Overview of vitiligo research
April 2012 – June 2012

This is a review of research results in the vitiligo field which were indexed in the PubMed database (www.ncbi.nlm.nih.gov/pubmed) in a period from April 2012 till June 2012. Abstracts of papers were retrieved from PubMed database with search term “vitiligo” with filter set up to retrieve records with creation date between March 15, 2012 and June 30, 2012. The search was done on August 02, 2012. As the methodology of search has been changed (earlier records were retrieved based on the publication date limit, search limits were extended from April 01 to March 15 to assure that no record is missing between previous and present quarterly overviews due to alteration of search limit parameter.

Retrieved 82 records were manually checked for a relevance to vitiligo research field, publication date (manuscripts published prior 2012 were excluded), and novelty in terms of not being reviewed in previous quarterly overviews. Out of 82 records initially retrieved, 41 records have been selected for inclusion into this quarterly overview of vitiligo research.

Reviews & consensus papers

Among remaining relevant records, 4 papers were reviews.

Picardo and co-authors in their review summarized experimental approaches already used and emerging in vitiligo research. The review aims to propose “innovative multidisciplinary options for the vitiligo understanding”, with the hope to be “a basis for a more coordinated and productive approach to the biological questions”.

Disease stability is of a great importance in vitiligo treatment, especially when surgical methods are used. Yet there is a little consensus in evaluation of this parameter. Lahiri & Malakar reviewed existing concepts of disease stability, and emphasized that clinical stability might not truly reflect stability on molecular level, and that novel approaches in addressing vitiligo stability question should be introduced such as analysis of perilesional and non-lesional skin biopsies and serological tests including cytokine/growth factor profile assessment.

Lee authored a review manuscript focused on the role of keratinocytes in vitiligo pathogenesis. Summarizing available data, it was emphasized that keratinocytes in depigmented epidermis are more vulnerable to apoptosis, and impaired PI3K/Akt signaling pathway along with increased TNFα level is a mechanism of keratinocyte death. Importantly, apoptotic keratinocytes in depigmented epidermis produce lower levels of keratinocytes-derived growth factors required for melanocytes (including SCF), thus resulting in melanocyte death. This review ultimately points on the importance of growth factor milieu in vitiligous skin as a potential factor in pathogenesis.

One review summarized data on efficiency of relevantly new vitiligo treatment modality, monochromatic eximer light (MEL) phototherapy. In summary, MEL was found to be superior over nbUVB phototherapy, and successfully used as both monotherapy or in combination with other vitiligo treatments, with eximer laser and eximer lamp showing equal efficiency in head-to-head comparison.

Case reports

A case of squamous cell carcinoma arising from vitiligo macula has been reported. Although authors suggest that decreased photoprotection in depigmented skin might be responsible for this phenomenon, skin cancer incidence is at least not increased in vitiligo
patients compared to general population, even despite frequently used ultraviolet therapy. Thus this assumption is hardly justified.

Quality of life (QoL)

Manzoni et al reported results of comparative study of QoL of pediatric patients with atopic dermatitis, psoriasis and vitiligo, with the finding that vitiligo affected QoL to lesser degree than atopic dermatitis and psoriasis.

Results of comparative study of Willingness to pay versus Quality of life of patients affected by rosacea were published, which included comparison with historical data on vitiligo. While rosacea patients were willing to pay €2880 (median €500) for complete healing, this figure was €7360 (median €3000) for vitiligo. These data demonstrate commercial potential of effective vitiligo treatment, far exceeding those for rosacea.

Comorbidities

Thyroid dysfunction is one of the most common vitiligo comorbidity. Subba et al assessed thyroid function (by measuring thyroid-associated hormones) in 66 Nepalese vitiligo patients and found thyroid abnormalities in almost 40% of patients studied. This emphasizes the need of undergoing thyroid function test for vitiligo patients to rule out thyroid disorder and to prevent long-term complication. Yet low frequency of newly diagnosed comorbid thyroid dysfunction in vitiligo patients has been reported previously for Netherland population, thus likely indicating that necessity of thyroid disease screening of vitiligo patients is population-specific and likely depends on quality and accessibility of general medical care.

