were positive in 12 (15%). With regards to the group of patients with vitiligo, an overt hyperthyroidism was found in four patients (5%), while a subclinical hyperthyroidism was found in two (3%). Conversely, an overt hypothyroidism was found in three patients (4%), while subclinical hypothyroidism was present in two (3%). Due to the low number of patients with vitiligo showing a subclinical or overt hypo/hyperthyroidism, the statistical evaluation of possible correlations between vitiligo activity and ANA, Tg or TPO positivity was not performed, as the obtained results would not have been reliable.[27]

Of the 79 patients with vitiligo, 77 (97%) were positive for at least one type of THAb. In particular, 11 patients (14%) had T3-Ab, 10 (13%) had T4-Ab and 56 (71%) had both T3-Ab and T4-Ab; thus, only two patients (3%) were negative for any THAb (Fig. 1a). Concerning the classes of THAb, 39 patients (49%) had IgM-T3, 63 (80%) had IgG-T3, 58 (73%) had IgM-T4 and 37 (47%) had IgG-T4 (Fig. 1b). As for the control subjects, only one (1%) was THAb positive, with IgG-T3.

Because of the disproportionate size of the two relevant vitiligo subgroups of patients (THAb positive, n = 77; THAb negative, n = 2), the two subgroups could not be compared. Other statistically significant comparisons, for given indices within the THAb-positive subgroup, are reported in Figure 1c, d. IgM T3-Abs were associated with leucotrichia ($P = 0·005$), and IgG T3-Abs were associated with both leucotrichia ($P = 0·039$) and TgAb positivity ($P = 0·058$, trend to significance) (Fig. 1c). The presence of both IgM and IgG T3-Ab was also associated with leucotrichia ($P = 0·033$) and TgAb positivity ($P = 0·031$) (Fig. 1c). The absence of IgG T3-Ab and (IgG+IgM) was related to personal history of cancer ($P = 0·039$ and $P = 0·021$, respectively) (Fig. 1c). IgM T4-Ab was associated with vitiligo activity ($P = 0·037$), IgG T4-Ab with vitiligo duration ($P = 0·013$) and the presence of both IgM and IgG T4-Ab with vitiligo activity ($P < 0·001$) (Fig. 1d). The frequency and percentage of the evaluated

Table 2 Thyroid function in patients with vitiligo

	Reduced TSH, n = 4	Normal TSH, n = 72	Increased TSH, n = 3
Reduced FT3	0	1 (1)	3 (4)
Reduced FT4	0	1 (1)	2 (3)
Normal FT3	3 (4)	70 (84)	0
Normal FT4	0	70 (84)	1 (1)
Increased FT3	1 (1)	1 (1)	0
Increased FT4	1 (1)	1 (1)	0
Overt hyperthyroidism	Four patients (5%): 0 ANA+, 1 Tg+, 2 active vitiligo		
Subclinical hyperthyroidism	Two patients (3%): 0 ANA+, 1 Tg+, 1 active vitiligo		
Overt hypothyroidism	Three patients (4%): 0 ANA+, 2 Tg+, 1 active vitiligo		
Subclinical hypothyroidism	Two patients (3%): 1 ANA+, 1 Tg+, 0 active vitiligo		

Values are n (%). TSH, thyroid-stimulating hormone; FT3, free triiodothyronine; FT4, free thyroxine; ANA, antinuclear antibodies; Tg, thyroglobulin.

