Molecular Genetic Studies of Alopecia Disorder in Pakistani Families

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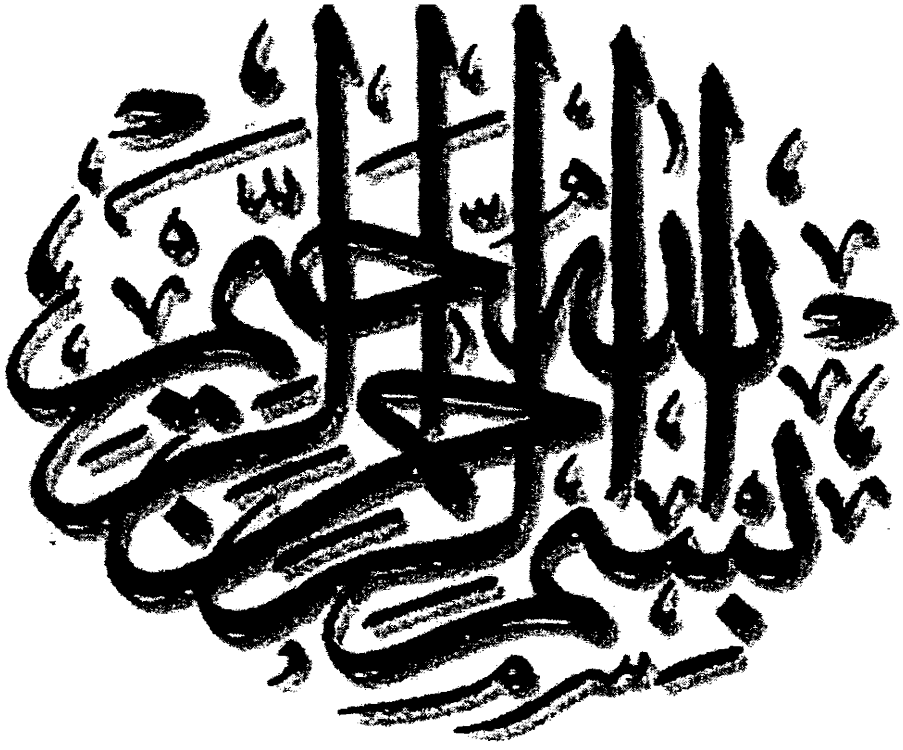

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FINAL APPROVAL

It is certificate that we have read the thesis submitted by Mr. Muhammad Saad Ahmed and it is our judgment that this project is of sufficient standard to warrant its acceptance by the International Islamic University, Islamabad for the MS Degree in Biotechnology.

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He is the One, Who created the heavens and the earth, truthfully. Whenever He says, "Be" it is. His word is the absolute truth. All sovereignty belongs to Him the say the horn is blown. Knower of all secrets and declarations, He is the Most Wise, the Cognizant.

(Al-Quran 6:73)

DEDICATIONS

"Truly my prayer and my service of sacrifice, my life and my death, are (all) for Almighty Allah, theabb (Cherisher and Sustainer) of the Worlds"

(Qur'an, 6:163)

"Our Rabb (Cherisher and Sustainer)! Accept (this service) from us. Verily, You are the All-Hearing, the All-knowing."

(Qur'an, 2:127)

This minor piece of my research work is dedicated to Almighty Allah and His Prophet Hazrat Muhammad (PBUH).

DECLARATION

I hereby declare that the work present in the following thesis is my own effort, except where otherwise acknowledged and that the thesis is my own composition.

No part of the thesis has been previously presented for any other degree.

Date: 2012-11-04

Muhammad Saad Ahmed

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All praises for the Supreme Almighty "Allah" The all Knowing, The most Merciful, and The most Mighty. Who, created the beautiful biological living and non-living world and Almighty Allah, offers the man to unveil the legends of the nature by donating the eternal judgement, inspect the things around them, and choose the path of Holy Prophet Hazrat Muhammad (PBUH) for the guidance of humanity. All regards, respects and blessings be upon the Holy Prophet Hazrat Muhammad (PBUH), whose teachings and blessings flourished my thoughts and thrived my ambitions.