In a review of Franks & Slansky focused on cross-talk between autoimmune diseases, chronic inflammation and cancer, a link between vitiligo and melanoma has been overviewed, with vitiligo being considered as a favorable prognostic factor in melanoma patients, likely due to reflection of induced anti-melanoma immunity, and with the induction of autoimmune conditions to fight tumors as a therapeutic option which although should be considered with the care due to potential severe side effects.

Of the other comorbidities, coincidence of vitiligo with autoimmune hepatitis/primary biliary cirrhosis (2 patients, 2.8% incidence), inflammatory bowel disease and Noonan syndrome were reported.

Epidemiology

No manuscripts were published on this topic in an overviewed period.

Understanding of mechanisms of vitiligo pathogenesis

Kluger focused on tattoo as a potential vitiligo trigger with the conclusion that tattoo is unlikely to be a causative reason for tattoo-associated vitiligo. However patients with vitiligo could be advised to take into account potential risk of Koebner phenomenon occurrence at the site of tattoo, and wait for disease stabilization before undergoing tattooing.

Study of Toosi and co-authors aimed to link oxidative stress as a vitiligo trigger to autoimmune reaction contributing to disease progression. Based on a hypothesis that oxidative stress triggers accumulation of misfolded proteins and unfolded protein response, authors demonstrated that phenols indeed up-regulated expression of unfolded protein response in melanocytes, including X-box binding protein 1 (XBP1) in melanocytes. The latter induced secretion of pro-inflammatory IL-6 and IL-8 by melanocytes, which could be attenuated by
XBPl inhibitors. Notably, XBPl gene allele conferring stronger promoter activity has been previously link to vitiligo. Thus, XBPl emerges as a potential target in vitiligo treatment, linking oxidative stress and autoimmune reaction.

Two studies aimed to pinpoint systemic abnormalities in immune system in vitiligo patients. Zhou et al. when analyzing blood immune system cells in 43 vitiligo patients with active disease found no difference in CD4\(^+\), CD8\(^+\) T-cells and in regulatory T-cells (T\(_{regs}\)) compared to healthy subjects, which is in line with previously reported data. However, percentage of peripheral invariant natural killer T-cells (iNKT) was significantly lower in vitiligo patients suggesting contribution of defects in iNKT to vitiligo pathogenesis. At the same time, Lili et al. reported obvious expansion of CD8\(^+\) cytotoxic T-cells and decrease in T\(_{regs}\) in patients with generalized vitiligo. In perilesional skin, both CD8\(^+\) cytotoxic T-cell and T\(_{reg}\) load was increased, but peripheral T\(_{reg}\) have impaired ability to suppress proliferation and cytolytic activity of CD8\(^+\) T-cells. Authors conclude that reduced number and impaired functions of T\(_{regs}\) in generalized vitiligo patients might be responsible for widespread activation of CD8\(^+\) cytotoxic T-cell leading to melanocyte destruction. Apparent contradiction with results of Zhou et al potentially might be explained by different characteristics of patient enrolled in studies.

It has been shown previously that levels of catecholamine and their metabolites are increased in blood and urine. This observation has been confirmed again in study of Shahin et al. Reimann et al. found that expression of some genes associated with dopamine pathway is altered in vitiligo patient’s skin both on mRNA and protein levels. These findings provide a basis for an increased toxic to melanocytes dopamine level documented in vitiligo patients. In particular, authors observed decreased DDC (key enzyme in dopamine synthesis) mRNA level in vitiligous skin while DDC protein level was increased. This controversial observation might indicate deregulation of some mechanism controlling DDC protein synthesis/stability which contributes to increased dopamine level and vitiligo development owing to toxic effect of dopamine on melanocytes.

Stylianos et al. undertaken analysis of epithelial histology of the skin in the center and at the aged of vitiligious macula, with the finding of higher thickness of epithelium at the center of lesions, which was frequently accompanied by increased vascularization. Authors speculate that this might reflect protective changes in depigmented skin after melanin loss.

Shi et al. compared transcriptomes of Smyth line chicken feathers at different stages of depigmentation, with the differentially expressed genes associated not only with innate and adoptive immune response but also with disturbed redox balance and apoptosis, supporting paradigm of vitiligo being a disease with multifactorial etiology.

Abdou et al. investigated tenascin C distribution in vitiligous skin and found profound differences between lesional and perilesional/normal skin, with generally increased tenascin C expression. In addition, intense expression of keratinocyte-derived tenascin C hallmarked more active disease. These results confirm previously reported data and suggest that increased expression of tenascin C might contribute to melanocyte loss due to melanocyte adhesion loss owing to capability of extracellular tenascin C to inhibit melanocyte adhesion to fibronectin.