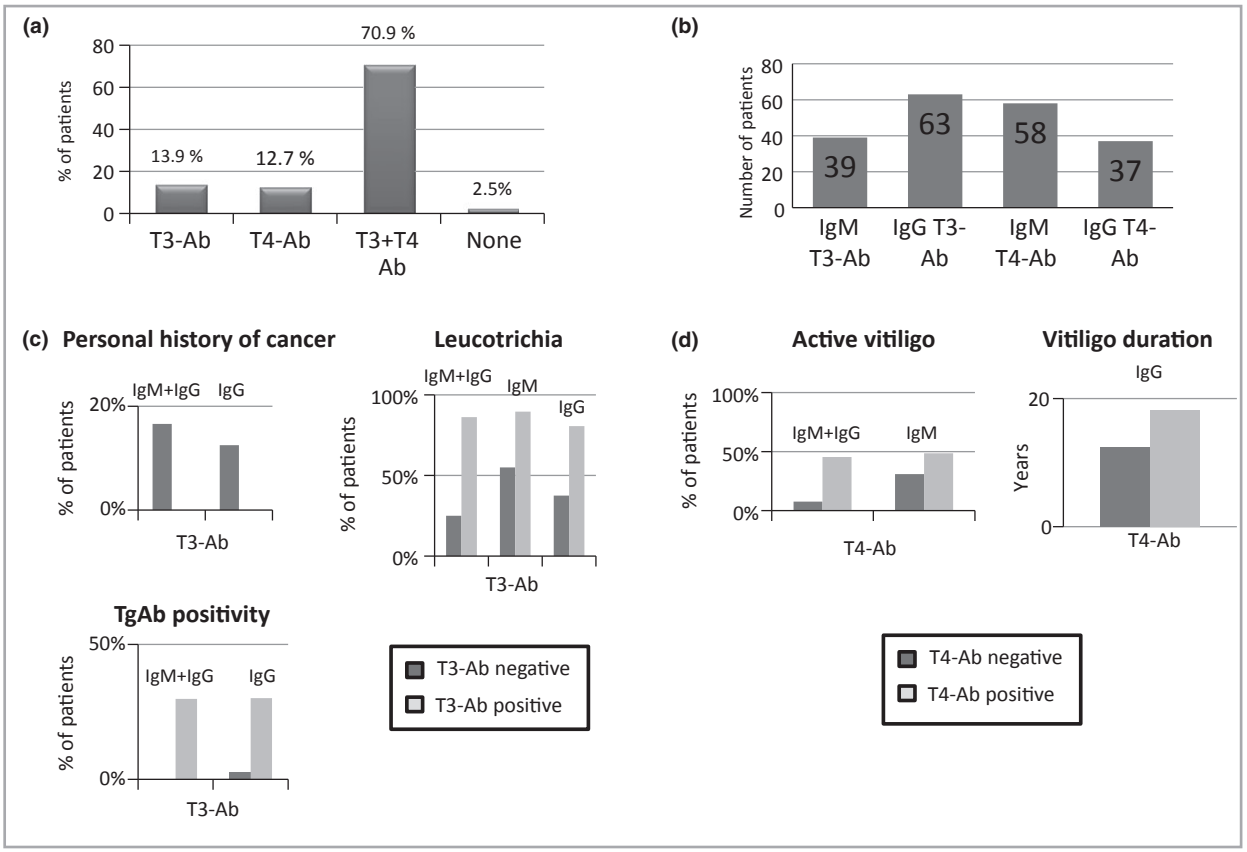

Fig 1. (a) Specificity of autoantibodies against thyroid hormones (THAbs) detected in 79 patients. The most commonly detected THAbs were against both triiodothyronine (T3) and thyroxine (T4), followed by those against T3 alone and T4 alone. Only two patients were negative for the presence of THAbs. (b) Classes of THAb detected in 79 patients. The most frequent class of T3 antibody (T3-Ab) was IgG, while for T4-Ab it was IgM. (c) Significant results of the statistical analysis of T3-Ab. A significant association was found between IgM+IgG T3-Ab and both leucotrichia and thyroglobulin antibody (TgAb) positivity. The IgM T3-Ab group was significantly associated with leucotrichia, while the IgG group was associated with both TgAb and leucotrichia. The absence of IgG T3-Ab and IgG+IgM T3-Ab was related to a personal history of cancer. (d) Significant results of the statistical analysis of T4-Ab. IgM+IgG T4-Ab and IgM alone were significantly associated with active vitiligo, while IgG T4-Ab was associated with vitiligo duration.

parameters with regards to T3-Ab and T4-Ab are reported in Tables 3 and 4.