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Muhammad Saad Ahmed

LIST OF ABBREVIATIONS

AA	Alopecia Areata
AD	Autosomal Dominant
AGA	Androgenetic Alopecia
AH	Autosomal Recessive Hypotrichosis
APL	Atrichia with Papular lesions
APMR	Alopecia with Mental Retardation
AR	Autosomal Recessive
AR	Androgenetic Receptor
ARHS	Autosomal Recessive Hypotrichosis Simplex
AT	Alopecia Totalis
AU	Alopecia Universalis
BOS	Board of Study
bp	Basepair
°C	Centigrade
CA	Congenital Atrichia
CDH	Cadherin
cDNA	Complementary Deoxyribonucleic Acid
CDSN	Corneodesmosin
cm	Centimeter
cM	Centimorgan
DNA	Deoxyribonucleic Acid

dNTP	Deoxynucleoside Triphosphate
Del	Deletion
DCS	Desmocollin
DSG	Desmoglein
ED	Ectodermal Dysplasia
EDTA	Ethylenediamine Tetra Acetic Acid
FLG	Filaggrin Gene
HDACs	Histone deacetylases
HF	Hair Follicle
HGMD	Hypotrichosis with Juvenile Macular Dystrophy
HNP	Human Nude Phenotype
HR	Hairless
HS	Hypotrichosis Simplex
HSS	Hypotrichosis Simplex of the Scalp
IRB	Institutional Review board
kb	Kilo basepair
KTH	Khyber Teaching Hospital
LAH	Localized Autosomal Recessive Hypotrichosis
LIPH	Lipase-H
LEKTI	Lymphoepithelial Kazal-type 5 (gene)
LPAR6	Lysophosphatidic Acid Receptor 6
MAD	Matrix-associated deacetylase
mA	Milli Ampere

mg	Milligram
ml	Millilitre
mM	Milli Molar
MR	Mental Retardation
MUHH	Marie Unna Hereditary Hypotrichosis
NCBI	National Centre for Biotechnology Information
ng	Nanogram
NS	Netherton Syndrome
OMIM	Online Mendalian Inheritance in Men
PCR	Polymerase Chain Reaction
PK	Proteinase Kinase
P2RY5	Purinergic Receptor P2Y, G Protein-Coupled
rpm	Revolution Per Minute
SDS	Sodium Dodecyl Sulfate
SNP	Single Nucleotide Polymorphism
SPI	Serine Protease Inhibitor
SPINK5	Serine Protease Inhibitor kazal type-5 (gene)
TE	Tris-EDTA
TEMED	N, N, N′, N′-Tetramethylethylene-diamine
uORF	Upstream Open Reading Frame
µM	Micro Molar
µl	Micro Litre
WHN	Winged Helix Nude

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ABSTRACT

A plethora of genetic disorders affects humans. Alopecia is also one of such genetic disorders. Collectively distinct human hair loss forms are recognized as Alopecias, which probably represent a dysregulation of hair growth. All hair disorders are normally hair follicle disorders. The hereditary alopecias are having great biological interest because they can normally be connected to a particular malfunctioning gene product. In less than a decade, the genetic basis of almost 15 monogenic forms of inherited hairs loss has been clarified. Partial or total loss of hairs occurs, either alone or in association with other aberrations. The inheritance pattern of alopecias is normally autosomal recessive, but families with X-linked recessive and autosomal dominant inheritance has been observed. Several genes have been recognized in which the mutations are responsible for the human hair loss. These genes encode structural proteins, transcription factors and associated molecules that are mainly concerned with hair follicle differentiation and its cycling.

In the present study, three Pakistani families (A, B, C) having isolated type of hereditary alopecia were recruited to find out genetic causes for alopecia. Affected individuals in two families i.e. A and B have no hairs on the scalp and other parts of body i.e. alopecia universalis while we observed phonotypical features of hypotrichosis in family C.

So far, 43 different mutation of HR gene have been recognized and it has been cleared that HR gene plays a main role in alopecia disease. The sequence analysis of the HR gene in the affected and normal individuals of family A reveals no functional pathogenic sequence variant. This predicts that the mutation may lie in the regulatory region of HR gene. Complete Exome sequencing is required to find the genetic cause in this family.

In family B, sequence analyses of the HR gene show us the missense novel mutation in exon 3 at position 285 (GsubstA). The substitution mutation changes the glycine to serine.

In family C, the affected individuals have autosomal recessive hypotrichosis for which the responsible gene to be reported is P2RY5/LPAR6. The sequence analyses of the P2RY5/LPAR6 gene show us the substitution mutation in exon 2, at position 565 (GsubstA). This specific missense mutation was previously reported in other family as well.

Identification of novel mutation in family B and subsequent characterization of proteins they encode will further increase our knowledge of molecular mechanisms underlying the disease. This and further studies in this field will help getting rid of such genetic anomalies and securing human beings.

1. INTRODUCTION

A plethora of genetic disorders affects humans. Some of which are very common and few are very rare. Scientists are in continuous efforts to find cures for these disorders. Alopecia is also one of such genetic disorders. The word "Alopecia" is derived from Greek language meaning "hair loss or baldness". Collectively distinct human hair loss forms are recognized as Alopecias (Green and Sinclair, 2000) which probably represent a dysregulation of hair growth that show a discrepancy in onset age, severity and interconnected ectodermal deformities. All hair disorders are normally hair follicle disorders, as hair formation and growth takes place in hair follicle structure.

