Dermatology Practical & Conceptual

Circulating Podocalyxin, Tumstatin/Col-IVα3 and Chitinase 1: New Culprits in Vitiligo Occurrence

Şule Gençoğlu

Department of Dermatology, Gozde Academy Hospitals, Malatya, Turkey

Key words: Vitiligo, inflammation, adhesion, angiogenesis, immunity

Citation: Gençoğlu S. Circulating Podocalyxin, Tumstatin/Col-IVα3 and Chitinase 1: New Culprits in Vitiligo Occurrence. *Dermatol Pract Concept.* 2024;14(1):e2024095. DOI: https://doi.org/10.5826/dpc.1401a95

Accepted: December 26, 2023; **Published:** January 2024

Funding: None.

Competing Interests: None.

Corresponding Author: Şule Gençoğlu, MD, Department of Dermatology, Gozde Academy Hospitals, Gozde Academy Hospital, Ankara Asfaltı 6. Km. No:219 (Makro Market Karşısı.) Malatya, Turkey. Phone: +9(0) 422 238 25 25 E-mail: sulegencoglu2309@gmail.com

ABSTRACT

Introduction: The roles of anti-adhesive podocalyxin (PODXL), anti-angiogenetic tumstatin/Col-IVα3 and neuro-inflammation and innate immunity modulator Chitinase 1 (CHIT-1) in the etiology of vitiligo have not been studied yet.

Objectives: This study was planned to detect changes in serum PODXL, tumstatin/Col-IVα3 and CHIT1 levels in vitiligo patients.

Methods: This case-controlled study was performed on a total of 50 patients, 25 with vitiligo and 25 healthy controls. Participants in the vitiligo and control groups were matched in pairs for age and sex. At least 8-10 hours of overnight fasting, venous blood samples were taken from the participants in both groups and serum levels of podocalyxin, tumstatin/Col-IVα3 and CHIT 1 levels were measured by sandwich enzyme immunoassay.

Results: In the classification made according to the vitiligo European Task Force evaluation criteria, 18 of 25 vitiligo patients were in the slowly progressive phase and 7 patients were in the active progressive phase. Serum podocalyxin levels increased significantly in the vitiligo group compared to the controls (7.03±2.09 ng/ml vs. 4.99±1.20 ng/ml, $p<0.02$). However, serum tumstatin levels in vitiligo patients showed a significantly lower course compared to controls (4.88±1.76 ng/ml vs. 6.05±2.19 ng/nl, $p<0.03$). Serum CHIT-1 levels of vitiligo patients (42.4±7.22 ng/ml) were found to be significantly higher than the serum levels of the control group (34.5±5.33 ng/ml) ($p<0.01$).

Conclusion: High podocalyxin and CHIT1versus low tumstatin levels are new biomarkers that support the role of anti-adhesive, anti-angiogenic and neuroinflammatory pathways in the formation of vitiligo.

Introduction

Vitiligo is the most common autoimmune, polygenic and multifactorial cutaneous depigmentation disease characterized by selective loss of melanocytes and melanin pigment [1]. Although it varies according to the population studied, the incidence in the general population approaches is 0.5%-2.9% [2]. The absence of melanocytes leads to depigmentation and a skin appearance characterized by chalky-white macules [3]. Although the critical role of inherent and environmental factors in the development of the disease is well known, a complex set of mechanisms consisting of oxidative stress, angiogenesis and proinflammatory pathway defects lie behind the autoimmune picture [4]. According to the convergence theory, the progressive loss of melanocytes is caused by more than one factor or mechanism, suggesting that the disease develops on a multifactorial basis [4–6]. As a result of a defect in the innate and adaptive immune system, the immune attack to the basal cell layer, where melanocytes are located, damages both melanocytes and neighboring basal cells. The close neighborhood of the dermis and basal layer cells contains abundant sensorial, vascular and neuronal components, which are necessary structures for melanocytes to be exposed to immune attack [1, 4].

Although the disease is characterized by the loss of melanocytes, all cells in the vicinity of the dermis and basal plate should be affected by the immune attack, albeit at different rates [6]. If the loss of melanocyte causes degeneration and detachment in neighboring cells, some degradation products must be going into the systemic circulation.

