ANALYSIS OF GENETIC ASSOCIATION OF *TGFB1* AND *SMAD4* GENES WITH KELOID IN MALAY POPULATION

By

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ANALISA PERKAITAN GENETIK BAHAGIAN GEN *TGFB1* DAN *SMAD4* DENGAN PENYAKIT KELOID DALAM POPULASI MELAYU

oleh

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Thesis yang deserahkan untuk

Memenuhi keperluan bagi

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Universiti Sains Malaysia

February 2012

DEDICATION

Firstly, I would like to thank Allah for blowing energy unto my feeble frame for completing this study. I dedicate this thesis to my mother and father for their unconditional love, support and prayer throughout my life and to my husband and son for their patience and understanding during the course of my studies. My love goes to all of you.

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LIST OF ABBREVIATIONS

^{0}C	**:** Degree Celsius
µl	**:** Micro liter
bp	**:** Base pair
mg/ml	**:** Milligrams per milliliter
TGFβ	**:** Transforming growth factor beta
SMAD4	**:** Mothers against decapentaplegic homolog 4
UV	**:** Ultraviolet
pH	**:** Potential of hydrogen
KD	**:** Keloid Disease
HLA	**:** Human leukocyte antigen
OMIM	**:** Online Mendelian Inheritance in Man
ECM	**:** Extracellular matrix
PDGF	**:** Platelet-derived growth factor
TGFA	**:** Transforming growth factor, alpha
FGF	**:** Fibroblast growth factors
VEGF	**:** Vascular endothelial growth factor
MMP	**:** Matrix metalloproteinase
ATP	**:** Adenosine-5'-triphosphate
RSTS1	**:** Rubinstein-Taybi syndrome1
CREBBP	**:** CREB-binding protein
cAMP	**:** Cyclic adenosine monophosphate
UCMD	**:** Ullrich congenital muscular dystrophy
TKCR	**:** Torticollis, keloids, cryptorchidism and renal dysplasia
EDS	**:** Ehlers-Danlos syndrome

PDGF-alpha : Platelet derived growth factor alpha

siRNA : Small interfering RNA

STR : Short tandem repeat

UTR : Untranslated region

NEDD4 : Neural precursor cell expressed, developmentally down-regulated 4

BMP : Bone Morphogenic Proteins

DAXX : Death-domain associated protein

EDTA : Ethylenediaminetetraacetic acid

DNA : Deoxyribonucleic acid

dH_2O : Distilled water

LB : Litihium boric acid buffer

PCR : Polymerase chain reaction

Taq : Thermophilus aquaticus

$MgCl_2$: Magnesium chloride

dNTP : Deoxy nucleotide triphosphate

dATP : Deoxy adenosine triphosphate

dGTP : Deoxy guanine triphosphate

dTTP : Deoxy thymine triphosphate

dCTP : Deoxy cytosine triphosphate

NCBI : National Center for Biotechnology Information

RFLP : Restriction fragment length polymorphism

SNP : Single nucleotide polymorphism

dbSNP : Data base single nucleotide polymorphism

SS : Submitted single nucleotide polymorphism

rs : Reference single nucleotide polymorphism

RE : Restriction enzyme

ARMS : Amplification refractory mutation system

PB : DNA binding buffer

BSA : Bovine serum albumin

HiDi : Highly deionized formamide

MAF : Minor allele frequency

EM : Expectation-maximization

OR : Odds ratio

LCL : Left confidence limit

RCL : Right confidence limit

Freq : Frequency

LRT : Likelihood Ratio Test

RR : Relative risk

CI : Confidence interval

LD : Linkage disequilibrium

LOD : Logarithm of odds

mRNA : Messenger RNA

HWE : Hardy Weinberg Equilibrium

u-PA : Urokinase-type plasminogen activator

t-PA : Tissue plasminogen activator

PCNA : Proliferating cell nuclear antigen

cM : CentiMorgan

ANALISA PERKAITAN GENETIK BAHAGIAN GEN *TGFB1* DAN *SMAD4* DENGAN PENYAKIT KELOID DALAM POPULASI MELAYU

ABSTRAK

Pembentukkan keloid adalah sebabkan oleh faktor persekitaran and genetik**.** Dua jenis gen iaitu *TGFβ1* dan *SMAD4* yang terletak pada signal yang sama dan sangat tinggi di ekpresi di dalam fibroblast sel keloid. Kajian ini bertujuan untuk menyiasat kaitan di antara varian *TGFβ1* dan *SMAD4* gen menggunakan kaedah genotyping PCR-RFLP. Frekuensi alel, genotip dan haplotaip jenis ini berbanding antara 100 kes keloid dan 100 kawalan sihat biasa. Tiada kaitan signifikan di temui di dalam kes-kawalan di kalangan populasi Melayu bagi allel dan genetik variasi untuk *TGFβ1.* Manakala untuk *TGFβ1* haplotaip keputusan yang berbeza di tunjukkan, iaitu penyumbangan kepada risiko memperolehi keloid. C-C haplotaip yang mengandungi c.29C>T dan -509 T>C varian kerap di temui di kalangan kes keloid (*p* - nilai yang telah di ubah = 0.037, OR = 2.07, 95% CI = 0.87-4.93), menunjukkan 4.5 kali berisiko untuk memperolehi keloid. AG genotaip untuk SMAD4 iaitu c5131A>G varian menunjukkan statistik signifikan (*P*-nilai = 0.0573, OR = 1.75, 95% CI = 0.99-3.13). Di samping itu juga, C-C haplotaip untuk varian TGFβ1 menunjukkan peningkatan risiko ke arah keloid apabila bergabung dengan *SMAD4* c.5131A>G varian. Ini menunjukkan interaksi di antara gen kepada perkembangan keloid. Varian berlaku samaada di peringkat ekpresi ataupun di dalam ketidakseimbangan hubungan dan bergabung dengan faktor persekitaran boleh menyumbang kepada keaadaan tersebut. Setakat ini, hanya satu laporan di berdasarkan populasi orang berkulit putih yang menunjukkan hubung kait *TGFβ1* dan

keloid, manakala tiada laporan di buat bagi *SMAD4*. Oleh sebab itu, kajian ini merupakan kajian pertama yang menunjukkan kaitan yang positif antara *TGFβ1* dan *SMAD4* varian dengan keloid di kalangan populasi Melayu.