Discussion

Our study is the first to analyse the presence of THAbs in patients with vitiligo. We showed that THAbs have a surprisingly elevated prevalence in vitiligo (97%), higher than in any other disease investigated so far. Moreover, we found that the presence of these autoantibodies significantly correlates with some clinical aspects of vitiligo, historical parameters and serological markers of ATD. Even if the prevalence of vitiligo seems to be unaffected by sex,[1,2] in our study we enrolled more women than men, probably due to their stronger attention to the aesthetic aspects of vitiligo.[26] ATDs are reported to be more frequent in women,[9] but THAbs do not seem to be affected by sex;[11,13–17,22] thus, our female preponderance should not have affected our results. The overall prevalence of THAbs in the normal population is very low,[11–14] while the prevalence rises in patients affected by ATDs or extrathyroid autoimmune diseases.

In our patients with vitiligo, approximately two-thirds had both T3-Ab and T4-Ab, which contrasts with the large prevalence of T3-Ab reported in ATDs or non-ATDs,[11,16,18,22] and the predominant class is IgG, which is in line with the literature.[11,13,15] As mentioned in the introduction, THAbs may cause spurious measurement of serum thyroid hormones, most frequently overestimating the concentration of hormone bound by THAb. Indeed, this interference in the thyroid hormone assay very likely occurred in some of our patients, as otherwise unexplainable elevated FT4 or FT3 occurred in approximately 10% of our patients.

As no other study regarding the presence of THAbs in vitiligo exists, and their pathogenetic role is still unknown, it is impossible at present to clarify the physiopathological mechanisms underlying this association. Therefore, we can make only reasonable hypotheses aiming to explain the presence of THAbs in patients with vitiligo. THAbs seem to be a particular

Table 3 Frequency and percentage of the evaluated parameters with regards to T3-Ab

Parameter[a]	T3-Ab IgM+ IgG+ (positive, n = 67) vs. T3-Ab IgM− IgG− (negative, n = 12)	IgM T3-Ab+ (positive, n = 39) vs. IgM T3-Ab− (negative, n = 40)	IgG T3-Ab+ (positive, n = 63) vs. IgG T3-Ab− (negative, n = 16)
Age (years), mean ± SD			
Positive	39·45 ± 15·98	39·94 ± 15·32	39·47 ± 16·22
Negative	32·83 ± 16·27	37·00 ± 16·89	34·43 ± 15·43
Vitiligo duration (years), mean ± SD			
Positive	15·11 ± 11·13	14·78 ± 12·27	14·80 ± 11·32
Negative	13·50 ± 11·49	14·95 ± 10·73	15·12 ± 12·27
Age of onset (years), mean ± SD			
Positive	27·82 ± 15·08	28·49 ± 15·20	27·99 ± 15·35
Negative	21·42 ± 9·65	25·25 ± 13·84	22·43 ± 9·82
Phototype I			
Positive	2 (3)	1 (3)	2 (3)
Negative	2 (17)	3 (8)	2 (12)
Phototype II			
Positive	45 (67)	25 (64)	3 (5)
Negative	5 (42)	25 (62)	7 (44)
Phototype III			
Positive	19 (28)	12 (31)	17 (27)
Negative	5 (42)	12 (30)	7 (44)
Phototype IV			
Positive	0	0	0
Negative	0	0	0
Phototype V			
Positive	1 (1)	1 (3)	1 (2)
Negative	0	0	0
Koebner phenomenon			
Positive	33 (49)	17 (44)	30 (48)
Negative	7 (58)	23 (58)	10 (62)
Leucotrichia			
Positive	54 (81)	35 (90)	51 (81)
Negative	3 (25)	22 (55)	6 (38)
Odds ratio	4·12	5·25	3·332
P value	0·033	0·005	0·039
Stress at onset			
Positive	47 (70)	28 (72)	44 (70)
Negative	7 (58·3)	26 (65)	10 (62)
Disease activity: active			
Positive	28 (41)	15 (38)	24 (38)
Negative	3 (25)	16 (40)	7 (44)
Disease activity: stable			
Positive	21 (31)	14 (36)	20 (32)
Negative	3 (25)	10 (25)	4 (25)
Disease activity: borderline			
Positive	19 (28)	10 (26)	19 (30)
Negative	5 (42)	14 (55)	5 (31)
Previous repigmentation			
Positive	42 (63)	24 (62)	40 (63)
Negative	8 (67)	26 (65)	10 (62)
Inflammation			
Positive	17 (25)	11 (28)	15 (24)
Negative	5 (42)	11 (28)	10 (62)
Personal history of hair greying			
Positive	5 (7)	4 (10)	5 (8)
Negative	0	1 (2)	0
Family history of hair greying			
Positive	2 (3)	0	2 (3)
Negative	0	2 (5)	0