The main initiator in the formation of both segmental and non-segmental forms of vitiligo is an external or internal injury. This is followed by an increase in the release of inflammatory cytokines and neuropeptides and a widespread vasodilation process at the dermis-stratum basale border. Changes in the vascular bed cause a large number of immune cells from the dermal segment to target melanocytes and neighboring cells in the basal segment [5, 6]. As a result, the chain of autoimmune reactions that occur in the affected area leads to the loss of melanocytes, leading to the emergence of the disease picture characterized by depigmentation [4, 6, 7]. This study was designed considering that proinflammatory reactions occurring at the dermis-stratum basale border provide detachment and degeneration in melanocytes, keratinocytes, basal and dermis cells through (i) vascular endothelial damage, (ii) apical/basal membrane protein degradation and (iii) innate immunity [3–6]. To confirm the presence of vascular endothelial destruction, we measured the levels of tumstatin, the NC1 domain of type IV collagen alpha 3, which is widely expressed in the vascular basement membrane.

Tumstatin is a breakdown product of type 4 collagen and prevents endothelial proliferation and angiogenesis [8, 9]. Basal membrane expression of tumstatin is controlled by matrix metalloproteinase-9, and when it binds to alphaV beta3 integrin it also blocks new endothelial formation [8]. To demonstrate apical/basal membrane and keratinocytes damage, we evaluated the levels of Podocalyxin (PODXL), an anti-adhesive molecule. PODXL is a glycosylated sialomucin of the CD34 family expressed on apical cell surfaces [10, 11]. The expression of PODXL levels in the vascular endothelium, mesothelial cells and neurons in addition to the apical membranes may help us to explain the membrane damage in the dermis-stratum basale region [12].

Objectives

The aim of this study was We tried to determine whether there is a defect in innate immunity by measuring serum Chitinase 1 (CHIT-1) levels. CHIT1 is a member of a chitinase family produced and secreted by macrophages and neutrophils and has a critical role in the homeostasis of innate immunity [13]. Its levels increase in autoimmune reactions and Th2-mediated inflammation [14]. There is no study investigating serum PODXL, tumstatin and CHIT-1 levels in studies on etiology in vitiligo patients. Since vitiligo is thought to develop at the dermis-stratum basal border as a result of melanocyte-keratinocyte separation due to endothelial damage and basement membrane disruption, PODXL, Col-IVα3 and 1 CHIT-1 protein analysis was performed to represent each parameter [3-6].

Methods

This case-controlled study was performed on a total of 50 patients, 25 with vitiligo and 25 healthy controls. Participants in the vitiligo and control groups were matched in pairs for age and sex. Patients over 45 years of age were not included in the study due to possible age-related changes in the dermis vascular endothelium. A total of 25 patients diagnosed with nonsegmental vitiligo (NSV) or segmental vitiligo (SV) according to the 2011 international vitiligo classification constituted the study group [15]. Mucosal, acrofacial, generalized, universal and mixed localized forms formed NSV, while unisegmental, bisegmental or multisegmental vitiligos formed the SV group. The control group was selected from 25 healthy individuals without systemic disease and depigmented skin lesions. The diagnosis of vitiligo was made by two experienced dermatologists based on the typical clinical appearance of the depigmented lesions and the images obtained in the Wood ‘s lamp examination.

Vitiligo was diagnosed in the presence of an amelatonic, sharp-edged and chalky-white macular lesion. We strengthened our diagnosis in the presence of a sharply demarcated, bright blue-white fluorescent lesion on Wood’s light

examination. Since no biopsy or laboratory test was required for the diagnosis of vitiligo, these procedures were not performed. All applications in the study were carried out in accordance with the principles of the Declaration of Helsinki. The study was initiated after the protocol was approved by the Malatya turgut özal universty ethics review board and patient consent was obtained.

Due to the close relationship between vitiligo and other autoimmune diseases, patients diagnosed with Hashimoto thyroiditis, Addison disease, psoriasis, diabetes mellitus, systemic lupus erythematosus or melanoma-associated leucoderma and those with other endocrinopathy were excluded from the study. Patients with a history of systemic or topical drug-induced depigmentation were also excluded from the study. Patients who were pregnant or lactating, those who had acute viral or bacterial infections and those who had an additional dermatological disease were not included in the study. Cases with a family history of depigmented skin lesions and a tendency to depigmentation due to topical or systemic drug application were not included in the control group. Similarly, those with a history of autoimmune and endocrine diseases were not included in the control group.