The hereditary alopecias are having great biological interest because they can normally be connected to a particular malfunctioning gene product, which gives insight into the convoluted mechanisms of the epithelial mesenchymal interactions comprising but not restricted to hair follicle proliferation, differentiation, morphogenesis, maintenance and cycling. These interactions are the reasons, because of the effects of more than hundred genes. OMIM (Online Mendalian Inheritance in Men) have greater than 300 heritable diseases that having a prominent feature of hair loss. Knowledge regarding molecular mechanism of hair follicle morphogenesis in men and rats has drastically enhanced and the recent years have witnessed remarkable advances in our understanding of congenital alopecia. In less than a decade, the genetic basis of almost 15 monogenic forms of inherited hairs loss has been clarified (McKusick, 2000).

Partial or total loss of hairs occurs, either alone or in association with other aberrations as some of abnormalities are of very diverse nature. The main known aberrations associated with partial or total hair loss comprise of nail dystrophy, mental retardation, epilepsy, impaired sweating, cataracts and retinas pigmentation (Pinheiru and Freire, 1985; Feinstein *et al.*, 1987; John *et al.*, 2006a; Wali *et al.*, 2006b, 2007). The inheritance pattern of alopecias is normally autosomal recessive, but families with X-linked recessive and autosomal dominant inheritance has also be observed from the study (Ahmad *et al.*, 1993; Anzari *et al.*, 1996; Ahmad *et al.*, 1998a; Kljuic *et al.*, 2003a; Wali *et al.*, 2007).

1.1 Skin

The body's largest organ is skin that makes up at least 6% of an individual's total body weight, which comprise of three layers, epidermis, dermis and subcutaneous layer (Fig. 1.2). The outermost layer of skin is the epidermis comprised of mainly Merkel, keratinocytes, melanocytes and langerhans cells. Cells of the epidermis are nourished through diffusion from the blood capillaries present beneath the epidermis in the deepest layers, as the epidermis is devoid of blood vessels. Starting with the outermost layer of epidermis, it can be further divided into stratum corneum, stratum lucidum (only in palms and bottoms of feet), stratum granulosum, stratum spinosum and stratum basale (Fig. 1.1) (Matoltsy, 1958).

The second layer dermis is structurally divided into papillary region, region adjacent to the epidermis and the reticular region. This reticular region is thicker and is composed of irregular connective tissue, sweat glands, blood vessels, receptors and sebaceous glands (Fig. 1.2). The subcutaneous layer is below the dermis and contains fats (Fig.1.2) (Proksch, 2008; Madison, 2003; Stücker, 2002).

1.1.1 Hair, its Structure and Function

Hair is multipart tissue whose biology and structure is less understood. It is a heterogeneous fibre comprised of keratinized cells stick to the cell membrane complex, present almost all over the body surface, except on the soles, palms, the side of toes and fingers, nipples, clitoris and the glans penis. The "Keratin" protein that makes the hair is also responsible for the outer layer of skin and nails formation. Keratin accounts for 85% of the cellular proteins of dead cells and 30 % of the cellular proteins of all the living cells. By weight of the total hair fibre these protein comprises 65-95%. These keratin proteins are subdivided into two groups (i) hard keratin and (ii) soft keratin (Marshall and Orwin, 1991).

About 5 million hair follicles cover the body of an adult human of which 1 million cover the scalp (Shimomura and Christiano, 2010).

Each stand of hair consists of three layers: (Fig. 1.3b)

1) Cuticle: the outermost layer serves as the protector of the cortex.
2) Cortex: the middle layer provides colour, texture and strength to the hair.
3) Medulla: the innermost layer that is only present in large thick hair.

