Review Article

Immunohistochemical expression of cytochrome P450 in skin of vitiligo patients

Abstract

Vitiligo is an acquired skin depigmentation that affects all races but is far more disfiguring in blacks. The precise cause of vitiligo is unknown. An autoimmune process targeting melanocytes is considered to mediate its pathogenesis. Consistent with this hypothesis histological studies have reported the absence of melanocytes in the affected ski. **(1)**

In addition to cellular immunity, multiple autoantibodies against melanocyte antigens including various enzymes and other substances have been detected in the sera of some patients with vitiligo. Since genetic factors appear to play a role, 20% to 30% of patients were reported with a positive family history of the disorder. Nevertheless, many vitiligo patients have neither a family history of vitiligo nor a history of other autoimmune diseases6. Consequently, many other hypotheses have been proposed to explain the pathogenesis of this disorder, including an inadequate defense from the toxic effects of free radicals and exposure of industrial chemicals. **(2)**

These effects were hypothesized to be controlled by the variable expression of cytochrome P450 (CYP or P450) genes that encode a superfamily of multi-functional monooxygenases, which comprise more than 6,000 individual enzymes9. CYPs play a major role in the metabolism of foreign lipophilic compounds, including drugs and chemical carcinogens, as

well as endogenous compounds such as steroids, fat-soluble vitamins, fatty acids, and biogenic amines9. In addition, CYP expression and activity can be in uenced by various factors such as genetic variations, presence of inhibitors or inducers, and disease states with differential tissue-specific expression pattern including the skin. [(3)]

The polymorphisms of important CYP450 genes such as CYP2C9, CYP2C19, CYP2D6, and CYP2E1 have been studied extensively in a large number of populations and showed a significant heterogeneity in the frequency of different alleles/genotypes and consequently in the resulting metabolizer phenotypes. Cytochrome P/450/2C9 (CYP2C9) is primarily localized in the liver but can be expressed in other tissues like the skin. This enzyme belongs to the subfamily cytochrome 2C, which comprise CYP2C9 and 3 isoenzymes, 2C8, 2C18, and 2C19. [(4)] Variations in CYP2C9 can be detected by real time polymerase chain reaction (PCR) using Taqman probes or probe-based melting curve analysis with the light cycler instrument. [(5)]

Introduction

Vitiligo is a pigmentary disorder of the skin, which is characterized by circumscribed depigmented macules and patches. It is a progressive disorder in which some or all of the melanocytes in the affected skin are selectively destroyed. While vitiligo may be more obvious in patients with darker skin, this disorder does not have a racial or ethnic predilection. [(6)]

Epidemiology

Vitiligo is the most prevalent pigmentary disorder, occurs worldwide, with an incidence rate between 0.1-2%, irrespective of age, race, ethnic origin, or skin color. [(7)]

Etiology

The exact etiology of vitiligo is unknown. It is frequently associated with multiple autoimmune diseases. There are various theories about its pathogenesis and the etiology is multifactorial. It is characterized by incomplete penetrance, genetic heterogeneity, and multiple susceptibility loci. Family and other twin studies have shown that inheritance is complex and also involves both environmental and genetic factors. Additionally, it is hypothesized that genetic factors can influence the age of onset of vitiligo. The inheritance of vitiligo may also include genes associated with the biosynthesis of melanin, regulation of autoantibodies, and response to oxidative stress. [8]

Recent research studies have not highlighted any associations with any certain HLA type. There is a strong reason to believe that segmental and nonsegmental vitiligo have a unique genetic mechanism, which can account for variable treatment responses. [9]

Epidemiology

Vitiligo is the commonest cause of depigmentation. It can appear at any age from child to adulthood but peak incidence is reported in the second and third decade. The age of onset usually varies between the sexes. Its prevalence is approximately 0.1% to 2% of people including adults and children worldwide and it affects all races equally. [9]

Pathophysiology

Vitiligo is commonly known as multifactorial polygenic disorder and has complex pathogenesis. It is commonly associated with both non-genetic and genetic factors. However various theories have been proposed about its pathogenesis but the exact etiology is still unknown. The generally agreed principles are the absence of melanocytes in vitiligo skin with melanocytes

loss, owing to their destruction. The destruction is most results in progressive melanocytes decreases. Theories about the melanocyte destruction include cytotoxic mechanisms, autoimmune mechanisms, intrinsic melanocyte defects, neural mechanisms, and oxidant-antioxidant mechanisms. [9]

In the neural hypothesis, a neurochemical mediator usually destroys the melanocytes and decreases the production of melanin. In oxidant and anti-oxidant mechanism intermediate or metabolic product of melanin synthesis causes the destruction of the melanocyte. In the intrinsic defect of melanocyte, there is an inherent abnormality that impedes their growth and differentiation. Another hypothesis is autoimmune or cytotoxic one where there is an alteration in humoral and cellular immunity that causes the destruction or dysfunction of melanocytes. This theory supports the hypothesis that nonsegmental vitiligo is commonly associated with autoimmune disorders than the segmental type of vitiligo. [10]