Table 3 (continued)

Parameter[a]	T3-Ab IgM+ IgG+ (positive, n = 67) vs. T3-Ab IgM− IgG− (negative, n = 12)	IgM T3-Ab+ (positive, n = 39) vs. IgM T3-Ab− (negative, n = 40)	IgG T3-Ab+ (positive, n = 63) vs. IgG T3-Ab− (negative, n = 16)
Extension of vitiligo (% of body surface area), mean ± SD			
Positive	6·9 ± 12·22	8·57 ± 15·84	6·40 ± 11·49
Negative	10·70 ± 26·98	6·55 ± 10·04	12·06 ± 24·06
Staging, mean ± SD			
Positive	5·05 ± 2·31	5·43 ± 2·57	4·93 ± 2·31
Negative	4·16 ± 2·48	4·42 ± 2·01	4·87 ± 2·55
Family history of vitiligo			
Positive	19 (28)	13 (33)	18 (29)
Negative	6 (50)	12 (30)	7 (44)
Family history of thyroid condition			
Positive	26 (39)	15 (38)	24 (38)
Negative	4 (33)	15 (38)	6 (38)
Family history of autoimmune comorbidities			
Positive	3 (4)	1 (3)	3 (5)
Negative	1 (8)	3 (8)	1 (6)
Family history of cancers			
Positive	10 (15)	6 (15)	9 (14)
Negative	2 (17)	6 (15)	3 (19)
Personal history of autoimmune comorbidities			
Positive	8 (12)	5 (13)	7 (11)
Negative	1 (8)	4 (10)	2 (12)
Personal history of cancers			
Positive	0	0	0
Negative	2 (17)	2 (5)	2 (12)
Odds ratio	0·131		0·182
P value	0·021		0·039
FT3 increased			
Positive	7 (10)	3 (8)	7 (11)
Negative	1 (8)	5 (12)	1 (6)
FT3 reduced			
Positive	1 (1)	1 (3)	1 (2)
Negative	0	0	0
FT4 increased			
Positive	6 (9)	3 (8)	6 (10)
Negative	1 (8)	4 (10)	1 (6)
FT4 reduced			
Positive	3 (4)	1 (3)	2 (3)
Negative	0	2 (5)	1 (6)
TSH increased			
Positive	1 (1)	1 (3)	1 (2)
Negative	0	0	0
TSH reduced			
Positive	3 (4)	2 (5)	3 (5)
Negative	0	1 (2)	0
Anti-TPO antibodies+			
Positive	13 (19)	8 (21)	12 (19)
Negative	2 (3)	7 (18)	3 (8)
Antithyroglobulin antibodies+			
Positive	20 (30)	13 (33)	19 (30)
Negative	0	7 (18)	1 (3)
Odds ratio	0·797		6·447
P value	0·031		0·058 (trend)
Anti-TSH-R antibodies+			
Positive	3 (4)	3 (8)	3 (5)
Negative	1 (8)	1 (2)	1 (6)

Table 3 (continued)

Parameter[a]	T3-Ab IgM+ IgG+ (positive, n = 67) vs. T3-Ab IgM− IgG− (negative, n = 12)	IgM T3-Ab+ (positive, n = 39) vs. IgM T3-Ab− (negative, n = 40)	IgG T3-Ab+ (positive, n = 63) vs. IgG T3-Ab− (negative, n = 16)
Antinuclear antibodies+			
Positive	8 (12)	6 (15)	8 (13)
Negative	4 (33)	6 (15)	4 (25)