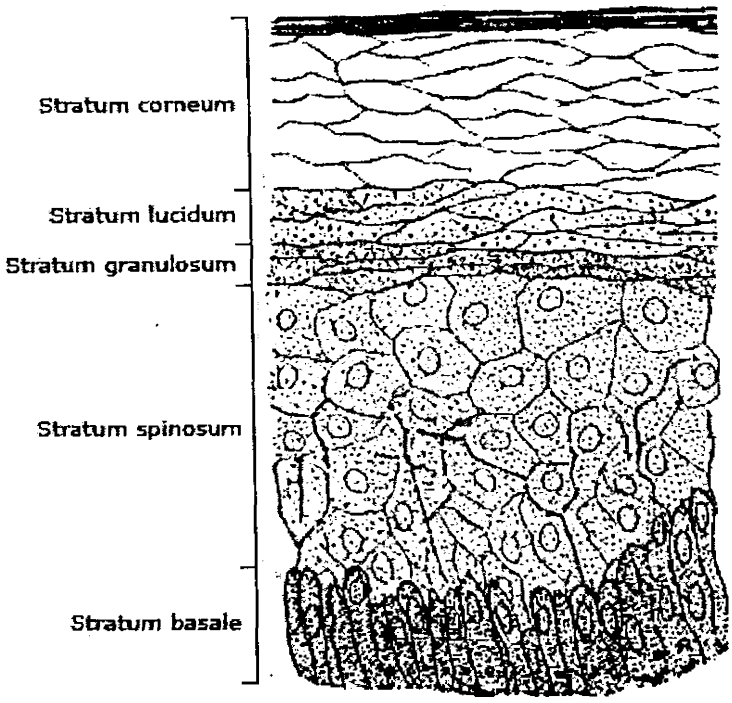

Figure 1.1 Epidermis of Skin layers in human

(From http://en.wikipedia.org/wiki/File:Skinlayers.png)

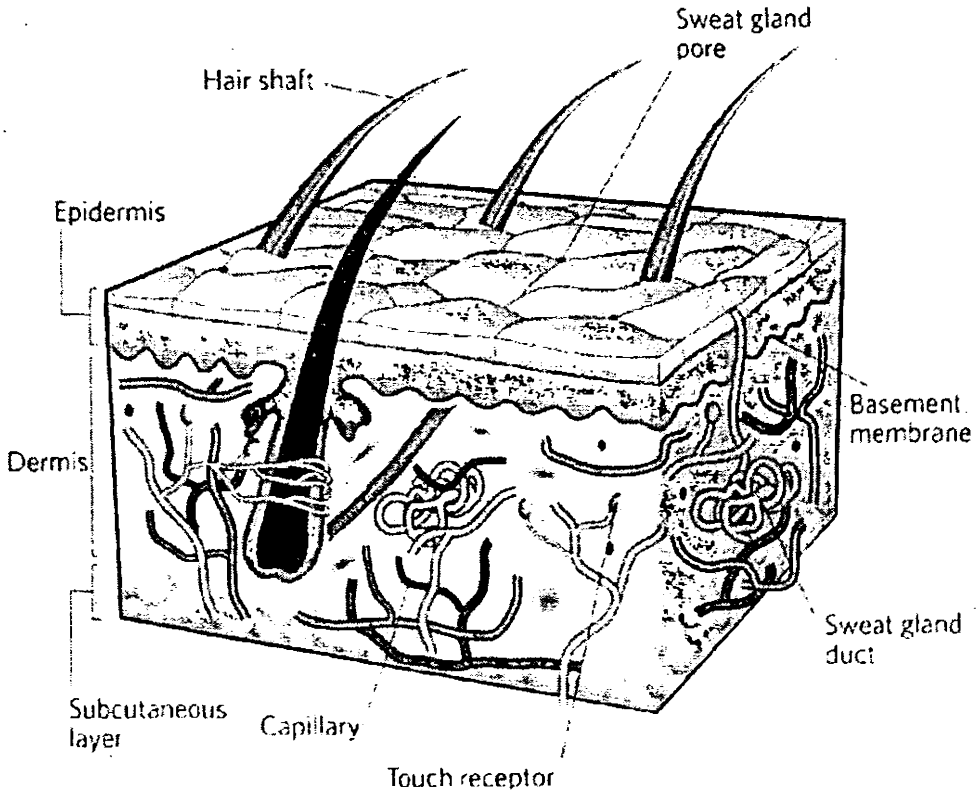

Figure 1.2 Structure of human skin. (MacNeil, 2007)

The 5-10 layers of the non-pigmented cuticle are accountable for high physical and chemical resistance. Hair grows in 3-5 mm down in the surface of skin, the hair follicles (Figure.1.3a). The matrix cells (melanocytes and keratinocytes), which is the germination centre present around the papilla. Dermal Papilla is considered, to control numeral matrix cells and is fed by blood stream for the nourishment of producing new hair. This is very vital for hair growth because it have the receptors for androgen and male hormones, which are responsible for hair growth regulation (Marshall and Orwin, 1991; Powell and Rogers, 1997). The bulge comprised of bunch of distinct cells in outer root sheath that are located near the insertion of the arrector of the pili muscle (Fig. 1.3a).Each hair bathed 2-3 days prior to reach the skin surface in an oily substance sebum, secreted by sebaceous glands, present in the upper part of the hair follicle. The sweat glands present in the dermis, wet the hair shaft (Fig. 1.3a) (Pragst *et al.*, 1998; Pecoraro and Astore, 1990). Melanocytes present at the ground layer in association with the basal layer are responsible for hair colour (Fig. 1.3c).

1.1.2 Hair Follicles and its Formation

Hair is the only organ in mammals that undergo lifelong cycles with rapid development. The hair follicle in its mature stage is convoluted structure and composed of concentric cylinders of epithelial cell, known as root sheaths that encircle the hair shaft (Sperling, 1991). Every hair follicle comprises of mesenchymal and epithelial parts and fluctuates noticeably in shape and size, depending on their area of persistence and they all have same basic structure. The hair follicles are always present in all phases from anagen to telogen, with principles that each follicles is not in a synchronous manner with its neighbouring follicles.