ANALYSIS OF GENETIC ASSOCIATION OF TGFB1 AND SMAD4 GENES WITH KELOID IN MALAY POPULATION

ABSTRACT

Keloid is a complex condition with environmental and genetic risk-contributing factors. Two candidate genes, *TGFβ*1 and *SMAD4*, located in the same signaling pathway are highly expressed in the keloid fibroblast cells. The present study investigated the association between variants of *TGFβ*1 and *SMAD4* genes using PCR-RFLP genotyping method. Allele, genotype and haplotype frequencies of these variants were compared between 100 keloid cases and 100 normal healthy controls. No statistical significant difference was found between alleles and genotypes of *TGFβ*1 variants in the current case-control study in a Malay population, while *TGFβ*1 haplotypes in contrast showed a strong association with the risk of keloid. The C-C haplotype, composed of both c.29C>T and -509 T>C variants was observed more frequently among cases (Corrected *p-value* = 0.037, OR = 2.07, 95% CI = 0.87-4.93), showing a 4.5-fold increased risk for keloid. The AG genotype of the *SMAD4* c.5131A>G variant showed a statistically significant trend (*P-value* = 0.0573, OR = 1.75, 95% CI = 0.99-3.13). The C-C haplotype of *TGFβ1* variants showed an increased risk when combined with both alleles of the *SMAD4* c.5131A>G variant, indicating possible interaction of these genes in keloid development. Either of these variants is most probably causative at the expression level or is in linkage disequilibrium with other causative variants in a complex pattern together with the environmental factors that contribute to the condition. There is only one documented

report on a relationship between *TGFβ*1 and keloid with no association in the Caucasian population, while there has been no report for *SMAD4*. Therefore, the present study is the first of its kind showing a strong positive association between *TGFβ*1 and *SMAD4* variants and keloids in the Malay population.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

The skin is the largest organ that supports the whole body from mechanical impact, thermal and chemical impact, UV radiation and water loss. In addition, the skin protects the body against microorganisms by its acidic pH value. One of the largest defense mechanisms that the body has is through the epidermis and dermis.

At microscopic level, the skin is composed of two main layers; epidermis and dermis. Epidermis contains several layers namely the basal cell layer, the spinous cell layer, the granular cell layer and the stratum corneum. The cells in the epidermis are called keratinocytes. The dermis with a variable amount of fat, collagen and elastic fibers provides strength and flexibility of the skin.

The skin can be affected by several conditions, but the most common skin diseases has been categorized into eight common types: rashes, bacterial infections, fungal infections, parasitic infections, pigmentation disorders, tumors and cancers and trauma (Kelly and Taylor, 2009).

Keloid and hypertrophic scars are benign proliferative diseases that caused by overproducing of collagen. In keloid scarring which also known as keloid

disease (KD), the scar may grow beyond the boundaries of the original injury that can occur even after a minor skin trauma. Hypertrophic scar in contrast is a cutaneous lesion that characterized by excess scar tissue and remains within the boundaries of the original injury. Hypertrophic scars are usually red, pink or purple in color, hypersensitive to touch and may itch or be generally painful. Hypertrophic scars usually occur soon after injury and improve over the time in contrast to the keloid scars. Keloid scars may occur few months after injury (Wolfram *et al.*, 2009).

In addition, keloid characterizes by a collection of atypical fibroblasts with excessive deposition of extracellular matrix components, especially collagen, fibronectin, elastin and proteoglycans. Keloids are benign hyperproliferative growths of dermal fibroblasts (Kelly, 2004). However, most of cases occur sporadically, but familial cases are common as well.

There are many risk factors involved in the development of this disease. Keloid is known with alterations in growth factors, collagen turnover and tension alignment. Genetics and environmental risk factors contribute to the etiology of this disease. Trauma, foreign-body reactions, infections and endocrine dysfunctions have been suggested as environmental risk factors of keloids (Russell *et al.*, 1988). In addition, keloid scars can be caused by surgery, an accident, by acne or, sometimes, from body piercings. Both sexes are susceptible to keloid with an equal proportion (Shaffer *et al.*, 2002, Kuflik, 1994, Child *et al.*, 1999).

Keloid can develop at every age but higher incidence is noticed between 10 and 30 years. Average age of first keloid diagnosis has been reported 22.3 years for women and 22.8 years for men (Cosman *et al.*, 1996). It has been suggested that hormones contribute in the keloid development. This evidence has been supported by data that showing an elevated androgen receptor level in the clinically active keloid tissue (Schierle *et al.*, 1997). A higher incidence of keloid has been reported during pregnancy and puberty (Oluwasanmi, 1974). Alterations in the hormone profile and increased neo-angiogenesis during pregnancy has been suggested as possible explanation.

The incidence of keloid varies among different races. In general, it is more common among peoples with a black skin compared with those with a white skin (McDonald, 1988, Fong *et al.*, 1999). Higher incidence of keloid in black peoples has been attributed to the thick and more seborrhoeic skin of them. The incidence of keloids in Caucasians in the United Kingdom is reported to be <1% (Bloom, 1956), while the incidence in Blacks and Hispanics varies from 4.5 to 16% (Oluwasanmi, 1974). The higher rate for earlobe keloids after piercing may be responsible for a slight female predominance (Kelly, 2004). Keloid is genetically heterogeneous and different genes may be involved in different populations (Yan *et al.*, 2007b, Brown *et al.*, 2008a, Nassiri *et al.*, 2009).

There is no single therapy procedure for keloid as yet. Treatment for this disease can be difficult, and is not usually successful. The common treatment procedures in keloid include surgery (Brown *et al.*, 1990, Salasche and

Grabski, 1983), pressure (Urioste *et al.*, 1999), topical silicone gel (Slemp and Kirschner, 2006), radiation (DeBeurmann and Gougerot, 1906), laser therapy (Alster, 1997), corticosteroids (Golladay, 1988), Imiquimod 5% cream (Berman and Villa, 2003) and onion extract (Zurada *et al.*, 2006).