Values are n (%) unless stated otherwise. Only significant P values (< 0·05) and odds ratios are shown. TPO, thyroid peroxidase; FT3, free triiodothyronine; FT4, free thyroxine; TSH, thyroid-stimulating hormone; TSH-R, TSH receptor; ANA, antinuclear antibodies. [a]Positive and negative refer to the parameters in the column headings.

type of TgAb, namely antibodies directed against iodinated epitopes of Tg itself, as demonstrated by the studies regarding their appearance after diagnostic thyroid puncture. Indeed, this technique is able to cause the prompt release into the bloodstream of iodinated and heterogeneous molecules of Tg, which otherwise would have remained confined in the colloid, making them accessible to the autoreactive T lymphocytes. Subsequent studies[22] with monoclonal antibodies against human Tg were consistent with the conclusion that THAbs are a particular subset of TgAb. In order to elucidate the association of THAbs with rheumatoid arthritis, some authors proposed that Tg might share some chemical similarities with the chondroitin sulfate units, which are prominent components of the cartilage.[15,28,29] Possible connections between thyroid proteins and melanocytes also exist and might imply a potential cross-reactivity. Namely, the cysteine-rich units of Tg share some structural similarities with the epidermal growth factor homologous repeats[29] that can be found also in tyrosinase and tyrosinase-related protein (TRP)-1 and TRP-2 (also called dopachrome tautomerase).[30,31] Thus, it is conceivable that THAbs, which are particular subsets of TgAb, might cross-react with tyrosinase or TRP-1 and TRP-2, leading to a possible impairment/inactivation of this melanocytic enzyme and therefore leading to depigmentation. Moreover, the 570 residues at the carboxylic acid terminus of Tg are evolutionarily connected to a superfamily of lipases and esterases,[29] containing also acetylcholinesterase (AChE) and serum butyrylcholinesterase (BChE).[29,32,33] The development of THAbs possibly also directed towards the above-mentioned carboxylic acid terminus of Tg might have a role on vitiligo pathogenesis. It has been demonstrated indeed that both AChE and BChE are present in vitiligo epidermis,[34,35] where their activity is reduced by the high amount of hydrogen peroxide typical of vitiligo skin.[34,35] Therefore, THAb might react also with epidermal AChE and BChE, leading to the inactivation of these enzymes and to a consequent increase in local acetylcholine concentration. As acetylcholine has an inhibiting effect on 3,4-dihydroxyphenylalanine oxidase,[36] this event might result in a blockade of melanogenesis and therefore in depigmentation.

In addition, it is important to emphasize that in autoimmune disorders such as vitiligo, the immune system creates a persistent inflammatory milieu in which reactive oxygen species (ROS) accumulate and exert a toxic effect on surrounding cells.[37,38] In vitiligo, this effect might be exacerbated by the intrinsic impairment of ROS production at both skin and systemic level.[3,5,39] Systemic ROS accumulation might exert a toxic effect on the thyroid, releasing high amounts of Tg proteins and making them more accessible to immune system attack. Some authors indeed previously demonstrated that human thyrocytes exposed to locally increased hydrogen peroxide levels are able to release immunoreactive Tg fragments.[40] In turn, evidence of increased oxidative stress has been reported also in ATDs such as Hashimoto disease and Graves disease,[41,42] with impairments in glutathione peroxidase, superoxide dismutase, catalase and arylesterase activities.[41,42] We could assume that increased ROS levels in patients with thyroid autoimmunity, eventually induced also by the presence of THAbs, could contribute to the formation of *ortho*-phenol substrates capable of binding tyrosinase. Mutated tyrosinase in vitiligo, according to the haptenation theory, could be a genetically controlled polymorphism able to accept the above-mentioned substrates, which covalently bind to the enzyme after conversion to reactive *ortho*-quinone, leading to autoimmunity.[43] According to this process, the presence of serum THAb and possible consequent ROS increase could modify tyrosinase into a neoantigen, leading to the appearance of vitiligo.[42] All of the interactions described above between the melanocytic and thyroid systems eventually create a vicious cycle in which thyroid autoimmune processes give rise to vitiligo lesions, and in turn the presence of vitiligo perpetuates the formation of thyroid autoantibodies, in this case THAbs (Fig. 2).