During embryogenesis, the hair follicle development depends on series of information between dermal cells and overlying surface epithelial cells, which results in cells population fate that results in dermal papilla, inner and outer hair sheaths, and hair shaft (Hardy, 1992). The first indication comes to the epidermis from the dermis to thicken, which form a placode, then a downward grown starts known as "hair plug". The upcoming indication comes from the epidermis instruct the dermis to make dermal papilla. This dermal papilla then stimulates the division of matrix cell in the hair plug. These cells divide quickly and distinguish into hair-shaft cell or internal root sheath cells (Figure 1.4).

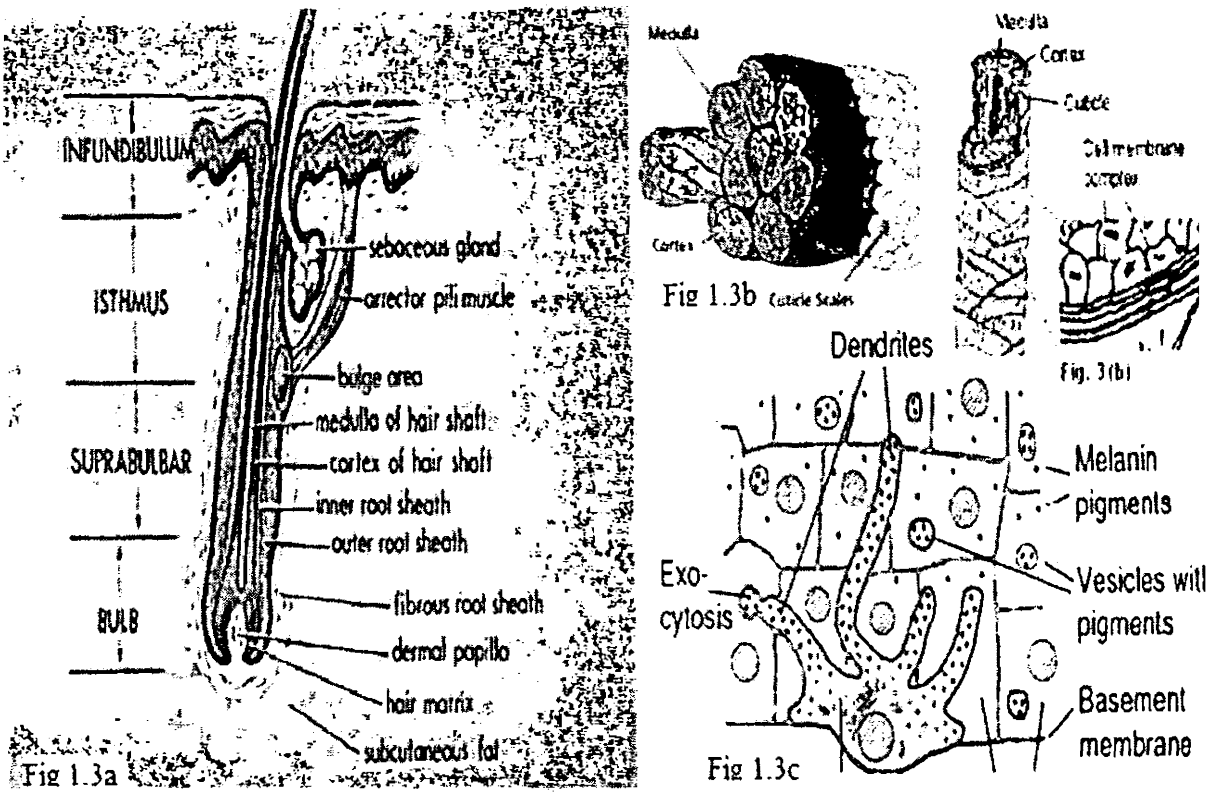

Figure 1.3 (a) Formation of hair in a follicle from matrix cells on the basement membrane to the mature hair shaft; (b) Structure and constituents of the human hair shaft; (c) Melanocytes (Pragst and Balikova, 2006)