**Candidate (bio)markers/disease classification**

Lee et al. addressed a question of severe leukotrichia in segmental vitiligo patients as a predictor of phototherapy outcome. All 9 patients studied with more than 90% of white hairs as revealed by examination with digital microscope showed poor response to phototherapy, suggesting and supporting that leukotrichia is a marker of poor response to phototherapy in segmental vitiligo. In addition, authors noticed that 2 patients developed leukotrichia within 1 year of vitiligo onset warranting early after onset treatment of the disease.
Another potential predictor of clinical profile and course of vitiligo has been revealed by van Geel et al. Presence of Koebner phenomenon (in general of its particular subtype(s)) was found to predict larger body area surface involved, increased disease activity, and early age of onset. Similarly, autoimmune component presence in non-segmental vitiligo (comorbid autoimmune diseases and/or autoantibodies) was reported also to be a potential factor correlating with some clinical characteristics of vitiligo such as disease duration.

When comparing pre- and post-pubertal at onset (i.e. prior of after age of 12) non-segmental vitiligo, association of post-pubertal onset vitiligo with stress as onset factor and autoimmune thyroiditis/presence of anti-thyroid antibodies. Pre-pubertal vitiligo was associated with generalized type, family history of vitiligo, other autoimmune diseases and premature hair graying, presence of halo nevi and concomitant atopic dermatitis. This study showed that pre- and post-pubertal vitiligo is likely to differ in predisposing factors for a disease onset, including genetic ones. Further on, halo nevi presence has been associated with family history of premature hair graying in vitiligo patients, suggesting that activation of autoimmune processes as evidenced by halo nevus presence might contribute to premature hair graying.

Two promoter polymorphisms in IL-4 gene associated with vitiligo as well as increased IL-4 level due to genetic variations are emerging as candidate biomarkers for vitiligo subtyping (see section Genetic studies below for details).

Genetic studies

Five papers were focused on mining genetically determined susceptibility to vitiligo, four of which were case-controlled studies, and one was genome-wide association study (GWAS).

Aygingoz et al studied vitamin D receptor (VDR) gene polymorphisms in association with vitiligo on Turkish population and found that presence of TaqI polymorphism confers 2.23-fold increased risk of vitiligo development. In addition, specific haplotype of 5 different polymorphisms in VDR gene was found to be overrepresented in vitiligo patients in this study on 98 vitiligo patients. Notably, this haplotype included SNP previously associated with vitiligo in small inbred Romanian community, although this association has not been confirmed by the data of GWAS analysis.

Bassiouny & Khorshied analyzed GSTM1 and GSTT1 polymorphisms in Egyptian women with and without non-segmental vitiligo and found that GSTM1-null genotype alone and combined GSTM1/GSTT1 double-null genotype were associated with risk of vitiligo, suggesting that defects in detoxication system might contribute to vitiligo risk. Similar results for association of GSTM1/GSTT1 null genotype with vitiligo risk were obtained earlier for Chinese and Korean populations, thus further supporting association of GSTM1/GSTT1 variations with risk of vitiligo in particular, and genetic defects in detoxification system in general.

Group of researchers leading by Richard Spritz reported identification of additional 13 new vitiligo susceptibility genetic loci, mostly linked to immune system and melanocyte components, thus further suggesting autoimmune nature of genetic susceptibility to vitiligo.

Korean researchers investigated found association of two cyclin-dependent kinase 5 regulatory subunit associated protein 1 (CDK5RAP1) gene polymorphisms with vitiligo age of onset and the difference in one haplotype between case and control groups derived from Korean population, thus suggesting CDK5RAP1 gene as a potential vitiligo susceptibility genes.