1.2 Problem statement

The increased familial aggregation, increased prevalence in certain races and increased concordance in identical twins provide a strong genetic predisposition to etiology of keloid. In addition, syndromic forms of keloid support the hypothesis of a genetic background for keloid (Hendrix and Greer, 1996, Goeminne, 1968). It seems that development of keloid controls by several loci under both autosomal recessive (Omo-Dare, 1975) and autosomal dominant mode of inheritance (Marneros *et al.*, 2001, Shaffer *et al.*, 2002). Several candidate loci have been identified in association with keloid including 15q22, 18q21.1, P53, *HLA-DRB1*, 19q13.1, 2q23, 7p11, Xq28, *TGF-β*, *SMAD* and Caspase family genes (Zuffardi and Fraccaro, 1982, Marneros *et al.*, 2004, Yan *et al.*, 2007b, Liu *et al.*, 2008b, Brown *et al.*, 2008b). Lu *et al.*, (2008) described a positive association of HLA-DQA1 and DQB1 alleles and haplotypes with keloids and Rossi and Bozzi (1989) proposed an association with the *HLA*-types, *HLA-DR5* and *HLA DQw3*. Transforming growth factor Beta (*TGFβ*) has previously been implicated in keloid pathogenesis (Bayat *et al.*, 2002, Bayat *et al.*, 2003b, Bayat *et al.*, 2005a, Bayat *et al.*, 2005b) and the high expression of *TGFβ*1 has been associated with keloid formation by several investigators (Peltonen *et al.*,

1991, Niessen *et al.,* 1999, Chin *et al.,* 2001). Therefore, the basis for keloid formation has not been fully clear and though different genes are said to be involved in the formation of keloids, the role of these in keloid formation has not been clearly established.

1.3 Justification of the study

The role of genetics in development of keloids has been accepted through various studies in different populations using expression analysis, linkage analysis and case-control association studies. Keloid is a heterogeneous disease at clinical and molecular level, as several genes and or loci has been recognized as candidate in its development. Therefore, the aim of this study is to investigate the role of two candidate regions namely *TGFβ* and Mothers against decapentaplegic homolog4 *(SMAD4)* in cases with keloid in comparison with controls. In both these regions, some strong candidate genes for Keloids are located which suggests interaction in the same molecular pathway. *TGFβ*1 (OMIM #190180) gene, one of the candidate genes for keloid, is located on 19q13.1 chromosomal region. *TGFβ*1, *TGFβ*2 (OMIM #190220), and *TGFβ*3 (OMIM #190230) genes act through the same receptor signaling systems (Massague, 2000).

Transforming growth factor beta (*TGFβ*) plays an important role in wound healing and many fibrotic diseases including cirrhosis, chronic hepatitis, glomerulonephritis, scleroderma and pulmonary fibrosis (Border and Noble, 1994, Massague, 2000). Many biological functions have been suggested for

TGF-β family genes including; regulation of cell proliferation, migration, differentiation and development, tissue turnover and repair (Massague, 2000).

*TGFβ*1 and *TGFβ*2 are supposed to be the most important cytokines responsible for keloid scarring (Niessen *et al.*, 1999). The *TGFβ*1 gene expression plays a critical role in development of fibrosis in keloid (Peltonen *et al.*, 1991).

Genetic linkage was found between 18q21.1 and keloid disease in a Chinese population (Yan *et al.*, 2007b). The *SMAD* 2, 4 and 7 genes are located in this region and these genes are candidate in the development of keloids. The importance of *SMAD* genes and their role in fibrotic disorders has been well documented. The signals from *TGFβ* family members transmit via cell surface receptors to the *SMAD* proteins. *SMAD* proteins act as transducer and transmit the signals to nucleus (Flanders, 2004). Overall, there is close interaction between *TGFβ* and *SMAD* family genes in the development of keloid scarring.

1.4 Objectives of the study

1.4.1 General objective

1. To determine the association between *TGFβ*1 and *SMAD4* Genes with keloid scarring

1.4.2 Specific objectives

1. To determine the association between the SNPs c.29C>T and -509T>C of TGFβ1 Gene and Keloid in Malay population in comparison with control group.

2. To determine the association between the SNPs c.686T>G and c.5131A>G of SMAD4 Gene and Keloid in Malay population in comparison with control group.

3. To determine the interaction between these SNPs of TGFβ1 and SMAD4 Genes in patients with Keloid

1.5 Research hypothesis

Genetic variants in the TGFβ1and SMAD4 Genes are involved in the etiology of Keloid formation in Malay population.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Skin structure and function

Skin is the largest organ in body with several functions in which protection against environmental factors and controlling the body's fluid are the most important role. Skin contains three structural layers; epidermis, the dermis and subcutis (Figure 2.1). Skin is a dynamic organ in which dead outer layer cells are continuously replaced by new cells that originate from basal layer. Epidermis is the outer layer of skin that is relatively thin and tough. Epidermis itself contains three sub-layer including stratum corneum (horny layer), keratinocytes (squamous cells) and basal layer. Stratum corneum contains mature keratinocytes which produce fibrous proteins namely keratins (Kelly and Taylor. 2009). This layer has a protective role against most foreign substances and fluid loss from the body. Most of the cells in the epidermis are keratinocytes that originate from basal layer, which is the deepest layer of the epidermis. The skin color is relevant to the melanocyte cells, which are located in epidermis as well (Kelly and Taylor. 2009).

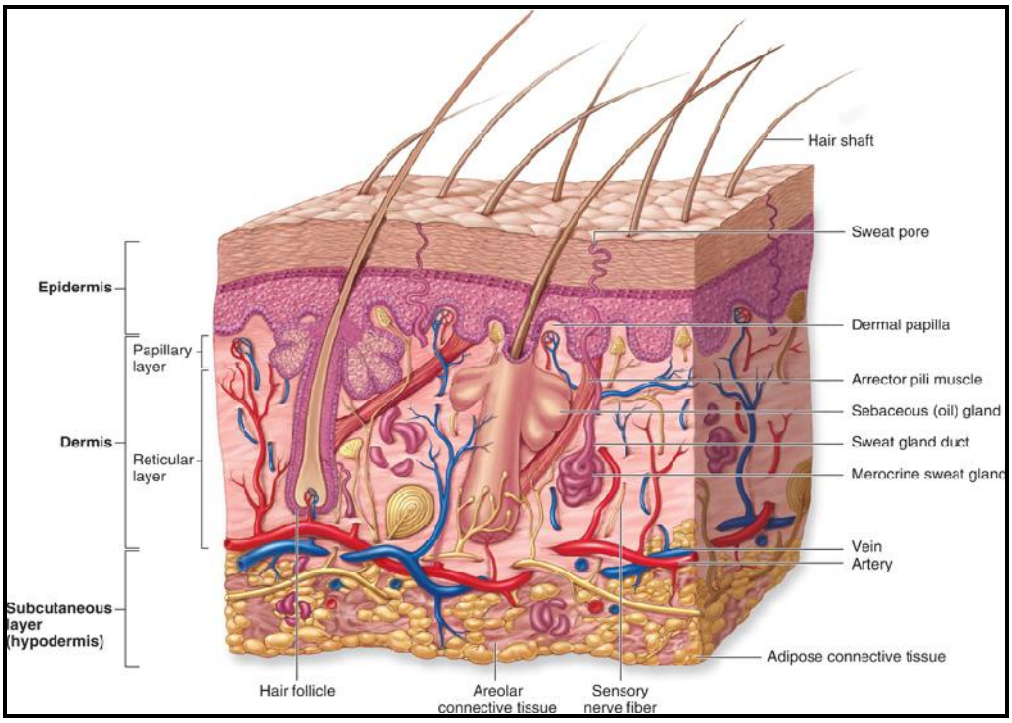

Figure 2.1 Structural layers of skin - epidermis, the dermis and subcutis (adapted from http://bioserv.fiu.edu/~walterm/FallSpring/Integument/integument_ex1_review.htm)