Histopathology

The basic histopathological findings in vitiligo is a total loss of functioning melanocytes in association with the complete loss of epidermal pigmentation (determined by Fontana-Masson stain or dohydrophenylalanine. Immunohistochemistry for melanocyte-specific markers like Melan-A and HMB-45 and specific electron microscopy can also be used). [11]

In the margins of lesions, perivascular and perifollicular lymphocytic infiltrate (CD4+ and CD8+ T lymphocytes) are seen, they have specific melanocytic toxicity. Degenerative changes in melanocytes are also visible like cellular enlargement, cytoplasmic vacuolization, and long dendritic processes filled with melanin granules. Other reported changes include

epidermal vacuolization, increase number of Langerhans cells, and basement membrane thickening. [12]

Vitiligo Clinical Presentation

1. Physical Examination

Vitiligo is almost always diagnosed clinically upon physical examination. Vitiligo manifests as acquired depigmented macules or patches surrounded by normal skin. The macules are chalk or milk-white in color and are well demarcated. Lesions can be rounding, oval, or linear in shape. The borders may be convex. Lesions enlarge centrifugally over time at an unpredictable rate. Lesions range from millimeters to centimeters in size. A Wood lamp may be necessary to see lesions on patients with lighter skin. [13]

The most common sites of vitiligo involvement are the face, neck, forearms, feet, dorsal hand, fingers, and scalp. When found on the face, lesions may favor a periocular or perioral distribution. In the setting of widespread or GV, lesions may also occur around the genital region, areola, and nipple. Additionally, lesions may occur in regions frequently subjected to trauma, such as bony prominences, elbows, and knees. Koebner phenomenon is defined as the development of vitiligo in sites of trauma, such as a cut, burn, or abrasion. Koebnerization may occur in as many as 20-60% of vitiligo patients. [14]

Body hair in vitiliginous macules may be depigmented. This is known as leukotrichia, and it may indicate a poor prognosis with regard regimentation therapy. Spontaneous regimentation of depigmented hair is unlikely to occur. [14]

2. Clinical Classifications of Vitiligo

Vitiligo Global Issues Consensus classified SV separately from all other forms of vitiligo, and the term vitiligo was defined to designate all forms of NSV. "Mixed vitiligo" in which SV and NSV coexist in one patient, is classified as a subgroup of NSV. Distinguishing SV from other types of vitiligo was one of the most important decisions of the consensus, primarily because of its prognostic implications. **(15)**

i. Segmental vitiligo

Segmental vitiligo (SV) has depigmented macules arranged in a dermatomal or quasi-dermatomal distribution, which does not cross the midline. It differs from NSV in terms of clinical features, natural history, and also treatment response. SV usually has an early onset in childhood in contrast to NSV, which predominantly affects adults. In SV, the lesions develop rapidly over short span of time in a localized area and then remain stable, whereas NSV has a highly variable course with periods of progression, remission, and stability.(88) SV responds poorly to medical treatment, and surgical methods are the treatment of choice. The characteristic feature of SV is the distribution pattern of the lesions. SV patterns are considered to be dermatomal or quasidermatomal, blaschkoid or following acupuncture lines. **(16)**

ii. Non-segmental vitiligo

Non-segmental vitiligo has served as an umbrella term to include all types of vitiligo that cannot be classified as SV. Of note, NSV is more strongly linked than SV to markers of autoimmunity or inflammation such as halo nevi and thyroid antibodies. **(17)**

Examples of NSV include the following:

- Focal vitiligo: refers to a small, isolated, depigmented lesion without an obvious distribution pattern and which has not evolved after a period of 1–2 years. It can evolve into SV or NSV. [15]

- Mucosal vitiligo refers to a depigmented lesion wherein a single or multiple mucosal sites are involved in the buccal or genital mucosa. If more than one mucosal site is involved, it is classified under NSV. However, an isolated mucosal vitiligo lesion is classified as unclassified vitiligo. [15]

- Acrofacial vitiligo: It typically involves the distal extremities and face. Characteristic involvement of fingers and facial periorificial areas, that is, perioral and periorbital areas are seen. This form has the potential for progression toward generalized or universal disease. Acro-facial vitiligo is generally a treatment-resistant form. [18]

- Generalized vitiligo: characterized by bilateral, often symmetrical, depigmented macules or patches occurring in a random distribution over the entire body surface. It often affects areas that tend to experience pressure, friction and/or trauma. It may begin in childhood or early adulthood. [15]

- Universal vitiligo: Complete or nearly complete depigmentation of the body occurs. [15]