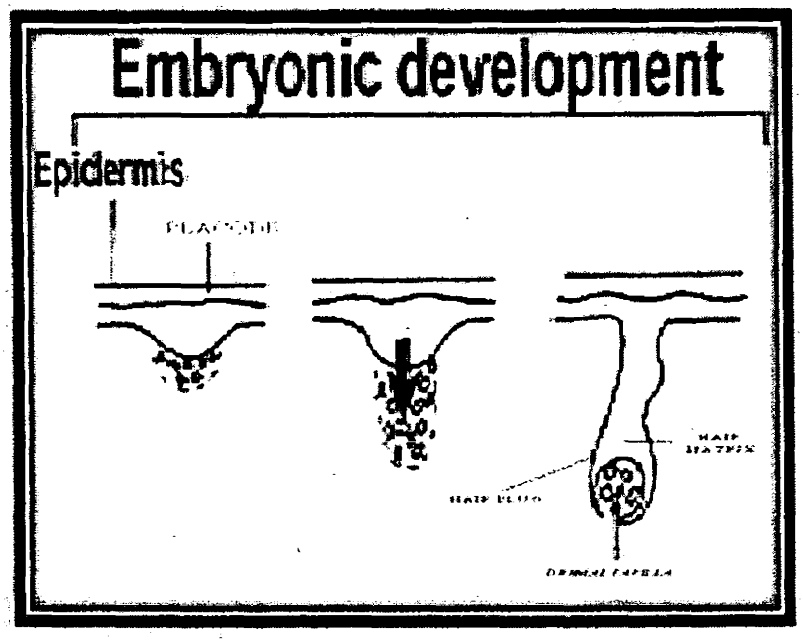

Figure 1.4 Embryogenesis (Adapted from Schneider *et al.*, 2009)

1.2 Types of Alopecias

Genetic conditions that affect human hair growth cycle or structure may come alone, or in combination with other abnormalities. Alopecias are of different types based on related disorders; syndromic alopecias and isolated alopecias or non-syndromic alopecias.

1.2.1 Syndromic Alopecias

Syndromic forms of alopecia shows hair loss condition in association with various clinical conditions i.e. nail dystrophy, mental retardation, epilepsy, impaired sweating, immunodeficiency, cataracts and retinas pigmentation. The inheritance pattern of these syndromic autosomal alopecia syndromes are both recessive and dominant type.

1.2.1.1 Alopecia With Mental Retardation (APMR) Syndrome

In APMR the affected individuals shows complete loss of scalp hairs, and in severe condition complete absence of full body hairs like eyebrows, eyelashes, axillary hair in association with mental retardation (mild to severe). However, the individuals have normal hearing and sweating (John *et al.*, 2006a; Wali *et al.*, 2006b, 2007).

1.2.1.2 Hypotrichosis Congenital with Juvenile Macular Dystrophy (HJMD)

For the first time in 1935, Wagner illustrated the relationship of congenital Hypotrichosis in association with macular dystrophy. Congenital Hypotrichosis with Macular Dystrophy is a rare disorder noticeable at birth where the patient having sparse and short hair. In the life's second decade, the patient gets progressive macular degeneration leads to almost complete blindness. The disease recognized to be caused by mutation in *cadherin 3 (CDH3)* gene, which spans 55kb on 16q22.1 chromosome comprising 16 exons.

1.2.1.3 Netherton Syndrome (NETH)

Netherton syndrome is a severe autosomal recessive disorder characterized by severe atopic manifestation, specific hair shaft defect, a specific hair shaft defect and congenital ichthyosis having deformative cornification (Netherton, 1958; Wilkinson

et al., 1964). Minors with this syndrome often don't develop life threatening complications, results in high postnatal mortality. Scalp hair is brittle and sparse and through microscopy, it specifies that the hair have nodes. Through homozygosity mapping and linkage analysis with Netherton syndrome in 20 families, Chavanas *et al.*, (2000) mapped the disease locus on 5q32 chromosome.

1.2.1.4 Human Nude Phenotype (HNP)

The Human Nude Phenotype is a recessive phenotype distinguished by severe immunodeficiency and congenital absence of hair. The patient in this disease having incomplete hair shafts and follicles, and the hair follicles are present in normal numbers (Koepf-Maier *et al.*, 1990). Mutation at the 'nude' locus of rats and mice interrupt thymus development and normal hair growth.

The human homologue of the nude mouse shares the characteristics of no eyelashes, no eyebrows, absence of hairs on full body and scalp and severe immunodeficiency that is quite lethal in some cases (Frank *et al.*, 1999).

Table 1.1 Different types of syndromic Alopecia, the genes responsible and the position/Loci

S/N	TYPES OF SYNDROMIC ALOPECIA	GENES RESPONSIBLE	POSITION/LOCIs	REFERENCES
1	Alopecia with Mental Retardation (APMR)	No genes yet identified	3q26.2-q26.31, 3q26.33-q27.3, 18q11.2-q12.2	John *et al.*, 2006a; Wali *et al.*, 2006b
2	Hypotrichosis Congenital with Juvenile Macular Dystrophy (HJMD)	*Cadherin 3* gene *(CDH3)*	16q22.1	Kjaer *et al.*, 2005; Indelman *et al.*, 2002
3	Netherton Syndrome (NETH)	*SPINK5* gene	5q31-q32	Magert *et al.*, 1999; Chavanas *et al.*, 2000
4	Human Nude Phenotype (HNP)	*WHN* gene, *Foxn1* gene	17q11-q12	Frank *et al.*, 1999; Adriani *et al.*, 2004