Age, gender, medical history, family history and body mass index (BMI) of the participants in both groups were recorded. In the classification made according to the vitiligo European Task Force evaluation criteria, 18 of 25 vitiligo patients were in the slowly progressive phase and 7 patients were in the active progressive phase. After at least 8-10 hours of overnight fasting, venous blood samples were taken from the participants in both groups and stored at - 20° C until analysis. Blood samples were collected on the third day of the menstrual cycle in female participants of reproductive age.

Measurement of Serum Podocalyxin, Collagen Type IV Alpha 3 and CHIT 1 Levels by Sandwich Enzyme Immunoassay

Podocalyxin, collagen type IV alpha 3 and CHIT 1 levels were measured in frozen-thawed serum samples using the quantitative sandwich enzyme immunoassay principle. Commercially available human podocalyxin ELISA kit (Sunred Biotechnology Company, Shanghai, CHINA), collagen type IV alpha 3 kit (Bioassay Technology Laboratory, Shanghai, CHINA) and human CHIT 1 ELISA kits (Sunred Biotechnology Company, Shanghai, CHINA) were used for the measurements. Measurements were made in accordance with the procedures specified in the catalogues. The absorbance of each sample was measured on the Bio-Tek ELx800 device at a wavelength of 450 nanometers. Concentrations of absorbances were calculated with the formula obtained using the standard curve graph.

The measuring range of the podocalyxin kit was 0.2–60 ng/mL, and the minimum measurable level was 0.153 ng/mL. The measuring range of the Collagen type IV alpha 3 kit was 0.2–70 ng/mL, and the minimum measurable level was 0.12 ng/mL. The measurement range of the CHIT 1 kit was 0.5–120 ng/mL, and the minimum measurable level (sensitivity) was 0.433 ng/mL.

Statistical Analysis

All analyses were performed on IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA). For the normality check, the Shapiro-Wilk test was used. Data are given as mean ± standard deviation for continuous variables according to normality of distribution and as frequency (percentage) for categorical variables. Continuous variables were analyzed with the independent samples t test or Mann Whitney U test. Categorical variables were analyzed with the chi-square test or Fisher's exact test. Pearson, Spearman correlation coefficients was calculated to evaluate relationships between serum markers and other variables. $P<0.05$ was accepted as statistically significant.

Results

Demographic data of both groups as well as serum podocalyxin, CHIT-1 and tumstatin levels are presented in Table 1 in detail. The number of participants in the vitiligo and healthy control groups was similar. Although the mean age of the vitiligo group was higher than the control group, the difference did not reach statistical significance. Similarly, the BMI values of both groups were similar and consistent with the overweight category values. The number of male participants in both groups was determined as 16 and the number of female participants as 9. Since there was male dominance

Table 1. Comparison of Demographic Characteristics and Potential New Serum Markers pf Patients in Vitiligo and Control Groups.

	Vitiligo	Control	p-values*
N (%)	25 (50%)	25 (50%)	0.23
Age (years)	40.12±6.33	38.92±5.13µ	0.08
BMI (kg/m2)	26.3±3.98	25.8±4.11	0.44
Gender (Female or male)	F:9 M:16	F:9 M:16	NA
Podocalyxin (ng/ml)	7.03±2.09	4.99±1.20	<0.02
Tumstatin (collagen type IV alpha 3) (ng/ml)	4.88±1.76	6.05±2.19	<0.03
CHIT-1 (ng/ml)	42.4±7.22	34.5±5.33	<0.01

*Results are given as Mean±SD. µP<0.05 was considered significant. NA: Not applicable.CHIT: Chitinase 1; BMI: Body Mass Index

in the vitiligo group, appropriate selection was made in the control participants as well.

Serum podocalyxin levels increased significantly in the vitiligo group compared to the controls (7.03±2.09 ng/ml vs. 4.99±1.20 ng/ml, p<0.02). However, serum tumstatin levels in vitiligo patients showed a significantly lower course compared to controls (4.88±1.76 ng/ml vs. 6.05±2.19 ng/nl, p<0.03). Serum CHIT-1 levels of vitiligo patients (42.4±7.22 ng/ml) were found to be significantly higher than the serum levels of the control group (34.5±5.33 ng/ml) (p<0.01). There was no significant correlation between demographic characteristics and serum ELISA markers in neither vitiligo nor control group. Figure 1 shows the distribution of serum podocalyxin, CHIT-1 and tumstatin levels between the groups.