- Recently rare variants of vitiligo have been described as hypochromic or minor vitiligo (observed in patients with dark skin, and partial facial and torso depigmentation), follicular vitiligo (which involves depigmentation of the hair without affecting the surrounding skin area, at least initially) and dotted vitiligo (which involves damage by dotted spots that may affect any skin segment. The macules have

dimensions between 1-1.5 mm and if they do not coexist with classical vitiligo macules, they should be classified as "dotted leukoderma or leukoderma punctata". [19]

iii. Mixed vitiligo

Mixed vitiligo is the co-existence of both SV and NSV and it is considered to be a superimposed segmental manifestation of a generalized polygenic disorder. In this case, SV usually precedes NSV with a period of 1–2 years and it is mostly more refractory to treatment. Leukotrichia and the presence of halo nevi at the onset may be the risk factors for progression toward mixed vitiligo. Halo nevus/Sutton nevus refers to the loss of pigmentation around the pre-existing nevus, leading to a halo. Numerous halo nevi are an indicator of autoimmunity against nested pigment-producing cells which increases the risk of developing vitiligo. [18]

3. Clinical Variants

Trichrome vitiligo is a clinical variant characterized by the presence of a narrow to broad intermediate color zone between a vitiligo macule and normal pigmented surrounding skin. Hann et al. [20] had highlighted its clinical and histopathological characteristics and concluded that it is a variant of unstable vitiligo. Cockade like vitiligo is a variant of trichrome vitiligo. A cockade is an oval –shaped symbol of distinctive colors normally worn on a hat. [21]

Quadrichrome vitiligo is another variant of vitiligo, which reflects the presence of a fourth color (dark brown), usually seen in darker skin phenotypes at sites of perifollicular repigmentation. A macular perifollicular or marginal hyperpigmentation is its salient feature and denotes a repigmenting disease. [22]

Penta-chrome vitiligo: It is an infrequently encountered variant in which there is a sequential display of white, tan, brown, blue-gray hyperpigmentation and the normal skin. Black-skinned individuals are predisposed to have this disorder. [23]

Marginal inflammatory vitiligo: It is a very rare variant in which an erythematous, raised border in a vitiligo macule with frequent itching and / or burning. These changes could be induced by aggressive therapy. [22]

Blue vitiligo: It usually corresponds to vitiligo macules occurring at the site of postinflammatory hypermelanosis. Ivkar et al. [106] reported the development of extensive blue vitiligo following the simultaneous progression of vitiligo and postinflammatory hyperpigmentation in an acquired immunodeficiency syndrome patient. [23]

Halo nevus: It is benign skin condition with a central melanocytic nevus, surrounded by an area or halo of depigmentation. It is the result of immunological response of the body toward the nevus, which destroys the melanocytes in surrounding skin, leading to the depigmented halo. An increased frequency of halo nevi in patients with vitiligo is observed. It is more commonly seen in children or young adults of either sex, particularly on the trunk, less commonly on the face, neck, and limbs. [23]

Vitiligo activity

1. Vitiligo Signs of Activity Score (VSAS):

The reported clinical signs of vitiligo activity are as follows: Koebner's phenomenon, confetti-like depigmentations, tri- and hypochromic lesions (including poorly defined borders), inflammatory borders/areas, presence of new lesions, extension of old lesions and itching. This urges the vitiligo community to come forward with consensus-based definitions as

well as a reliable scoring system (VSAS) to assess these clinical signs and to design optimal trials to investigate their true predictive value. The overall VSAS is based on the presence of (at least one) visible clinical signs within the 15 predefined areas resulting in a score between 0 and 15. [24]

A similar score (0-15) can be performed for each clinical sign separately generating the subscores. The grading reflects estimation of the intensity of each clinical sign within a specific area as follows: [25]

a. c-VSAS (confetti-like lesions): This is the estimated number of confetti-like depigmentations around a representative lesion (grade 1, < 10; grade 2, 10–50; grade 3, > 50).

b. k-VSAS (Koebner phenomenon) and h-VSAS (hypochromic areas/borders): This is the presence (estimated number of signs) per demarcated area: grade 1, 1; grade 2, 2–5; grade 3, > 5.

The grades correspond overall to 'somewhat present' (grade 1), 'clearly present' (grade 2) or 'very clearly present' (grade 3). Based on the grading per area, in addition one 'global grade' (total body grade) per sign can be assigned, which can be considered as the grade that is most evident on average for a specific sign. [25]

Dermoscopy:

Under dermoscopy, a key indicator is the retention or loss of perifollicular pigment as follows: [26]

a. Dermoscopic features of unstable vitiligo:

Progressive or unstable lesions present with perifollicular pigmentation, starburst, comet tail, salt and pepper, or trichrome patterns, particularly those vitiligo patches with irregular margins. [27]

An additional feature, “tapioca sago,” may be appreciated in perilesional skin of active disease; on dermoscopy, roughly 1-mm white, structureless macules are appreciated in areas that appear to be unaffected clinically. “Tapioca sago” was first described by Jha et al. [26]

b. Dermoscopic features of stable vitiligo:

