# Immunohistochemical Study of Protein Gene Product 9.5and Single Strand DNA in Generalized and Segmental Vitiligo

Thesis
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# LIST OF ABBREVIATIONS

| | |
|---|---|
| **4-TBP:** | 4-tertiary butylphenol |
| **5-MOP:** | 5-methoxypsoralen |
| **8-MOP:** | 8-methoxypsoralen |
| **AD:** | Anno Domini |
| **ADCC:** | Antibody dependant cell mediated cytotoxicity |
| **AISL:** | Autoimmune susceptibility locus |
| **APC:** | Antigen presenting cell |
| **Bax:** | B cell lymphoma-2 associated X protein |
| **BC:** | Before Christ |
| **BCL2:** | B cell lymphoma-2 |
| **bFGF:** | basic fibroblast growth factor |
| **C:** | Complement |
| **CAT:** | Catalase gene |
| **CD:** | Cluster of differentiation |
| **cDNA:** | Complementary DNA |
| **CGRP:** | Calcitonin gene related peptide |
| **CLA:** | Cutaneous lymphocyte associated antigen |
| **COMT:** | Catechol-O-methyl transferase |
| **CTLA:** | Cytotoxic lymphocyte antigen 4 |
| **DHICA:** | 5,6-Dihydroxyindole-2-carboxylic acid |
| **EDTA:** | Ethylenediaminetetraacetic Acid |
| **EGM:** | Extra cellular granular material |
| **ET** | Endothelins |
| **FGF:** | Fibroblast growth factor |
| **$H_2O_2$:** | Hydrogen peroxide |
| **H&E:** | Haematoxylin and Eosin |
| **HCV:** | Hepatitis C virus |
| **HIV:** | Human immune deficiency virus |
| **HLA:** | Human leucocytic antigen |
| **ICAM:** | Intracellular adhesion molecule |
| **IDDM:** | Insulin dependent diabetes mellitus |
| **IKP:** | Isomorphic Koebner phenomenon |
| **IL:** | Interleukin |
| **INF:** | Interferon |

**KDa:** Kilo Dalton
**KUVA:** Khellin plus UVA
**LAK:** Lymphokine activated killer cell
**LC:** Langerhans' cells
**LSAB:** Labeled StreptAvidin Biotin
MAO: Monoamino oxidase
**MBEH:** Monobenzyl ether of hydroquinone
**MCHR:** Melanin concentrating hormone receptor
**MHC:** Major histocompatibility complex
**MITF:** Microphthalmia-associated transcription factor
**MSH:** Melanocyte-stimulating hormone
**NGF:** Nerve growth factor
**NK:** Natural killer cell
**NPY:** Neuro peptide Y
**PBS:** Phosphate Buffered Saline
**PGP 9.5:** Protein geneproduct 9.5
**ROS:** Reactive oxygen species
**SCF:** Stem cell factor
**ssDNA:** Single stranded DNA
**TAP1:** Transporter associated with antigen-processing
**TCR:** T-cell receptor
**TGFβ1:** Transforming growth factor β
**Th:** T helper cell
**$TiO_2$:** Titanium dioxide
**TNF:** Tumor necrosis factor
**TRP:** Tyrosinase related protein
**VKHS:** Vogt-Koyanagi-Harada syndrome

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# A. INTRODUCTION

Vitiligo is an acquired dermatologic disorder characterized by loss of functioning melanocytes, resulting in depigmentation of the skin. ***(Tobin et al., 2000; Solano et al., 2006; Van Geel et al., 2014).***

The mechanisms underlying the destruction of functioning melanocytes and the absence of melanin in vitiligo lesions remain unclear. Nevertheless, certain theories have been suggested and studied including; the genetic hypothesis, the autoimmune hypothesis, the neural hypothesis (involving neuropeptides, adrenergic and cholinergic neurotransmitters), the apoptotic theory, the viral hypothesis, the self destruction hypothesis (including the significant contribution of oxidative stress through the accumulation of H2O2), and convergence theory (which combines previous theories). ***(Cucchi et al.,2000; Dell'Anna et al.,2003; Gauthier et al.,2003; Ortonne,2003; Hasse et al.,2004; Schallreuter et al.,2006; Solano et al.,2006).***

Developmentally, melanoblasts are derived from the neural crest, and so it is not surprising that an association between neurological disorders and changes in skin pigmentation can often be found. The segmental distribution of vitiligo, and the association of vitiligo with peripheral nerve injury, viral encephalitis, horner's syndrome and diabetic neuropathy, supports the neurological theory in vitiligo (***Al'Abadie et al.,1994; Liu et al.,1999).***

Protein gene product 9.5 (PGP 9.5) is a general marker for all cutaneous sensory and autonomic nerve fibers. It has been studied in skin biopsies of various dermatologic disorders ***(McArthur et al.,1998; Omdal et al.,2002; Antunes et al.,2003; Ebnezer and Daniel,2004).***

Studies of PGP 9.5 in vitiligo have been performed. One study showed a minimal increase in PGP 9.5 positive nerve fibers at the dermoepidermal junction and lower malpighian layers in patients with vitiligo at the periphery of the lesion relative to normal skin

(***Al'Abadie et al.,1994).*** Other reported no difference in PGP 9.5 positive nerve fibers between lesional, nonlesional, and normal skin in patients with vitiligo ***(Liu et al.,1999).*** However, recently ***Aroni et al.,2008*** detected a statistically significant difference in the number of PGP 9.5-positive nerve fibers/axons in the papillary dermis between the centre and periphery of the lesions of vitiligo (i.e. increased at the center in comparison with the periphery).

A few controversial theories have been studied concerning the role of apoptosis in vitiligo. The lack of evidence for the involvement of this process has been reported in several studies (***Tobin et al.,2000; Van den Wijngaard et al.,2000a).*** However vitiligo as a manifestation of apoptosis is supported by its histopathological findings, and is particularly evident from the changes at the border between the depigmented and clinically normal (uninvolved) skin (***Kovarik et al., 2009).***

A monoclonal immunoglobulin M (IgM) antibody was used by ***Aroni et al., 2008*** against single strand DNA (ssDNA), which specifically stains the apoptotic cells and has been applied in vitiligo to differentiate between apoptotic and necrotic cells.

On the basis of dermal PGP 9.5-positive nerve fibers and ssDNA-positive (apoptotic) cells, ***Aroni et al.,2008*** concluded that there is a relationship between the autonomic nerve system function and apoptosis, supporting the hypothesis that the destruction of functioning melanocytes in vitiligo could be the end result of different interacting pathogenic mechanism, such as apoptosis and accumulation of neural fibers/axons.

# B. AIM OF THE WORK

The aim of this work is to study the possible contribution of either the neural mechanism or apoptotic mechanism or both together in the etiopathogenesis of generalized and segmental vitiligo variants. This was done through immunohistochemical study of PGP9.5 as evidence of neural mechanism and ssDNA as an evidence of apoptotic mechanism in vitiligo.