Conclusions

Although studies on the etiology and treatment of vitiligo have gained weight in the last three decades, the data on the diagnosis, treatment and how this disease occurs dates back to ancient times [16, 17]. The fact that autoimmune diseases such as Hasimoto, diabetes mellitus, alopecia and Addison are more common in vitiligo patients suggested that vitiligo is also an autoimmune pathology [18]. However, a general consensus has not yet been reached on the mechanisms that trigger autoimmunity. Many factors have been held responsible in the etiology. Melanocyte adhesion defect and melanocyte degeneration due to oxidative stress, microvascularization defects due to vascular endothelial damage, uncontrolled neuropeptide release from local nerve endings, and inherent defects in innate immunity were the most frequently accused mechanisms [16, 18].

In the current study, we will focus focused on three of the possible mechanisms of vitiligo etiology through different serum markers that have not been studied before. These are, respectively, melanocyte adhesion defect, endothelial dysfunction characterized by vascular collagen degradation, and inherent defect in innate immunity. Our first focus is on melanocyte degeneration and depigmentation due to adhesion defect of melanocytes. For this purpose, we evaluated serum levels of podacalyxin (PODXL), an anti-adhesive molecule. PODXL was significantly higher in vitiligo patients compared to healthy controls. In the presence of high PODXL, strong adhesion of melanocytes to keratinocytes does not occur because the conditions are not suitable for a healthy adhesion. Oxidative stress, inflammation and mechanical effects can cause melanocytes to separate and disappear easily in the presence of high anti-adhesive PDXL [19]. It has been previously reported that the expression of anti-adhesive molecules such as Tenascin is increased in vitiligo patients [20]. Because PDXL is expressed on apical ? epidermal surfaces, basal membrane, vascular endothelium, and neurons, its increased expression in vitiligo patients may affect all cells in these regions [12]. Especially since the keratinocytes are affected by the increase in PDXL will prevent the adhesion of melanocyte and keratinocyte, melenocytes will lose their attachment sites. Since keratinocytes and melanocytes together form an "epidermal-melanin unit", damage to one may cause damage to the other [21]. The risk of oxidative stress is higher because the keratinocytes of vitiligo patients are defective in terms of mitochondria content [22]. In the presence of increased reactivated oxygen species, the melanin-keratinocyte unit will be damaged, and melanocyte loss and depigmentation will also accelerate. Since there are no knock out models of the anti-adhesive effects of PODXL, we do not have clear data on the defective expression of this molecule in vitiligo.

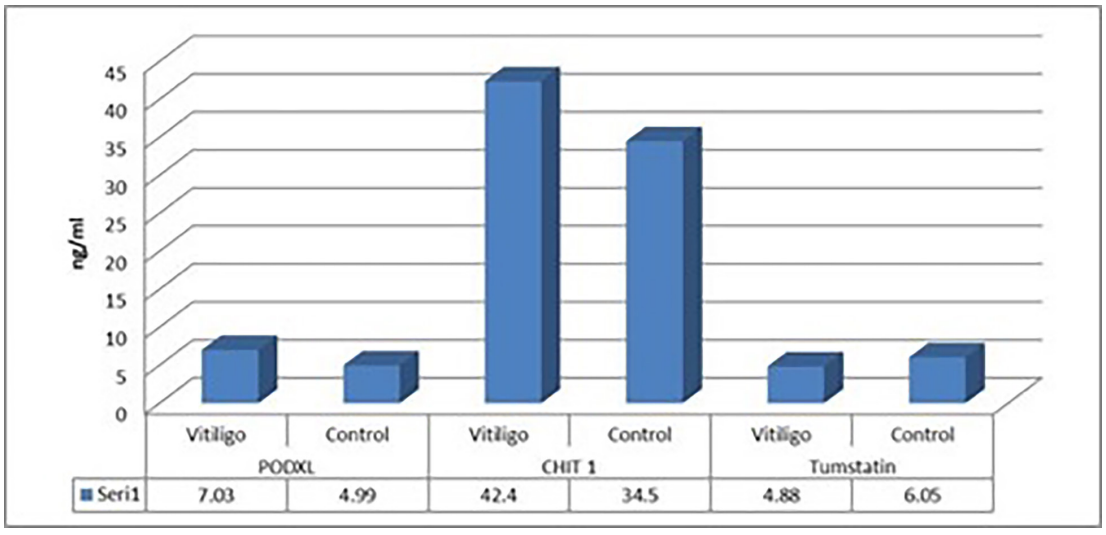

Figure 1. Graphical representation of the distribution of podocalyxin, CHT-1 and tumstatin levels in vitiligo and control groups.