Perifollicular depigmentation typifies the stable or remitting vitiligo. Leukotrichia can be also seen in stable vitiligo, correlated with treatment refractoriness. Perilesional hyperpigmentation and intralesional or perilesional erythema and telangiectasias are exclusively seen in patients responding to treatment (repigmenting vitiligo). [26] of perifollicular pigmentation (black arrow) are also appreciable. [27]

5. Reflectance Confocal Microscopy (RCM):

In vivo RCM is a real-time repetitive imaging tool that provides non-invasive images at a nearly histological resolution. Active stage of vitiligo had an apparent loss of melanin in lesional skin, disappearance or loss of integrity of the bright dermal papillary rings normally seen at the dermo-epidermal junction level, unclear border between lesional and normal skin, and highly refractile inflammatory cell infiltration within the papillary dermis at the edge of the part of the patient's lesions. Also studies demonstrated that highly refractile inflammatory cells seen within the papillary dermis at the edge of a vitiligo lesion could be a good marker to assess the stability. [28]

The stable stage of vitiligo had a complete loss of melanin in lesional skin, a clear lesional-normal skin border, and no inflammatory cell infiltration at the edge of vitiligo lesion. [29]

6. Histopathological evaluation and immunohistochemical examination:

Microscopic examination of involved skin (H&E) shows a complete absence of melanocytes in association with a total loss of epidermal pigmentation, thinning of epidermis with flattening of dermal papillae. Superficial perivascular and perifollicular lymphocytic infiltrates may be observed at the margin of active vitiliginous lesions, consistent with a cell-mediated process destroying melanocytes. Degenerative changes have been documented in keratinocytes and melanocytes in both the border lesions and adjacent skin. Other histopathological findings as epidermal spongiosis, basal vacuolar degeneration, and increased numbers of dermal melanophages are commonly observed in active lesions. Loss of pigment and melanocytes in the epidermis is highlighted by Fontana-Masson staining and immunohistochemistry testing. **(30)**

Histochemical and immunohistochemical examination further confirm a large number of CD8+ T lymphocytes infiltration at the edge of active stage vitiligo lesions. Since CD8+ T cell-mediated melanocyte destruction is thought to cause the occurrence of vitiligo, indicating the status of lymphocyte infiltration by RCM might be an asset for judging the vitiligo activity. **(31)**

7. Test grafting:

Stability of the disease process in vitiligo is the most important parameter to achieve a successful outcome in surgical treatment. Stability is defined as the absence of new lesions and absence of the spread of existing lesions for a defined period. However, there is no consensus on the period of stability, and it varies from 4 months to 2 years, according to different authors.[120, 121, 122] Regarding stability history provided by the patients which may not be entirely reliable, other methods of establishing stability have been

proposed, such as, test grafting and VIDA scoring. Falabella et al., [32] proposed the test graft method which consisted of placing six to eight punch grafts within a vitiligo lesion and observing the repigmentation over the next 12 weeks. Unequivocal repigmentation occurring beyond 1 mm from the border of the test graft indicates a positive test and is taken as an indicator of stability. However, doubts have been expressed over its utility as it has been seen that even when the disease may be unstable the minigraft test is positive and also the test may only confirm the stability of the lesion tested and not necessarily of the disease process in the patient. [32]

Vitiligo Treatment & Management

1. Approach Considerations

Various types of medications, phototherapy, laser therapy, and surgical therapy exist. However, it is important to note that in patients with lighter skin, no intervention may be needed. Instead, diligent sun protection may be the best strategy in order to avoid the surrounding normal skin from becoming more tan and making the lesions more obvious. When therapy is necessary, topical steroids, topical calcineurin inhibitors, and narrowband ultraviolet–B (NB-UVB) phototherapy are widely used and are now considered the mainstays of treatment. However, treatment must be individualized and patients should be made aware of the risks associated with therapy. No single therapy for vitiligo produces predictably good results in all patients, and the response to therapy is highly variable. [33, 34]

Choice of treatment depends on several factors including: the subtype of the disease, the extent, distribution and activity of disease as well as the patient's age, photo-type, effect on quality of life and motivation for treatment. The face, neck, trunk and mid-extremities respond best to therapy, while the lips and distal extremities are more resistant. SV and an age of

onset younger than 14 years have been associated with more refractory disease. [35]

During therapy, pigment cells arise and proliferate from the pilosebaceous unit, spare epidermal melanocytes, and the border of lesions, and migrate up to 2-4 mm from the edge. [36]

The Vitiligo subcommittee of the European Dermatology Forum has reported guidelines for the management and treatment of vitiligo based on best available evidence combined with expert opinion. Treatments were graded from first- to fourth-line options. First-line treatments consist of topical treatments (corticosteroids and calcineurin inhibitors). Second-line treatments consist of phototherapy (NB-UVB and psoralen and UVA [PUVA]) and systemic steroid treatment. Third-line treatments consist of surgical grafting techniques and fourth-line of depigmenting treatments. [37]