Table 1.2 Different types of Isolated Alopecia, the genes responsible and the position/Loci

S/N	TYPES OF ISOLATED ALOPECIA	GENES RESPONSIBLE	POSITION/LOCIs	REFERENCES
1	Monilethrix	*hHb3* gene, *hHb6* gene	Substitution of glu407lys	Horve *et al.*, 2003; Van Steensel *et al.*, 2005
2	Congenital Atrichia	*Murine hr* gene, *human HR* gene	Mouse chr 14, 8p21-p22	Cichon *et al.*, 2006; Betz *et al.*, 2007
3	Hereditary Hypotrichosis	---	---	---
3 (i)	Hypotrichosis Simplex		18p11.32-p11.23	Baumer *et al.*, 2000
3 (i) a	Generalized Hypotrichosis	No genes yet identified	No Locus identified	---
3 (i) b	Hypotrichosis simplex of the scalp	*Corneodesmosin (CDSN) gene*	6p21.3	Levy-Nissenbaum *et al.*, 2003; Davalos *et al.*, 2005

3 (i) c	Localized Autosomal Recessive Hypotrichosis	*desmoglein gene complex, LIPH* gene	18q12.1, 3q26.33-q27.3, 13q14.11-q21.32	Kazantseva *et al.*, 2006; Ali *et al.*, (2007); Wali *et al.*, (2007)
3 (ii)	Marie Unna Hereditary Hypotrichosis	*MUHH* gene	8p21, 1p21.1-1q21.3	He *et al.*, 2004; Yang *et al.*, 2005
4	Androgenetic Alopecia	*AR* gene	Xq12	Levy-Nissenbaum *et al.*, 2005; Hillmer *et al.*, 2005;
5	Alopecia Areata	*FCRL3* gene, *Sox21* gene, *ULBP3* gene	HLA-A*02, 03, HLA-B*18, 27, 52, DRB1*0301, 1104, HLA-CW*0704, 6q25.1	Morling *et al.*,1991; Xiao *et al.*, (2005); DeAndrade *et al.*, 1999; Bettina, 2007; Lyn, 2009; Welsh *et al.*, 1994
5 (i)	Alopecia Totalis	The gene encoding the lymphoid protein tyrosine phosphatase	6q25.1	Kemp *et al.*, 2006
5 (ii)	Alopecia Universalis	*HR* gene	8p2l, 8p21-22	Ahmad *et al.*, 1998a; Nothen *et al.*, (1998)

1.2.2 Isolated Alopecias

The genetic studies of several forms of hypotrichosis or alopecia in inherited families led to the identification of novel genes/loci controlling hair growth. Several genetic defects recognized for the isolated alopecias. The inheritance pattern in isolated forms of alopecias is also observed as both autosomal recessive and dominant.

1.2.2.1 Monilethrix

It is a hereditary hair shaft defect in which, the affected individuals have normal hair at birth and within the first few months of life, develop brittle and fragile hair that tends to fracture and produce different degrees of dystrophic alopecia. In the severe condition, the disease involves eyelashes, eyebrows, secondary hairs and even the entire scalp may be affected. Monilethrix is normally autosomal dominant disorder and in some cases, autosomal recessive transmission has been observed (Salamon and Schnyder, 1962).

1.2.2.2 Congenital Atrichia (CA)

The congenital atrichia is an uncommon form of isolated alopecia having autosomal recessive pattern of inheritance. The affected individuals have the clinical features of shedding of normal scalp hair. Several families with this condition got the development of papular lesions as an extra phenotypic feature, which describes a related phenotype designated as atrichia with papular lesions (APL) (Ahmad *et al.*, 1998a; Panteleyev *et al.*, 1998). At two years of age, many follicular papules develop which spread over different parts of body. Patients having normal nails, hearing, teeth, sweating with normal growth and development (Ahmad *et al.*, 1998a; Paradisi *et al.*, 2003).

1.2.2.3 Hereditary Hypotrichosis (HH)

Hereditary hypotrichosis is an explanatory medical term for a variety of phenomena related to different thinning of scalp hair, without or with any gross abnormality of the hair shaft morphology or associated anomalies. The inheritance pattern of the hereditary hypotrichosis is autosomal dominant and in few cases autosomal recessive pattern observed. The unpredictability observed in clinical

characteristics like severity of the disease, age of onset, and involvement of eyelashes and eyebrows.

i) Hypotrichosis Simplex (HS)

The patient show short and sparse thin hair, in few cases long and less sparse hair been enunciated. This sparse hair only observed for the scalp and body and no abnormalities enunciated for the beard, eyebrows and eyelashes. The affected individuals having no other associated abnormalities like mental retardation.