Since PODXL inhibits binding via adapter proteins in many cells, it may cause loss of melanocytes by disrupting the integrity of the epidermal-melanin unit [23]. While PODXL is necessary for the continuation of the functions of many organs such as kidney, pancreas, and breast, its pathological expression may lead to the emergence of different diseases, including cancer [11, 24]. Since these functions of PODXL in cell adhesion under physiological conditions will be impaired in case of PODXL overexpression, it may contribute to melanocyte loss and disease progression in vitiligo patients. Therefore, PODXL can be a new marker that may contribute to the formation of vitiligo, or it can be used as a potential biomarker in the differentiation of segmental and nonsegmental vitiligo. In addition, it may offer an opportunity to develop new treatment options if the relationship of PODXL with vitilgo pathogenesis and subtypes is understood more clearly.

The second etiological mechanism we focus on is endothelial dysfunction and increased neovascularization due to type IV collagen alpha 3 destruction in the vascular basement membrane. For this purpose, we measured serum tumstatin levels. We encountered significantly reduced serum tumstatin levels in vitiligo patients compared to the control group. Tumstatin is a molecule derived from the destruction of type IV collagen alpha 3 (Col-IVα3) in the vascular basement membrane and is an angiogenesis inhibitor that blocks new endothelial formation and neovascularization [25, 26]. Decreased tumstatin levels in vitiligo patients may cause increased endothelial proliferation and subsequent new and irregular neovascularization. In the presence of increased vascularity and defective endothelium, melanocytes will be more exposed to cytotoxic T cells, as there will be more blood flow to the vitiligo area. Consistent with our findings, Doppler flowmetry studies have reported that blood flow in segmental vitiligo areas is increased by more than three times compared to healthy areas [27]. With increased blood flow, especially melanocyte-specific cytotoxic T cells reaching the epidermal-melanin unit and binding to melanocytes may lead to rapid depigmentation and the emergence of vitiligo clinic [28].

The basic mechanisms that initiate vascular basal membrane damage and lead to neovascularization in vitiligo are unknown. The most accepted theory is that catecholamines and neuropeptides released uncontrollably from sympathetic neurons initiate both vascular damage and vasodilation [16]. Our study suggests that vascular damage occurs because the conversion of type 4 collagen in the vascular basement membrane to tumstatin is prevented due to immune attack. Under normal conditions, tumstatin levels are increased by breaking down type 4 collagen in the vascular wall and neovascularization is prevented [8, 25]. Matrix metalloproteinase 9 is involved in the formation of tumstatin from Col-IVα3. For tumstatin to act, αVβ3 integrin receptors are needed in newly formed pathological vessels [26]. Decreased tumstatin levels in vitiligo patients may increase neovascularization and blood flow by stimulating endothelial proliferation. Increased angiogenesis and detection of VEGFR2 positive endothelial cells in animals with inherentdeletion of Col-IVα3/tumstatin support the role of tumstatin in the vascularization defect in vitiligo patients [26]. When our results and literature data are evaluated together, we can suggest that defective serum tumstatin release in vitiligo patients may contribute to neoangiogenesis and lesion spread. More clear results can be obtained by staining tumstatin, MMP-9 and integrin receptors in biopsy samples from vitiligo lesions.