We would like to consider the association between THAbs and vitiligo as additional support to confirm that patients with vitiligo have a sort of 'thyroid autoimmune diathesis', which we could define as a strong predisposition of patients with vitiligo to develop ATD. This predisposition is sustained by literature[8,44–47] and is also confirmed by our serological analysis, showing that 19%, 25% and 5% of examined patients were respectively positive for TPOAb, TgAb and TSH-R-Ab. Endocrinologists report that thyroid hormones can be considered as haptens and therefore are not immunogenic in the free form,[11] as they need to be conjugated with carrier macromolecules to elicit antibodies.[11,17] Some studies suggest that human Tg could be the antigenic carrier for THAb,[11,17] and in our assay we found a positive association between some THAbs and TgAbs, which might imply the possibility of a shared autoimmune process towards THAb and TgAb and suggest the possible antigenic/carrier role of Tg.

Table 4 Frequency and percentage of the evaluated parameters with regards to T4-Ab

Parameter[a]	T4-Ab IgM+ IgG+ (positive, n = 66) vs. T4-Ab IgM− IgG− (negative, n = 13)	IgM T4-Ab+ (positive, n = 58) vs. IgM T4-Ab− (negative, n = 21)	IgG T4-Ab+ (positive, n = 37) vs. IgG T4-Ab− (negative, n = 42)
Age (years), mean ± SD			
Positive	37·45 ± 15·61	37·72 ± 15·92	36·51 ± 16·43
Negative	43·53 ± 18·19	40·47 ± 16·80	40·16 ± 15·79
Vitiligo duration (years), mean ± SD			
Positive	15·66 ± 10·87	15·36 ± 11·34	18·24 ± 9·18
Negative	10·80 ± 13·76	13·50 ± 11·88	12·48 ± 11·89
Odds ratio			2·90
P value			0·013
Age of onset (years), mean ± SD			
Positive	26·20 ± 14·65	25·52 ± 14·88	27·42 ± 15·55
Negative	30·15 ± 13·93	30·52 ± 15·14	26·35 ± 13·73
Phototype I			
Positive	3 (5)	2 (3)	2 (5)
Negative	1 (8)	2 (10)	2 (5)
Phototype II			
Positive	40 (60)	36 (62)	22 (59)
Negative	10 (77)	14 (67)	28 (67)
Phototype III			
Positive	22 (33)	19 (33)	12 (32)
Negative	2 (15)	5 (24)	12 (29)
Phototype IV			
Positive	0	0	0
Negative	0	0	0
Phototype V			
Positive	1 (2)	1 (2)	1 (3)
Negative	0	0	0
Koebner phenomenon			
Positive	33 (50)	29 (50)	18 (49)
Negative	7 (54)	11 (52)	22 (52)
Leucotrichia			
Positive	46 (70)	39 (67)	28 (76)
Negative	11 (85)	18 (86)	29 (69)
Stress at onset			
Positive	48 (73)	43 (74)	26 (70)
Negative	6 (46)	11 (52)	28 (67)
Disease activity: active			
Positive	30 (45)	27 (47)	18 (49)
Negative	1 (8)	4 (19)	13 (31)
Odds ratio	8·25	3·70	
P value	0·001	0·037	
Disease activity: stable			
Positive	19 (29)	17 (29)	10 (27)
Negative	5 (38)	7 (33)	14 (33)
Disease activity: borderline			
Positive	14 (21)	14 (24)	9 (24)
Negative	5 (54)	10 (48)	15 (36)
Previous repigmentation			
Positive	42 (64)	37 (64)	25 (68)
Negative	8 (62)	13 (62)	25 (60)
Inflammation			
Positive	20 (30)	15 (26)	12 (32)
Negative	2 (15)	7 (33)	10 (24)
Personal history of hair greying			
Positive	3 (5)	2 (3)	1 (3)
Negative	2 (15)	3 (14)	4 (10)
Family history of hair greying			
Positive	0	0	0
Negative	2 (15)	2 (10)	2 (5)