Dermis, the middle layer of skin contains blood and lymph vessels, hair follicles, sweat glands, collagen bundles, fibroblasts and nerves. Dermis has a vascular structure composed of collagen, elastin, and ground substanc, and various glands. Dermis cells originate from primitive mesenchymal cells, including fibroblasts and several other cells such as histiocytes, mastocytes, lymphocytes, plasma cells, and eosinophils. Fibroblast cells produce collagen, elastin, and matrix. Dermis is known with multinucleated and large fibroblasts cells. Black ethnicities have shown the highest rate of keloid and interestingly have larger fibroblasts with more than two nucleuses in the skin (Montagna and Carlisle, 1991).

It has been known that there is a difference between structure and the function of the skin among various ethnicities (Kelly and Taylor, 2009). Most of studies had their focus on the thickness, density and compactness of the stratum corneum between the different races (Table 2.1). Difference in the skin structure and consequently susceptibility to the skin disorders could be the result of difference in both genetic and environmental factors. In the following table 2.1, the major differences between dermal structures of black and white skin have been summarized.

Table 2.1 Comparison of dermal structures between Black and White skins

	White skin	**Black skin**
Dermis	Thin and less compact	Thick and compact
Papillary and reticular layer	More distinct	Less distinct
Collagen fiber bundles	Large	Small, close stacking
Fiber fragments	Sparse	Prominent and numerous
Melanophages	Few	Numerous and larger
Lymphatic vessels	Moderate, dilated	Dilated empty channels
Fibroblasts	Few	Numerous and larger, binucleated and multinucleated
Elastic fibers	Several, elastosis	Few, elastosis uncommon
Superficial blood vessel	Sparse to moderate	Numerous, mostly dilated
Glycoprotein	Variable	Numerous in the dermis

(Adapted from Kelly and Taylor. 2009)

In addition, dermis is more susceptible to develop keloid (Taylor, 2002). Hence, keloid is called a benign dermal fibroproliferative tumor with plenty of extracellular matrix (ECM) proteins, which result in overabundance of

collagen formation (Bayat and McGrouther, 2006, Kose and Waseem, 2008). Hence, keloid is also called as collagenous tumors of the dermis that form during a prolonged wound-healing process, because, large amount of scar tissue grow out from the wound site, and consequently cause an increase of collagen production and decrease in collagen lysis.

The subcutis is the deepest layer of skin, which contains a network of collagen and fat cells. It has a mechanical and thermal protection role and serves as energy storage. During skin injury, both skin cells and fibroblast cells begin multiplying to repair the damage. Fibroblast cells provide a network for skin cells to migrate to the injured area to repair the wound (Singer and Clark, 1999). There is a balance between proliferation of fibroblast and skin cells in repairing the wound. In normal skin, the skin cells proliferate faster than fibroblast cells and the wound will be repaired as usual. In keloid after wound healing, fibroblast cells continue to replicate and form a large scar tissue around the wound (Singer and Clark, 1999). In general, keloid forms if wound-healing processes become prolonged. Alterations in the cellular signals that control growth and proliferation could be the related to the process of keloid formation. It has been known that several growth factors including epidermal growth factors (EGF), transforming growth factors (TGF) and their receptors and downstream proteins play a critical role in keloid formation.

2.2 Wound healing and the role of *TGFB1*

Wound healing has been known to be a dynamic, interactive process, in which several factors such as soluble mediators, blood cells, extracellular matrix, and parenchymal cells are involved (Singer and Clark, 1999).

In addition, three overlapping phases of inflammation, tissue formation (proliferation) and tissue remodeling has been characterized in wound healing.

Immediately after injury, coagulation system triggers a cascade of downstream processes that result in inflammation such as clotting cascade, vasoconstriction and vasodilation, polymorphonuclear neutrophils, macrophages, and decline of inflammatory phase (Singer and Clark, 1999). Without inflammation, wound healing never happens. During inflammation, the movement of plasma and leukocytes from the blood into the injured tissues increase (Figure 2.2). Innate immune system has a critical role in the initial stages of inflammation. Coagulation process is a critical part of body homeostasis in which injured blood vessels covered by a platelet and fibrin-containing clot to stop bleeding, and begin repair of the damaged vessel and subsequently wound healing. A complex interplay between several growth factors and mediators trigger this process. Platelets secrete several mediators of wound healing, such as platelet-derived growth factor (PDGF) and TFGβ1 that activate the fibroblast cells (Singer and Clark, 1999). In addition, activated macrophages secrete several growth factors such as TGFβ1, TGFA, FGF, PDGF and VEGF in order stimulate the fibroblast cells of dermis and wound vascularization (Figure 2.2). Macrophage and monocyte cells play a pivotal role in inflammation, such that their secreted growth factors are most important in wound repair. Macrophage depleted animals show an impaired wound healing (Leibovich and Ross, 1975). Inflammation stage, *TGFβ* family members are mainly secreted by macrophages and platelets, in which *TFGβ1* and 2 play a role in epidermal-cell motility, chemotaxis of macrophages and fibroblasts, extracellular matrix synthesis and remodeling, while *TGFβ3*, that is mainly

secreted by macrophage cells play an anti-scarring role (Singer and Clark, 1999). Approximately two or three days after injury, before ending up the inflammation phase, wound re-epithelialization (proliferation) phase begins with entry of fibroblast cells into the wound site (Falanga, 2005) (Figure 2.3).