Various types of topical and systemic medications, phototherapy, laser therapy, and surgical therapy are used for the treatment of vitiligo. Topical treatment, modalities include corticosteroids, calcineurin inhibitors, and vitamin-D analogs. Phototherapy is an effective treatment option. It induces repigmentation in most of the patients with early and localizes the disease. Narrowband UV-B is widely used, mostly two to three times in a week with 311-312nm wavelength. It has largely replaced the psoralen photochemotherapy because of its toxic side effects. Excimer Laser is used to treating limited, stable patches of vitiligo. In segmental vitiligo which is resistant to most of the treatments, Tacrolimus and systemic corticosteroids can be combined with it. Afamelanotide and JAK inhibitor therapy are emerging treatments. Topical ruxolitinib was also found to be very effective(in 2019 randomized placebo-controlled, double-blind prospective trial). [38]

Surgical treatment options are limited to segmental or localize vitiligo that is limited to a small area. Five basic methods of repigmentation include non cultured epidermal suspensions, thin dermo-epidermal grafts, suction epidermal graft, punch grafting, and cultured epidermis with melanocytes. The following patients are good candidates for surgical treatment. [39]

Cytochrome P450 (CYP)

Cytochrome P450 (CYP) is a hemeprotein that plays a key role in the metabolism of drugs and other xenobiotics. Understanding the CYP system is essential for advanced practitioners, as the consequences of drug-drug interactions can be profound. [40]

Numerous CYP proteins have since been discovered and found to be widespread throughout the body, demonstrating significant involvement in chemical activation, deactivation, and carcinogenesis. CYP enzymes are key players in the phase I-dependent metabolism of drugs and other xenobiotics, mostly catalysing oxidations of the substrate, but occasionally also reduction reactions. [40]

Classification

Cytochrome P450 pathways are classified by similar gene sequences; they are assigned a family number (e.g., CYP1, CYP2) and a subfamily letter (e.g., CYP1A, CYP2D) and are then differentiated by a number for the isoform or individual enzyme (e.g., CYP1A1, CYP2D6). Drugs that share a common pathway have the potential for drug-drug interactions. Not all drugs have CYP activity. However, drugs with CYP activity may be inhibitors, inducers, or substrates for a specific CYP enzymatic pathway, thus altering the metabolism of concurrently administered agents. Drugs that inhibit an enzymatic pathway of CYP may cause increased concentrations of other

drugs metabolized by the same pathway, resulting in drug toxicity. Likewise, drugs that induce an enzymatic pathway of CYP may reduce concentrations of drugs metabolized by the same pathway, leading to subtherapeutic drug levels or treatment failure. [41]

The polymorphic xenobiotic metabolizing CYP enzymes can be mainly divided into two classes:

Class I, composed of CYP1A1, CYP1A2, CYP2E1 and CYP3A4, which are well conserved, do not have important functional polymorphisms, and are active in the metabolism of precarcinogens and drugs.

Class II, composed of CYP2B6, CYP2C9, CYP2C19 and CYP2D6, which are highly polymorphic and active in the metabolism of drugs, but not of precarcinogens. [42]

A 2008 review of the most common drugs reported that the majority of hepatically cleared drugs involved the CYP enzymes from families 1, 2, or 3. The most common pathways involved CYP3A4/5, CYP2C9, CYP2D6, and CYP2C19, accounted for approximately 79% of these drugs' oxidation. Although only one chemotherapy agent was listed in the top 200 list, many patients have medical comorbidities that warrant concomitant drug therapies, which can then lead to drug-drug interactions. Therefore, it is important to understand the CYP system for both chemotherapy and nonchemotherapy agents. [43]

Cytochrome P450 enzymatic cycle

Knowledge of the CYP reaction cycle plays a crucial role in the understanding of how CYP enzymes can generate ROS and increase oxidative stress. The initial step (1) in the reaction is the binding of the substrate (R-H) to the ferric iron (Fe^{3+}) of the home-thiolate group. The iron

of the heme-thiolate group is then reduced (step 2), from Fe^{3+} to Fe^{2+}, through the one electron reduction by NAPDH cytochrome P450 reductase. This facilitates the binding of oxygen (O_2) to iron (step 3). Cytochrome P450 reductase then reduces the Fe^{2+}-O_2 complex with the addition of a second election (step 4), activating the oxygen in the complex (Fe^{2+}-O_2^{-}). Addition of two protons (H^+) cleaves the O-O bond and releases H_2O (step 5 & 6). The Fe^{3+}-O complex then removes a proton from the substrate (R), leaving a reactive intermediate, $RFe^{3+}.OH^{-}$ (step 7). The hydroxyl group is then transferred to the substrate radical (step 8) and then the oxidized substrate is then released (step 9). **(44, 45)**