Table 4 (continued)

Parameter[a]	T4-Ab IgM+ IgG+ (positive, n = 66) vs. T4-Ab IgM− IgG− (negative, n = 13)	IgM T4-Ab+ (positive, n = 58) vs. IgM T4-Ab− (negative, n = 21)	IgG T4-Ab+ (positive, n = 37) vs. IgG T4-Ab− (negative, n = 42)
Extension of vitiligo (% of body surface area), mean ± SD			
Positive	6·75 ± 12·34	6·75 ± 12·62	5·78 ± 6·30
Negative	11·59 ± 25·57	9·74 ± 20·96	9·11 ± 19·97
Staging, mean ± SD			
Positive	5·01 ± 2·27	5·01 ± 2·35	4·75 ± 2·22
Negative	4·46 ± 2·75	4·66 ± 2·35	5·07 ± 2·46
Family history of vitiligo			
Positive	22 (33)	18 (31)	11 (30)
Negative	3 (23)	7 (33)	14 (34)
Family history of thyroid conditions			
Positive	26 (39)	19 (33)	18 (49)
Negative	4 (31)	11 (52)	12 (29)
Family history of autoimmune comorbidities			
Positive	4 (6)	4 (7)	3 (8)
Negative	0	0	1 (2)
Family history of cancer			
Positive	12 (18)	10 (17)	8 (22)
Negative	0	2 (10)	4 (10)
Personal history of autoimmune comorbidities			
Positive	8 (12)	7 (12)	5 (14)
Negative	1 (8)	2 (10)	4 (10)
Personal history of cancer			
Positive	1 (2)	1 (2)	0
Negative	1 (8)	1 (5)	2 (5)
FT3 increased			
Positive	1 (2)	8 (14)	5 (14)
Negative	0	0	3 (7)
FT3 reduced			
Positive	8 (12)	1 (2)	0
Negative	0	0	1 (2)
FT4 increased			
Positive	6 (9)	6 (10)	2 (5)
Negative	1 (8)	1 (5)	5 (12)
FT4 reduced			
Positive	3 (5)	1 (2)	3 (8)
Negative	0	2 (10)	0
TSH increased			
Positive	1 (2)	1 (2)	0
Negative	0	0	1 (2)
TSH reduced			
Positive	3 (5)	3 (5)	1 (3)
Negative	0	0	2 (5)
Anti-TPO antibodies+			
Positive	12 (18)	11 (19)	6 (16)
Negative	3 (23)	4 (19)	9 (21)
Antithyroglobulin antibodies+			
Positive	18 (27)	18 (31)	9 (24)
Negative	2 (15)	2 (10)	11 (26)
Anti-TSH-R antibodies+			
Positive	4 (6)	4 (7)	2 (5)
Negative	0	0	2 (5)
Antinuclear antibodies+			
Positive	10 (15)	8 (14)	7 (19)
Negative	2 (15)	4 (19)	5 (12)

Values are n (%) unless stated otherwise. Only significant P values (< 0·05) and odds ratios are shown. TPO, thyroid peroxidase; FT3, free triiodothyronine; FT4, free thyroxine; TSH, thyroid-stimulating hormone; TSH-R, TSH receptor; ANA, antinuclear antibodies. [a]Positive and negative refer to the parameters in the column headings.