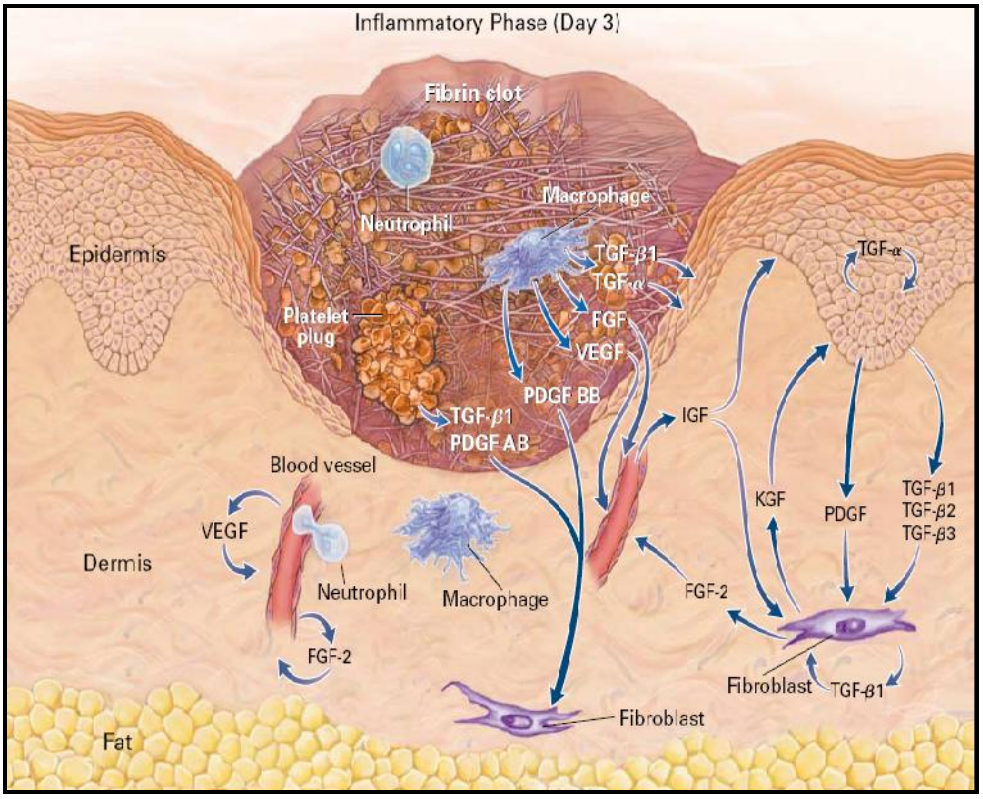

Figure 2.2 Inflammatory phase of wound in day 3 which has been triggered by several cytokines (Adapted from Cutaneous wound healing. Singer and Clark, 1999)

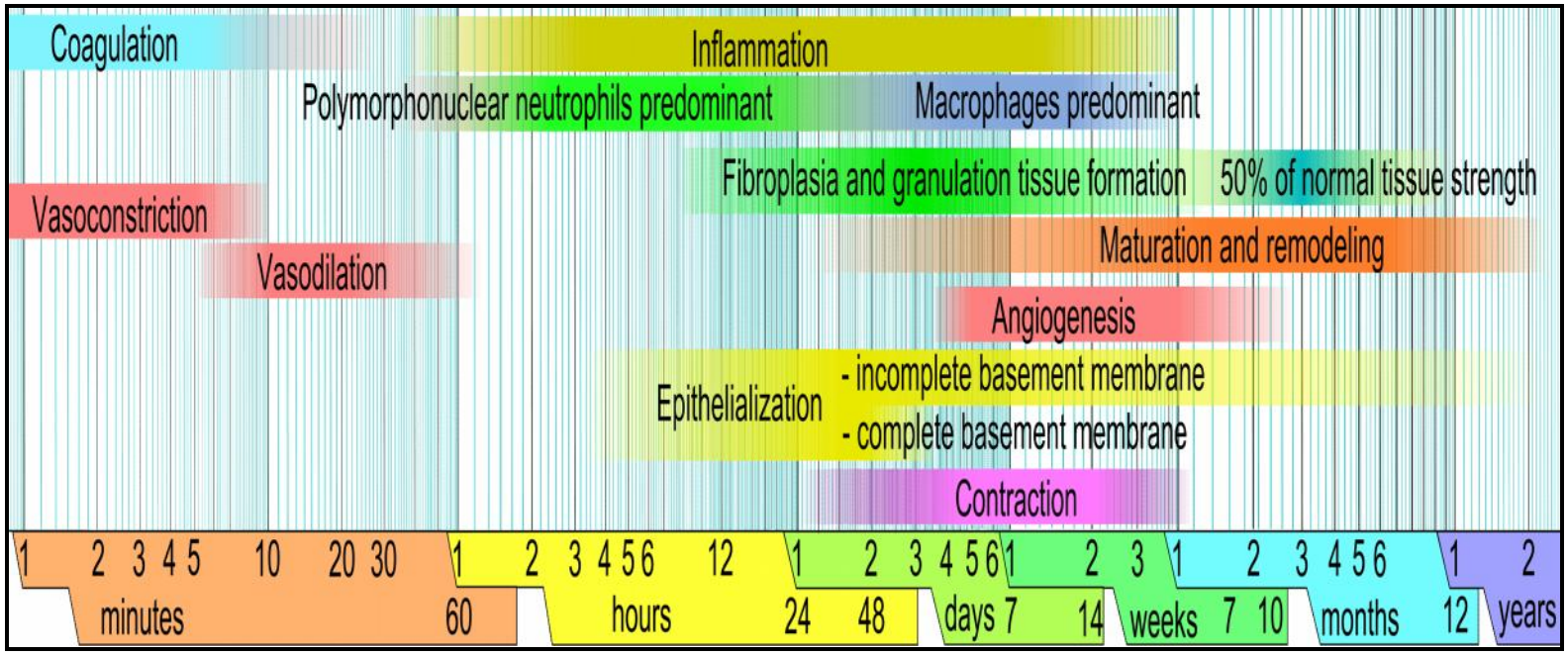

Figure 2.3 Approximate times of the different phases of wound healing

(Adapted from http://en.wikipedia.org/wiki/Wound_healing#cite_note-7)

This phase also includes several overlapping stages such as angiogenesis, fibroplasia and granulation tissue formation, collagen deposition, epithelialization and contraction (Figure 2.3). Cell migration, proliferation and angiogenesis are major processes of this phase; therefore digestion of clot is necessary to allow cells to migrate. Hence, several proteinases such as collagenases [collagenase 1 (MMP-1), gelatinase A (MMP-2), stromelysin 1 (MMP-3), and collagenase 3 (MMP-13)] and plasminogen activator [urokinase-type plasminogen activator (u-PA) and tissue plasminogen activator (t-PA)] are needed to digest basement membrane and ECM (Figure 2.4). Most of these enzymes such as plasminogen activator, collagenases, gelatinase A, and stromelysin secreted by fibroblast cells facilitate the cell migration (Singer and Clark, 1999).