Variation among patients

Genetic polymorphisms can have a significant impact on drug therapy and should be taken into consideration in clinical practice, especially when unexpected outcomes arise. For example, intermediate and poor metabolizers are at increased risk for toxicity and adverse effects due to drug accumulation. These patients demonstrate hypersensitivity or low tolerance to particular drugs and subsequently may require reduced doses or avoidance of the drug altogether. Conversely, prodrugs, defined as inactive parent drugs that require enzymatic conversion to the active metabolite, may exhibit low drug efficacy in poor metabolizers. These patients may need higher doses of drugs to produce the same response as extensive metabolizers. **(46)**

Ultra-rapid metabolizers represent the opposite end of the spectrum but may also be disposed to drug toxicity when the metabolite is more active than the parent drug. Alternatively, effects of certain drugs may be diminished or short-acting due to rapid metabolism and deactivation in these patients. **(46)**

Genotype Testing

Genotyping for CYP450 polymorphism has primarily been used for research purposes or clinical drug trials. Recently, the FDA approved the first genotype test designed for use by physicians to guide the selection of medications metabolized by CYP450 enzymes. The Amplichip CYP450 test is a DNA microarray that can detect 29 polymorphisms of CYP2D6 and two polymorphisms of CYP2C19 using a blood sample. Although there is evidence of a link between adverse effects and polymorphisms coding for reduced CYP450 activity, large prospective clinical trials are needed to determine whether use of genotyping in clinical practice is cost-effective and improves clinical outcomes by preventing adverse drug effects or identifying poor responders. [47]

Medical Researches on Cytochrome P450

General medical diseases:

i. Breast cancer

The association between CYP expression and cancer risk, tumorigenesis, progression, metastasis and prognosis has been widely reported in basic and clinical studies. CYP2C8, CYP19A1 and CYP1B1 gene polymorphisms are associated with breast cancer, and screening for these genes polymorphisms can be used to prognosticate disease, prevent disease progression, and to use appropriate therapeutic. [48]

ii. Hepatocellular carcinoma (HCC)

Cytochrome P450 activity values expressed as intrinsic clearance (CL_{int}) differed between HCC patients and control subjects. HCC patient samples show increased CLint for CYP2C9, CYP2D6, and CYP2E1 compared to controls. Meanwhile, CYP1A2, CYP2C8, and CYP2C19 CLint

values decreased and CYP2A6, CYP2B6, and CYP3A4/5 activity is unchanged relative to controls. For patients with HCC accompanied by fibrosis or cirrhosis, the same activity changes were seen for the CYP isoforms, except for CYP2D6 which has higher values in HCC patients with cirrhosis. [49]

iii. Colon Tumors

Cytochrome P450 2W1 (CYP2W1) is expressed predominantly in colorectal and also in hepatic tumors, whereas the levels are insignificant in the corresponding normal human adult tissues. CYP2W1 has been proposed as an attractive target for colorectal cancer therapy by exploiting its ability to activate duocarmycin prodrugs to cytotoxic metabolites. However, its endogenous function, regulation and developmental pattern of expression remain unexplored. [50]

iv. Primary Congenital Glaucoma

Cytochrome P4501B1 (CYP1B1) belongs to the CYP450 superfamily of heme-binding mono-oxygenases which catalyze oxidation of various endogenous and exogenous substrates. The expression of CYP1B1 plays an important role in the modulation of development and functions of the trabecular meshwork (TM). Mutations in CYP1B1 have been reported in patients with primary congenital glaucoma (PCG). Mice lacking CYP1B1 also exhibit developmental defects in the TM similar to those reported in PCG patients.

Dermatological diseases:

Enzymes of the CYP450 super family are the most versatile and important class of drug metabolizing enzymes that are induced in mammalian skin in response to xenobiotic exposure. At the same time, CYP have numerous important roles in endogenous and exogenous substrate

metabolism in the skin. For example, they participate in the metabolism of therapeutic drugs, fatty acids, eicosonoids, sterols, steroids, vitamin A and vitamin D. In addition, in some skin diseases, as basal cell carcinoma and psoriasis, many CYP are elevated. CYP are the target of special interest in the development of drugs for skin diseases because most, if not all, drugs available in the armamentarium of the dermatologists are substrate, inducer, or inhibitor of this enzyme family. The functional significance of drug metabolism in skin and the implication of CYP in skin pathology and therapy is an area for future investigation. A detailed insight into the mechanism of action of various cutaneous CYP, being capable of modulating the drug bioavailability, will be helpful in the development of better strategies for novel therapy against constantly increasing skin disorders. **(51)**

i. Psoriasis

The localization of CYP1A1 and CYP1B1 in human skin is different and may be related to keratinocyte differentiation. CYP1A1 is found to be primarily localized in the basal cell layer of the epidermis in non-UVB-exposed skin, whereas CYP1B1 is localized in the epidermal cells other than the basal cell layer. Further, UVB exposure to solar-ultraviolet-protected skin (buttock site) is found to result in an UVB concentration-dependent (0–4 minimal erythema doses) and time-dependent (0–48 h) induction of both CYP1A1 and CYP1B1 in the epidermis. It is suggested that UVB-mediated induction of both CYP1A1 and CYP1B1 in human skin may result in enhanced bio-activation of polycyclic aromatic hydrocarbons and other environmental pollutants to which humans are exposed, which could make the human skin more susceptible to UVB-induced skin cancers , allergic or irritant contact dermatitis and various skin disorders. **(52)**

ii. Basal cell carcinoma

There are associations between numbers of basal cell carcinomas and CYP450 (CYP2D6) genotypes. CYP2D6 EM is associated with increased numbers of lesions. [53]