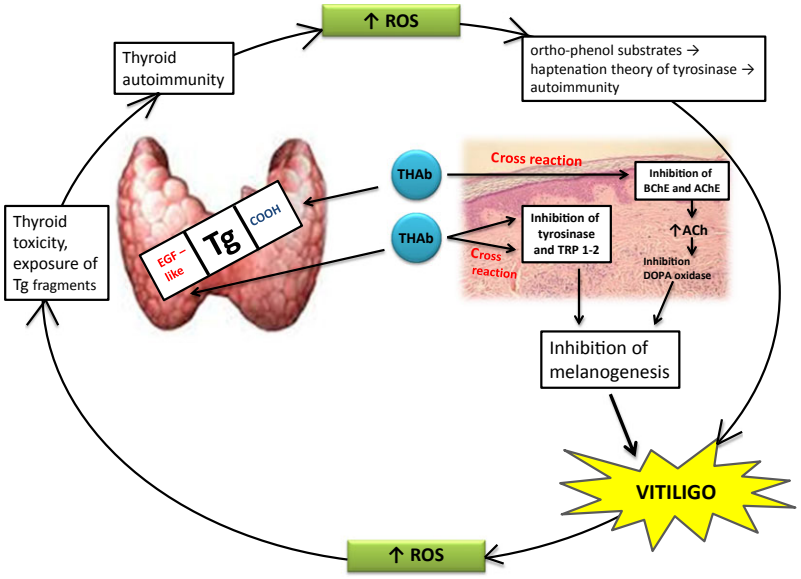

Fig 2. Proposed interplay between vitiligo and autoantibodies against thyroid hormones (THAbs). The melanocytic and thyroid systems might interact, developing a vicious cycle, in which thyroid autoimmune processes give rise to vitiligo lesions, and in turn the presence of vitiligo sustains the formation of thyroid autoantibodies, such as THAbs, which can inhibit melanogenesis. ACh, acetylcholine; AChE, acetylcholinesterase; BChE, butyrylcholinesterase; DOPA, 3,4-dihydroxyphenylalanine; EGF, epidermal growth factor; ROS, reactive oxygen species; Tg, thyroglobulin; TRP, tyrosinase-related protein.

With regards to clinical parameters of vitiligo, we observed a significant association between IgM T4-Ab and active vitiligo ($P = 0{\cdot}037$). It is conceivable that the autoimmune impairment in active vitiligo may be associated with a stronger autoimmune process than in stable vitiligo, possibly involving also thyroid antigens. Vitiligo duration instead is associated with IgG T4-Abs ($P = 0{\cdot}013$), which are produced secondarily in the immunological responses. Therefore, THAb production might persist during long-lasting vitiligo, possibly shifting from IgM to IgG classes.

We also found an association between both IgM+IgG T3-Ab and T4-Ab and the presence of leucotrichia, which is the clinical expression of damaged melanocytes of hair follicles and represents a more severe involvement of the melanocyte reservoir.[48,49] According to these data, the presence of both IgM and IgG THAbs seems to be more likely in patients with vitiligo who display a more severe depigmentation process. A significant association between the total extension of vitiligo macules and THAbs would therefore be expected, but unfortunately we did not obtain such a result, probably due to the low total mean extension of vitiligo ($7{\cdot}55 \pm 15{\cdot}19\%$) in our patients.

Finally, our results showed an association between a personal history of cancer and the absence of IgM and IgG T3-Ab ($P = 0{\cdot}021$) together or of IgG T3-Ab alone ($P = 0{\cdot}039$). This finding suggests a possible protective role of THAbs against the development of cancer. At present, no study has been performed regarding the role of THAbs in neoplastic patients, and only a few isolated case reports show their presence in cancer.[11,19–21] Also with regard to vitiligo, the current literature lacks studies evaluating its association with cancer. Only a few studies exist, and they report a lower incidence of both melanoma and nonmelanoma skin cancer in patients with vitiligo.[50–52] However, it is well known that an active immune system plays a fundamental role in preventing cancer development and progression,[53,54] and we could suggest that the coexistence of vitiligo and ATD implies a hyper-reactive and alert immune system that could exert a protective role against tumour cells.

In conclusion, we report for the first time the presence of THAbs in the vast majority of a population of patients with vitiligo. We found correlations with active vitiligo, leucotrichia, disease duration and TgAb positivity, while the absence of such autoantibodies was associated with personal history of cancer. The limitations of our study are the low number of patients negative for THAb, which prevented us from comparing them with THAb-positive patients, and the absence of adequate patient follow-up. In addition, we are not able to define the clinical significance of THAb in patients with vitiligo.

The surprisingly high prevalence of THAbs in vitiligo suggests a possible pathogenic role in the disease. An adequate follow-up of patients with vitiligo positive for THAb will be necessary, and might be useful to define a possible predictive role of such thyroid autoantibodies and potentially provide earlier diagnosis of thyroid disease.

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