Two promoter polymorphisms in IL-4 gene affecting gene expression were studied in Indian populations in respect with association with vitiligo in a case-controlled study. Indeed, genotype frequencies for both polymorphisms were different for case and control groups for both studied populations, with the concomitant increased level in IL-4 mRNA and serum level, with particular haplotypes associated with early age of vitiligo onset. These data reveal IL-4 gene variations as a potential genetic risk factor for vitiligo and further suggest the role of IL-4...
in vitiligo pathogenesis. Indeed, increased IL-4 expression was found in lymphocytes derived from patients with Hashimoto’s disease accompanied by vitiligo when comparing with Hashimoto’s disease patients without vitiligo, PubMed and increased level of IL-4 has been reported in vitiligo patients compared to healthy controls, PubMed. Yet in another study no difference in IL-4 level has been revealed between vitiligo patients and controls, PubMed and no increase in IL-4 has been observed in Smyth line chicken model, PubMed. Thus it is plausible that IL-4 level might hallmark specific subset of vitiligo patients and thus to be considered as a biomarker.

**Mechanisms of therapeutic interventions**

Tang et al PubMed addressed potential mechanism of beneficial UVB effect in vitiligo treatment. UVB is known to induce hydrogen peroxide generation in the skin, which at low (less than 0.3 mM) concentration as demonstrated by authors of the study, has beneficial effect on tyrosinase activity, melanin synthesis and melanosome transfer to keratinocytes, while higher concentration of hydrogen peroxide have opposite effect. However owing to intrinsically high (micromolar range) hydrogen peroxide concentration in vitiligous skin, this mechanism of UVB action seems to be weakly relevant to its effect in vitiligo treatment. In addition, one of the major milestones in vitiligo treatment is repopulation of depigmented skin by melanocytes, the process with the unknown effect of hydrogen peroxide on it.

Liu et al PubMed investigated mechanism of baicalein (5,6,7-trihydroxyflavone), a compound used in traditional Chinese medicine in vitiligo treatment, action. Being antioxidant, baicalein protected melanocyte cells in vitro from hydrogen peroxide-induced apoptosis, providing a rationale for baicalein use in vitiligo treatment.

Moreira et al PubMed investigated effect of *Pyrosteigia venusta* flower and leaf extract on melanogenesis using B16F10 mouse melanoma cells as an in vitro model and found that low extract concentration indeed stimulate melanogenesis which could be a rational for use of *P. venusta* extract in folk medicine to treat hypopigmentary disorders including vitiligo. On the other hand, effect of *P. venusta* extract flower extract in vitiligo treatment could also be attributed to its potent antioxidant properties. PubMed

**Novel treatment modalities**

Kim et al PubMed investigated possibility of circumcised foreskin use as a source of material for autologous non-cultured epidermal cell transplantation in vitiligo treatment, with excellent results obtained in 2 patients. PubMed Based on these results, circumcised foreskin can be recommended to be used as a donor material in epidermal cell transplantation treatment of vitiligo in non-circumcised males, offering large amount of donor material and no complication at donor site.

Durham & Orringer PubMed provided a case report of a successful use of 532 nm quality switched (QS) frequency-doubled neodymium doped yttrium aluminum garnet (Nd:YAG) laser for removal of recalcitrant pigmentation and recurrent pigmentation after full-body chemical depigmentation therapy for vitiligo, thus adding another treatment option in addition to being in use 694 nm QS ruby and 755 nm QS alexandrite lasers.

**Clinical trails**

**Methodology**

Repigmentation assessment is a critical issue in evaluation of vitiligo treatments. Linthors Homan et al PubMed compared evaluation of repigmentation after punch grafting performed by patients, clinical observers or using digital image analysis system. Similarly to as reported...
previously, there was a good correlation between results of evaluation by clinical observers and with the aim of digital image analysis system, while patient’s evaluation showed poor agreement with both. Despite almost perfect agreement between clinical observer’s and digital image analysis system results, use of the latter would help to avoid variations between clinical observers (3 observers were in the study) thus providing more precise data. However, patient’s satisfaction with treatment results was usually higher than those of physicians. This observation provides another rational to take into account patient’s satisfaction when evaluating treatment efficiency. This consideration is further supported by study of Eleftheriadou et al who revealed that although repigmentation is the most frequently used measurable in clinical trails outcome, patients and clinicians reported as the most desirable outcome being cosmetically acceptable repigmentation rather than its percentage.

**Clinical trails**

Hallaji et al in an open uncontrolled trail evaluated efficiency of nbUVB phototherapy in patients with recent (less than 4 year duration) versus long standing (more than 4 year duration) generalized vitiligo. Similarly to numerous reports on other types of vitiligo treatment, authors found that shorter disease duration predicts better outcome of the treatment, thus confirming the necessity of early after onset vitiligo treatment to obtain the best efficiency.

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