A previous Saudi study found that Cytochrome P450 *2C9* has been suggested to be very similar to epoxide hydrolase (sEH), which hydrolyzes a wide variety of endogenous and exogenous epoxides that are believed to be formed by cytochrome P450 epoxygenases. Moreover, sEH was found in various tissues including the epithelial cells in the skin. In active vitiligo patients, an increase in oxidative stress in the entire epidermal compartment has been demonstrated; in particular, the imbalance in catalase activity, reduced glutathione, and vitamin E levels was associated with hyperproduction of reactive oxygen species. Oxidative DNA damage in vitiligo patients manifested by DNA breakage in mononuclear leukocytes was shown to be comparatively higher. Since these factors might contribute to the susceptibility to vitiligo, we have undertaken this research to clarify the association of *CYP2C9* gene polymorphism with vitiligo among Saudi patients. [54]

Frequencies of CYP2C variants among normal Saudi subjects showed a relatively unique pattern in the form of high carrier rate of *2 allele (*1/*2, *2/*3, and *2/*2 genotypes), which was 29.1% of controls that was much higher than the carriage rate for the allele *3 (*1/*3, *2/*3, and *3/*3) which was 4.7%, thus resulting into allele frequencies of 17.44% and 2.33% for alleles *2 and *3, respectively. In this respect, we would note that normal Saudi subjects had a lower carriage rate and allele frequency of CYP 2C *3 when compared to Iranians and Caucasians, whose carriage rate was as high as 5% to 10%, but relatively closer to that of the Asian Indians, Korean,

Chinese, Hispanics, and African Americans whose carriage rate was 2% to 4%. [55]

Interestingly, the Saudi vitiligo patients showed a significantly higher frequencies of *3 allele carriage rate corresponding to 32.7% of cases with a significantly higher *3 allele frequency of 16.84% but with a lower *2 allele frequency (11.05%) that was statistically insignificant compared to controls. This suggests the potential association of CYP2C9 *3 with the susceptibility to vitiligo, in this particular population. [56]

Since degradation of drugs in humans is driven by detoxification mechanisms whose efficiency is influenced by genetic mutations, Weise et al. [57] studied the association between type 2 diabetes with mutations in prominent members of the *CYP* 450 2C9 isoenzyme family. [57]

Probable genetic contribution to the occurrence of vitiligo among Saudi subjects is relatively higher in patients with positive family history (41.1%) and consanguinity (33.7%). In contrast, gene frequencies related to allele *2 were not in accordance to the HWE that might be due to higher levels of consanguinity or due to the relatively small sized sample. So, this research probably needs to be investigated in a wider study by including other interactive haplotypes and genetic polymorphisms as suggested by other scientists. [58]

Veenstra et al. [5] found that genetic variation in CYP2C9 exons, rather than the promoter or other regulatory regions, is largely responsible for warfarin sensitivity associated with CYP2C9 variants in a European American population. Other studies have reported that CYP2C9 *3 genotype did not affect the required warfarin dose while it was associated with increased risk of bleeding when treated with routine dosage regimen during the initiation of treatment. [58]

Cytochrome P450 was found to be expressed in various autoimmune skin disorders like psoriasis and atopic dermatitis indicating its role in the pathogenesis of these dermatological diseases and related drug metabolism. **Akbulak et al.** found that levels of expression of CYP1B1 and CYP2E1 were found to be far higher in the pre-treatment and post-treatment psoriasis tissues than in the control tissues ($p < 0.05$*; $p < 0.05$*; $p < 0.05$*; $p < 0.05$*, respectively). **(59)**

Additionally, **Karadag et al.** found that levels of expression of CYP1A1 and CYP2E1 was significantly higher in pre- ($P = 0.003$ and $P = 0.001$, respectively) and post-treatment ($P = 0.003$ and $P = 0.001$, respectively) psoriatic tissues than in control tissues. Also, CYP1B1 levels were higher in post-treatment psoriatic tissue than in control tissue ($P = 0.045$). **(60)**

Hu et al. showed that the mRNA level of CYP1A1 in peripheral blood mononuclear cells of atopic dermatitis patients were higher compared with those of controls ($P = 0.002$*) which may contribute to the pathogenesis of atopic dermatitis. **(61)**