The third etiological mechanism we focus on to explain is the tinherent defect in innate immunity. For this purpose, we measured serum Chitinase 1 (CHIT-1) levels in vitiligo patients. We found a significant increase in CHIT-1 levels in vitiligo patients compared to the control group. CHIT-1 produced and released by macrophages and neutrophils is involved in the regulation of innate immunity [13]. CHIT-1 is elevated in autoimmune reactions and T hepler-mediated inflammatory reactions [29]. Increased serum CHIT-1 levels in vitiligo are compatible with the autoimmune and inflammatory nature of the disease and can be considered as evidence of impaired innate immunity. Although increased CHIT1 levels are observed in many disease groups requiring macrophage activation, CHIT1 also plays a critical role in the regulation of inflammation and innate immune response [30]. Although mammals do not have chitin or chitin synthases genes, they can synthesize this molecule by enzymatic activity [31]. It is known that the level of chitinases increases in neurodegenerative disorders such as multiple sclerosis, Alzheimer's disease or amyotrophic lateral sclerosis with neuroinflammation [30, 32]. As the increase in CHIT1 will increase the degradation of chitin, intermediate molecules such as chitinase-like proteins are formed and act in innate immunity similar to host defense enzymes [14]. Since abnormal sympathetic innervation and defective neuropeptide secretion are involved in the etiology of vitiligo [16], increased CHIT1 levels may trigger neuroinflammation in the epidermal-melanin unit, resulting in impaired innate immunity and loss of melanocytes. However, it may be possible to reach a clearer conclusion by demonstrating CHIT1 overexpression in biopsies obtained from areas affected by vitiligo.

Despite the small number of participants, our study has clinical value in terms of investigating three new molecules for vitiligo etiology as a possible etiological factor for the first time. This study may lead to the development of new drug options for the treatment of vitiligo by showing the anti-adhesive effect of PODXL, the anti-angioinherent effect of tumstatin and the effects of CHIT1 on neuroinflammation and innate immunity in vitiligo patients.