Angiogenesis occurs along with fibroblast proliferation. When the migration of cells facilitates, fibroblast cells enter into the wound site and at the end of first week, fibroblasts are the major cells in the wound site which is called fibroplasia (Stadelmann *et al.,* 1998). Growth factors, especially PDGF and *TGFβ*1 concurrently with extracellular matrix molecules are important to stimulate fibroblast proliferation from adjacent tissue towards the wound area (Adam *et al.,* 1999). Collagen production is one of the important roles of fibroblast cells in this stage of wound healing and subsequently, the temporary extracellular matrix gradually substitutes with a collagenous matrix, perhaps as a result of the action of *TGFβ*1 (Singer and Clark, 1999).

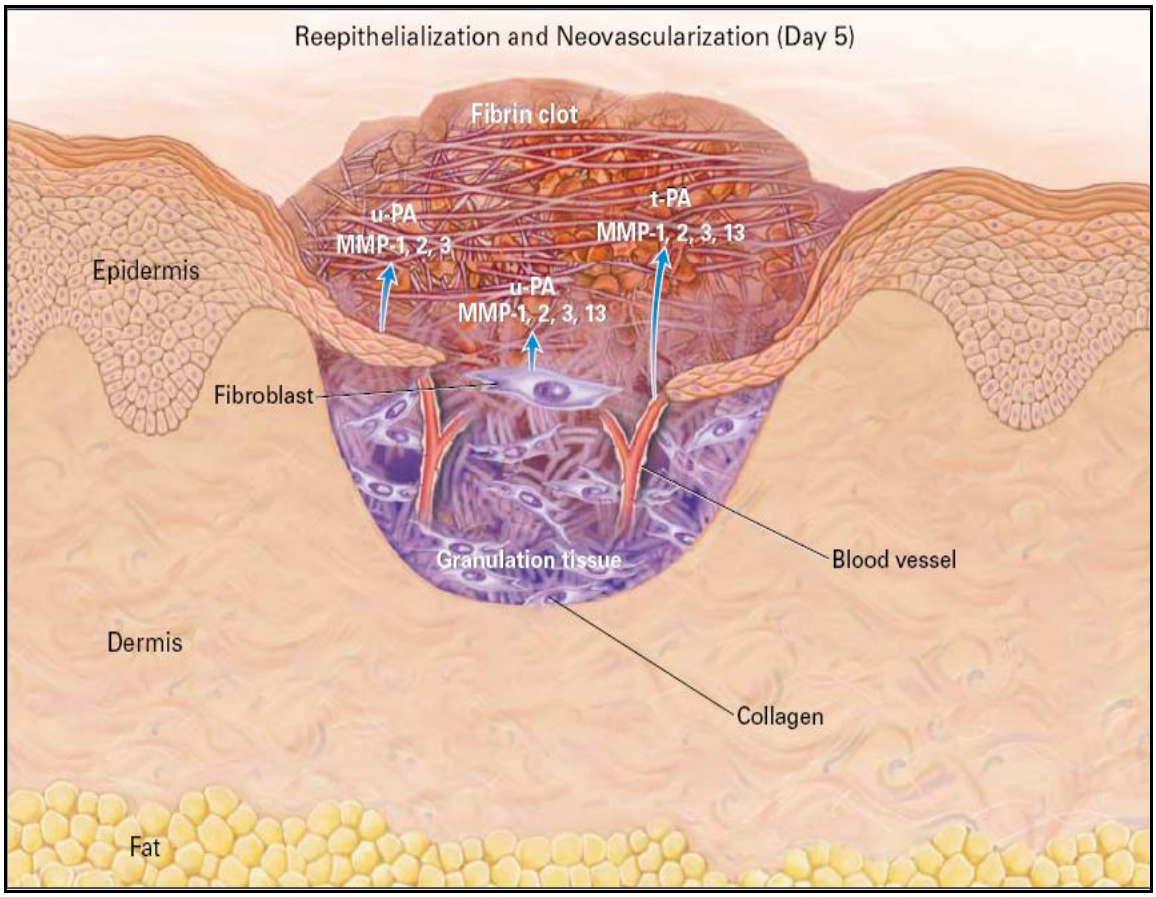

Figure 2.4 Pathway and major molecules that are involved in re-epithelialization and neovascularization at day 5. (Adapted from Cutaneous wound healing. Singer and Clark, 1999)

With enough amount of collagen, fibroblast cells stop collagen production and cells undergo apoptosis. Cell apoptosis disruption at this stage occurs in fibrotic disorders such as keloid (Singer and Clark, 1999).

Fetal skin reepithelializes more quickly than adult skin and fetal wound never produces scarring (Singer and Clark, 1999). Fetal skin is enriched with metalloproteinases and is poor in *TGFβ*1 (O'Sullivan *et al.*, 1996, Bullard *et al.*, 1997). In addition, evidence showed that down regulation of *TGFβ*1 reduces the scarring in adult rats (Shah *et al.*, 1995) and adding *TGFβ*1 to the fetal skin stimulates the development of scarring (O'Sullivan *et al.*, 1996). Therefore, lack of scarring in fetal skin has been attributed to the small amounts of *TGFβ*1 in the fetal skin (Singer and Clark, 1999).

2.3 Keloid and hypertrophic scar

Keloid and hypertrophic scars are most common fibrotic disorders which are due to extra production of collagen within the wound. Keloid and hypertrophic scar disorders occur usually after trauma, inflammation, surgery and burns. There are significant differences at both clinical and histological level between hypertrophic and keloid scars (Tables 2.2 and 2.3). Hypertrophic scars are confined to the wound area and never extend from the wound site. While in contrast, keloid scars are not restricted to the wound site and grow around the wound area (Plates 2.1 and 2.2).