Early work in CYP biology revealed that CYP enzymes were directly capable of generating superoxide and hydrogen peroxide.(266) It is now thought that two "shunts" exist within the CYP catalytic cycle which can generate ROS without completion of the substrate oxidation and is known as 'reaction uncoupling'. **(62)**

The first possible release of ROS is superoxide radical, due to the loss of reduced oxygen (O_2^-), which then quickly dismutates to H_2O_2 formation. The second possible release of ROS is after the addition of a proton to the reduced oxygen complex leading directly to H_2O_2 formation and not the

release of H_2O. There are many factors which determine the coupling efficiency of a given CYP reaction with the substrate and reaction environment (as pH, O_2 concentration). [63]

There is also evidence that the different CYP isoforms have different rates of reaction uncoupling, and they can also be dependent on the substrate. **Harskamp et al.** found that CYP1B1 and CYP1D1 in Danio rerio (zebrafish) had high rates of reaction uncoupling compared to other isoforms tested (CYP1A1/1C1/1C2), indicating that CYP1B1/1D1 are more prone to generating ROS due to reaction uncoupling. The differences in coupling efficiencies may be due, in part, to structural differences in the substrate binding pocket of the various CYP isoforms. [64]

There are many enzymatic sources of ROS, including CYP enzymes, that contribute to cellular oxidation-reduction (redox) balance, which play critical roles in normal cellular processes, including immune function and cell signaling. Disruption of the normal redox balance results in oxidative stress and is involved in a number of disease processes, including carcinogenesis and ageing. There are many types of ROS that are important to biology and medicine, including superoxide anion (O_2^-*), hydroxyl radial (OH*), hydrogen peroxide (H_2O_2), and even reactive carbonyls, as well as reactive nitrogen species, all of which can disrupt the redox balance and contribute to oxidative stress. [65]

ROS enacts biological function, and elicits cellular damage, through modifications of lipids, nucleic acids, and proteins. Lipid peroxidation products such as F2-isoprostanes, which result from non-enzymatic oxidation of arachidonic acid by oxygen radicals, are often used as surrogate markers of oxidative stress and ROS levels, but also have their own

biological effects. ROS also can modify DNA, which can create mutations and errors in replication [(66)], and are known to play major roles in cancer, particularly pro-tumorigenic signaling, pro-survival signaling, and drug resistance. CYP enzymes, notably CYP2E1, have been shown to generate ROS and lipid peroxidation and these lipid peroxidation products may interact with DNA, creating oxidative DNA adducts. Excessive ROS levels and increases in oxidative stress can also modify proteins, in particular amino acid cysteine, which can in turn damage proteins and/or lead to downstream signaling in toxic pathways. [(67)]

Reactive oxygen species that produced as the result of metabolizing xenobiotics by CYP450 enzymes are believed to play important role in pathophysiology of autoimmune diseases, ROS can alter the structure of cellular antigens producing a "neo-antigen" which could mount an autoimmune response against the original antigen by molecular mimicry, ROS are involved in apoptosis "ultimately melanocyte death", activation of antigen presenting cells, initiation or amplification of diverse immunologic reactions. So, this oxidative stress plays a central role in initiating the onset of vitiligo with melanocytes damage. It can also trigger the imbalance of redox homeostasis, manifested by excessive formation and inadequate scavenging of ROS. Not only ROS but also quinones which can be produced by CYP could be covalently bound to the catalytic centre of tyrosinase giving a "neo-antigen". [(68)]

During biochemical synthesis of melanin, specific quinone and indoles (produced by CYP enzymes) are generated as reactive intermediates. These melanin intermediates can themselves be cytotoxic to melanocytes. Therefore, elevated oxidative stress resulting from the increased generation of these intermediates is above the threshold that can be combated by

genetically susceptible vitiligo melanocytes and consequently cytotoxicity/cell death is induced. Finally, an autoimmune response can be initiated that facilitates melanocyte removal and perpetuates the disease. [(69)]

Cytochrome P450 gene expression is altered by various chemical compounds as nuclear erythroid 2-related factor 2-antioxidant response element (Nrf2-ARE). The reduced activity of Nrf2-ARE leads to the decrease of downstream antioxidant enzymes and the increase of oxidative stress damage, rendering melanocytes more vulnerable to oxidative stress damage, while overexpression of Nrf2 protects melanocytes against oxidative stress. This validated the correlation between the Nrf2-ARE pathway and oxidative stress in vitiligo and its role in melanocyte destruction, opening the possibility of targeting the Nrf2-ARE pathway as a promising therapeutic strategy against melanocyte oxidative damage. [(68)]

As regards clinical data, the present study showed that there was no statistically significant difference between the two studied groups regarding age and sex (p=1.000, 0.717 respectively). This was in agreement with the results of previous studies.[(70)]

Alzolibani et al concluded that CYP2C9 *3 is probably associated with the susceptibility to vitiligo among Saudi subjects regardless of the clinical pattern, gender, and presence of family history. [(71)]

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