Reference

1. Taïeb A, Picardo M. The definition and assessment of vitiligo: a consensus report of the Vitiligo European Task Force. *Pigment Cell Res*. 2007;20:27–35.
2. Burns T, Breathnach S, Cox N, Griffiths C. Rook's Textbook of Dermatology. Eight. Oxford: Blackwell Science. 2010. doi:10.1002/9781444317633.
3. Bergqvist C, Ezzedine K. Vitiligo: A Review. *Dermatology*. 2020;236:571–92.
4. Picardo M, Dell'Anna ML, Ezzedine K, Hamzavi I, Harris JE, Parsad D et al. Vitiligo. *Nat. Rev. Dis. Prim*. 2015;1:15011.
5. Sandoval-Cruz M, García-Carrasco M, Sánchez-Porras R, Mendoza-Pinto C, Jiménez-Hernández M, Munguía-Realpozo P et al. Immunopathogenesis of vitiligo. *Autoimmun. Rev*. 2011; 10:762–5.
6. Richmond JM, Frisoli ML, Harris JE. Innate immune mechanisms in vitiligo: danger from within. *Curr. Opin. Immunol*. 2013;25:676–82.
7. Attili V, Attili S. Segmental and generalized vitiligo: Both forms demonstrate inflammatory histopathological features and clinical mosaicism. *Indian J. Dermatol*. 2013;58:433.
8. Hamano Y, Kalluri R. Tumstatin, the NC1 domain of α3 chain of type IV collagen, is an endogenous inhibitor of pathological angiogenesis and suppresses tumor growth. *Biochem. Biophys. Res. Commun*. 2005;333:292–8.
9. Maeshima Y, Colorado PC, Torre A, Holthaus KA, Grunkemeyer JA, Ericksen MB et al. Distinct Antitumor Properties of a Type IV Collagen Domain Derived from Basement Membrane. *J. Biol. Chem*. 2000;275:21340–8.
10. Kerjaschki D, Sharkey DJ, Farquhar MG. Identification and characterization of podocalyxin--the major sialoprotein of the renal glomerular epithelial cell. *J. Cell Biol*. 1984;98:1591–6.
11. Doyonnas R, Kershaw DB, Duhme C, Merkens H, Chelliah S, Graf T et al. Anuria, Omphalocele, and Perinatal Lethality in Mice Lacking the Cd34-Related Protein Podocalyxin. *J. Exp. Med*. 2001;194:13–28.
12. Le Tran N, Wang Y, Nie G. Podocalyxin in Normal Tissue and Epithelial Cancer. Cancers (Basel). 2021;13:2863.
13. Boot RG, Blommaart EFC, Swart E, Ghauharali-van der Vlugt K, Bijl N, Moe C et al. Identification of a Novel Acidic Mammalian Chitinase Distinct from Chitotriosidase. *J. Biol. Chem*. 2001;276:6770–8.
14. Sutherland TE. Chitinase-like proteins as regulators of innate immunity and tissue repair: helpful lessons for asthma? *Biochem. Soc. Trans*. 2018;46:141–51.
15. Ezzedine K, Lim HW, Suzuki T, Katayama I, Hamzavi I, Lan CCE et al. Revised classification/nomenclature of vitiligo and related issues: the Vitiligo Global Issues Consensus Conference. *Pigment Cell Melanoma Res*. 2012;25:E1–13.
16. Katz EL, Harris JE. Translational Research in Vitiligo. *Front. Immunol*. 2021. doi:10.3389/fimmu.2021.624517.
17. Frisoli ML, Essien K, Harris JE. Vitiligo: Mechanisms of Pathogenesis and Treatment. *Annu. Rev. Immunol*. 2020;38:621–48.
18. Montilla AM, Gómez-García F, Gómez-Arias PJ, Gay-Mimbrera J, Hernández-Parada J, Isla-Tejera B et al. Scoping Review on the Use of Drugs Targeting JAK/STAT Pathway in Atopic Dermatitis, Vitiligo, and Alopecia Areata. *Dermatol. Ther*. (Heidelb). 2019;9:655–83.
19. Gauthier Y, Cario-Andre M, Lepreux S, Pain C, Taieb A. Melanocyte detachment after skin friction in non lesional skin of patients with generalized vitiligo. *Br. J. Dermatol*. 2003;148:95–101.
20. Le Poole IC, Van Den Wijngaard RMGJ, Westerhof W, Das PK. Tenascin is overexpressed in vitiligo lesional skin and inhibits melanocyte adhesion. *Br. J. Dermatol*. 1997;137:171–8.
21. Bastonini E, Bellei B, Filoni A, Kovacs D, Iacovelli P, Picardo M. Involvement of non-melanocytic skin cells in vitiligo. *Exp. Dermatol*. 2019;28:667–73.
22. Becatti M, Prignano F, Fiorillo C, Pescitelli L, Nassi P, Lotti T et al. The Involvement of Smac/DIABLO, p53, NF-kB, and MAPK Pathways in Apoptosis of Keratinocytes from Perilesional Vitiligo Skin: Protective Effects of Curcumin and Capsaicin. *Antioxid. Redox Signal*. 2010;13:1309–21.
23. Weinman EJ. New functions for the NHERF family of proteins. *J. Clin. Invest*. 2001;108:185-6.
24. Cipollone JA, Graves ML, Köbel M, et al. The anti-adhesive mucin podocalyxin may help initiate the transperitoneal metastasis of high grade serous ovarian carcinoma. *Clin. Exp. Metastasis*. 2012;29:239–52.
25. Maeshima Y, Sudhakar A, Lively JC, et al. Tumstatin, an endothelial cell-specific inhibitor of protein synthesis. *Science*. 2002;295(5552):140-143. doi:10.1126/science.1065298
26. Hamano Y, Zeisberg M, Sugimoto H, et al. Physiological levels of tumstatin, a fragment of collagen IV α3 chain, are generated by MMP-9 proteolysis and suppress angiogenesis via αVβ3 integrin. *Cancer Cell*. 2003;3:589–601.
27. Wu C-S, Yu H-S, Chang H-R, Yu C-L, Yu C-L, Wu B-N. Cutaneous blood flow and adrenoceptor response increase in segmental-type vitiligo lesions. *J. Dermatol. Sci*. 2000;23:53–62.
28. Westerhof W, d'Ischia M. Vitiligo puzzle: the pieces fall in place. *Pigment Cell Res*. 2007;20(5):345-359. doi:10.1111/j.1600-0749.2007.00399.x
29. Sutherland TE, Maizels RM, Allen JE. Chitinases and chitinase-like proteins: potential therapeutic targets for the treatment of T-helper type 2 allergies. *Clin. Exp. Allergy*. 2009;39:943–55.
30. Pinteac R, Montalban X, Comabella M. Chitinases and chitinase-like proteins as biomarkers in neurologic disorders. *Neurol. - Neuroimmunol. Neuroinflammation*. 2021;8:e921.
31. Guan S-P, Mok Y-K, Koo K-N, Chu K-L, Wong WS. Chitinases: Biomarkers for Human Diseases. *Protein Pept. Lett*. 2009;16:490–8.
32. Seibold MA, Reese TA, Choudhry S, et al. Differential Enzymatic Activity of Common Haplotypic Versions of the Human Acidic Mammalian Chitinase Protein. *J. Biol. Chem*. 2009;284:19650–8.