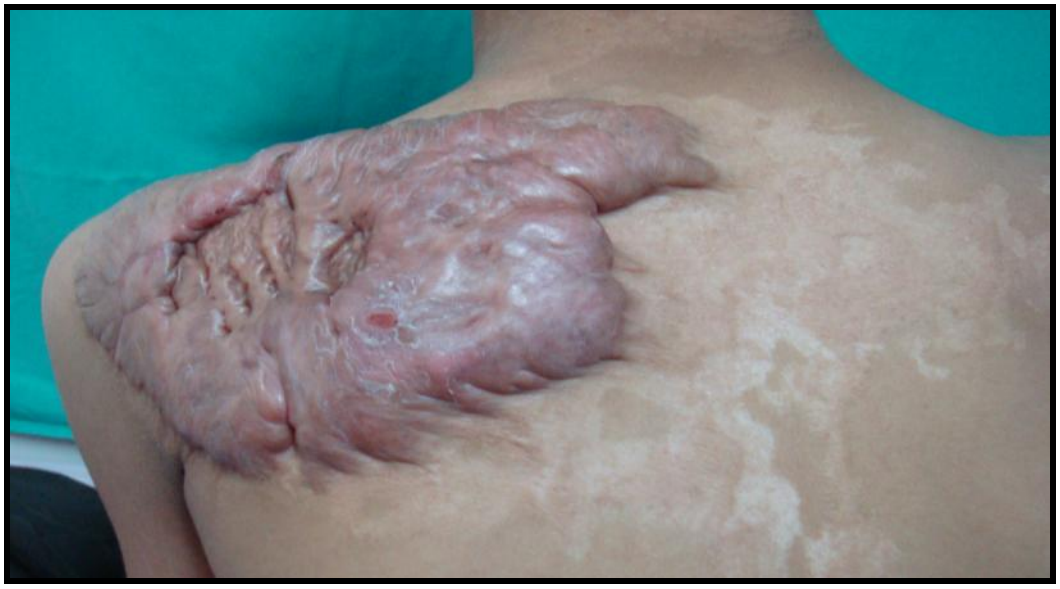

Plate 2.1 Keloid (Photo with permission, see appendix B, attachment 2)

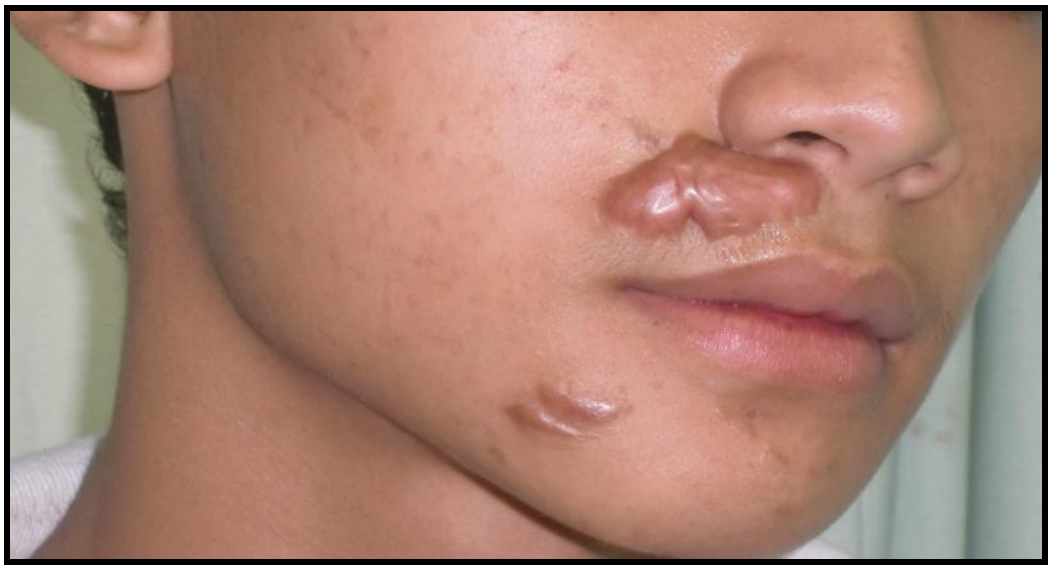

Plate 2.2 Hypertrophic scar (Photo with permission, see appendix B, attachment 2)

Table 2.2 Clinical differences of keloid and hypertrophic scars

Hypertrophic Scars	**Keloid**
Develop 4-8 weeks after surgery	May develop up to several years after the injury
Usually regress over the time	Rarely regress with time
Do not extend beyond the initial site of injury	Spread outside the boundaries of the initial lesion
Occur when scars cross joints or skin creases at a right angle	Occur predominantly on the ear lobe, shoulders, sternal notch, rarely develop across joints
Improve with appropriate surgery	Often worsened by surgery
Frequent incidence	Rare incidence
Have no association with skin color	Associated with dark skin color
40% to 70% following surgery, up to 91% following surgery	6% to 16% in African populations
Mostly on shoulders, neck, presternum, knees and ankles	Mostly on anterior chest, shoulders, earlobes, upper arms and cheeks
Low recurrence rates after excision of the original hypertrophic scar	High recurrence rates following excision

Adapted from (Wolfram *et al.*, 2009, Gauglitz *et al.*, 2011)

Table 2.3 Histological features of keloid and hypertrophic scars

Hypertrophic scar	Keloid
Primarily fine, well-organized, wavy type III collagen bundles oriented parallel to epidermis surface with abundant nodules containing myofibroblasts and plentiful acidic mucopolysaccharide.	Disorganized, large, thick, type I and III hypocellular collagen bundles with no nodules or excess myofibroblasts.
Large extracellular collagen filaments	Pale-staining hypocellular collagen bundles with no nodules or excess myofibroblasts
Plentiful acidic mucopolysaccharides	Poor vascularization with widely scattered dilated blood vessels.
Proliferating cell nuclear antigen (PCNA)/p53-level/ATP expression low	PCNA/p53-level/ATP expression high

Adapted from (Gauglitz *et al.*, 2011)

In addition, keloid and hypertrophic scars have significant differences at histological level as well. From histological viewpoint, both hypertrophic and keloid scars contain a large amount of collagen (Gauglitz *et al.*, 2011).

Keloid and hypertrophic scars have shown an equal sex distribution (Gauglitz *et al.*, 2011). The frequency of hypertrophic scar is estimated within 40-70% after surgery and up to 90% after burn. (Deitch *et al.*, 1983, Lewis and Sun, 1990) The genetic issue is more applicable for keloid and a study showed that approximately 50% of keloid patients had a positive family history of keloid (Bayat *et al.*, 2005a).

2.4 Evidence for the role of genetics in keloid

Several evidences have also shown that genetics play a critical role in keloid formation. Familial aggregation, occurrence in identical twins, Mendelian disorders, expression studies and high prevalence of keloid among different ancestries provide strong evidence in favor of genetic factors in keloid formation. Keloid is a complex condition in which several genetic and environmental factors could play a role in the disease formation (Figure 2.5). Evidence for the role of genetic factors emerged through several independent studies on populations with different ethnic backgrounds.

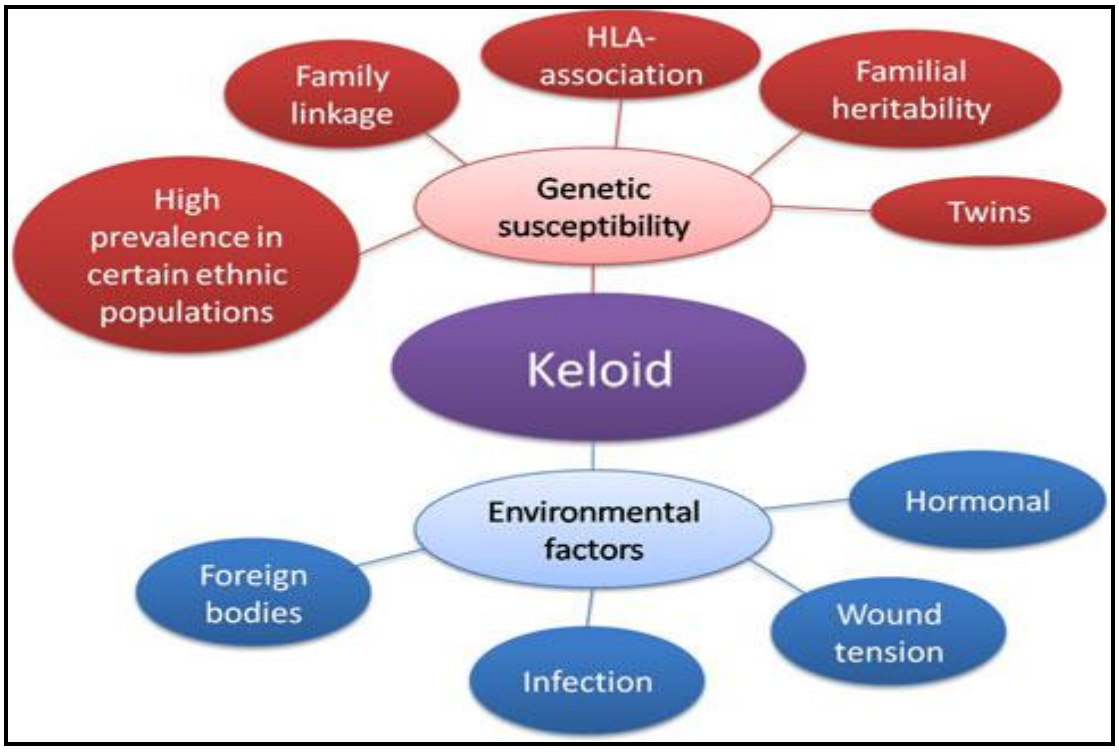

Figure 2.5 Genetic and environmental risk factors contributing to the development of keloid (Adapted from Shih and Bayat, 2010).

The incidence of keloid is different among populations reflecting different etiologic factors. Keloid is 5-15 folds more common among black people compared to white people (LeFlore, 1980). Keloid scar is the fifth most common skin disease in black adult patients in United Kingdom (Child *et al.*, 1999). Keloid is most common in Chinese population in Asia (Alhady and Sivanantharajah, 1969). Evidence for the role of genetic factors emerged through families that had higher frequency to develop keloid and identical twins and expression studies.

2.4.1 Evidence from families with keloid

Approximately one-third of keloid probands have first-degree relatives with keloid. Many reports have been published on cases with familial keloid (Bayat *et al.*, 2003b, Marneros and Krieg, 2004, Chen *et al.*, 2006b, Bella *et al.*, 2011) most probably reflecting the importance of genetic factors among these families. Occurrence of familial keloid was reported as 1.9% (19 out of 1000) among South Indians (Ramakrishnan *et al.*, 1974).

In an analysis conducted on fourteen families with familial keloid from different ethnicities including African American (n = 10), Japanese (n = 2), white (n = 1) and African Caribbean (n = 1), it was found to have an autosomal dominant mode of inheritance with incomplete penetrance and variable expression (Marneros *et al.*, 2001) .Among these, families with three to sixteen affected members have been seen. Bayat *et al.*, (2003a) described three cases with black Jamaican origin who had familial keloid scar. Furthermore, two large multigenerational families with Japanese and African-

American ancestries have been described in a genome-wide linkage study with an autosomal dominant inheritance of keloid (Marneros *et al.*, 2004). Another study reported familial keloid in six Han Chinese families that have shown autosomal dominant inheritance pattern with incomplete clinical penetrance and variable expression (Chen *et al.*, 2006a). Family history of keloid was reported in eight cases in a clinical examination of eleven Nigerian women with hypertrophic/keloidal lesions of the breast (Olasod, 2010). In another study, it has been reported to have autosomal recessive inheritance pattern in 34 Nigerian keloid families (Omo-Dare, 1975). More recently, 38 nuclear black families from Sudan have been reported who had familial keloid scars (Bella *et al.*, 2011).Keloid is a heterogeneous disease at both clinical and genetic level with a variable clinical expressivity between families and within the affected members of same family (Marneros *et al.*, 2001, Marneros *et al.*, 2004, Bella *et al.*, 2011).In general, an autosomal dominant mode of inheritance with incomplete penetrance and variable expressivity is well reported for keloid.

2.4.2 Evidence from identical twins with keloid

The presence of identical twins with keloid strongly supports the role of genetic risk factors in keloid formation. Marneros *et al.*, (2001) found four families with identical twins. All twins, developed keloids with a dominant inheritance pattern in African American population. Ramakrishnan *et al.* (1974) and Jacobson (1948) described a pair of twins with keloid formation in South India.

2.4.3 Evidence from Mendelian disorders with keloid

Several Mendelian disorders manifest keloid as part of clinical features. There is a possibility that individuals with a connective-tissue disorder develop keloid as part of the disease (Table 2.4). Almost all Mendelian syndromic forms of keloid have shown a dominant mode of inheritance which is consistent with the mode of inheritance among families with non-syndromic keloid (Table 2.4).

Rubinstein-Taybi syndrome1 (RSTS1) develop keloid in a high frequency. RSTS1 is caused by a contiguous gene deletion involving the CREBBP gene as well as other neighboring genes on the chromosome 16p13.3 (OMIM # 180849). In a questionnaire-based study on 61 adults with RSTS ranging in age from 18 to 67 years, Stevens *et al.* (2011) found that 57% of patients developed keloid. Siraganian *et al.* (1989) also detected keloid in 28 patients out of 574 individuals with RSTS. High incidence of keloid as a proliferative disorder and neoplasms in RSTS patients attributed to the function of CREBBP in cAMP-regulated cell immortalization (Petrij *et al.*, 1995).