Hereditary simplex is divided into main three types: **(a) Generalized Hypotrichosis;** (Universalis or Total) in which the hair loss defect, affects the whole body **(b) Hypotrichosis Simplex of the Scalp;** where the loss of hairs is only restricted to scalp and rest of body hairs are normal and **(c) Localized Hypotrichosis (LAH);** where hypotrichosis is limited to the specific parts of the body.

(a) Generalized Hypotrichosis

In this case the hair loss defect, affects the whole body.

(b) Hypotrichosis simplex of the scalp (HTSS)

HTSS is isolated type of alopecia and the inheritance pattern is autosomal dominant. Patients have normal hair in the childhood, which leads to total scalp hair loss in the first decade after birth. The teeth, nails armpit hairs, public hairs, beard, eyebrows and eyelashes all are normal.

(c) Localized Autosomal Recessive hypotrichosis (LAH)

LAH is a rare form of isolated alopecia, where hypotrichosis is limited to the arms, legs, chest and scalp. Eyelashes, eyebrows, beard, axillary and public hairs are meagre. Hairs are present on the head but after ritual shaving sparsely re-grow. Patients having normal teeth, nails, hearing, sweating and observe no deformation in growth or development.

ii) Marie Unna Hereditary Hypotrichosis (MUHH)

This diseases was first recognized by Marie Unna (Unna, 1925), a German Dermatologist. MUHH is autosomal, rare type of isolated alopecia whose inheritance

pattern is dominant. Affected individuals have abnormal hairs at the time of birth or short after birth that tends to proceed with increased hair loss; finally, in mature stage patients have varying degree of alopecia. The patients are normally born with no or little hairs and scratchy twisted hairs grown on the scalp in early childhood and are shed during the early stage of first decade of life. Axillary hairs, beard, body hairs and public hairs in men is sparse or absent. This MUHH is a genetically heterogeneous disorder.

1.2.2.4 Androgenetic Alopecia (AGA)

Androgenetic alopecia is androgen-dependant and a hereditary disease. In this, the patients get the progressive thinning of the scalp hair that leads to defined pattern normally in men got the M-shape on the scalp. In men the ordinary type of the early baldness has been thought to be autosomal dominant and in females autosomal recessive, who transmit the trait if heterozygous but are bald only if homozygous (Osborn, 1916; Snyder and Yingling, 1935). The other names of AGA in men are common baldness in men or male-pattern hair loss, pattern baldness in men and in females; it can be recognized as female-pattern hair loss or pattern baldness in females or female-pattern baldness. The prevalence of AGA is 50% in men by the age of 50 years and in the later stages, it leads up to 70 % in all men (Norwood, 1975). The prevalence of androgenetic alopecia assumed to be as 39.6% in women in one of the study in Iran, which is higher to world, reported data i.e. 20% (Fatemi *et al.*, 2010).

1.2.2.5 Alopecia Areata

Alopecia areata is an autoimmune, non-scaring and inflammatory hair loss of the scalp. It is one of the most common human autoimmune diseases. The prevalence rate of alopecia areata is ~2% of the total population with both sexes in all ethnic groups and at all ages (Green and Sinclair, 2000; McDonagh *et al.*, 2002; Gilhar and Kalish, 2006). It is characterized by patchy hair loss on the head, which in severe case leads to entire scalp hair loss, this condition known as "alopecia totalis" and in more severe case, it covers the entire body hair loss, recognized as alopecia universalis (Welsh and Guy, 2009). The patch of alopecia areata has a distinguishing border where ordinary hair demarcates the periphery of the lesion. The disease onset can be abrupt and its development is irregular.

i) Alopecia Totalis

It is a severe type of AA, in which the hairs from the full scalp removed and the eyelashes, eyebrows and all other body hairs like axillary hairs and public hairs all are normal. The onset of alopecia totalis can be sudden or it may come progressively because of AA, severe form of AA leads to alopecia totalis. The prevalence of alopecia totalis is one per million (1 in 100,000) (Robins, 2007; Letada *et al.*, 2007).

ii) Alopecia Universalis

It is the most severe type of AA in which the hairs from the full scalp and the eyelashes, eyebrows and all other body hairs like axillary hairs and public hairs all are absent. The onset of alopecia universalis can be sudden or in some cases, the onset of the alopecia universalis can to by birth or shortly after birth. The affected newborn babies have few sparse hairs at the crown of head, which fall off with in the few weeks and never re-grow. From the recent study, the scientist observed that 34 to 50 % of AA patients get back some or all of their hair within one year of onset on the other hand 15 to 25 % will progress to AT or AU (Tosti *et al.*, 2006). The prevalence rate of alopecia universalis is 0.001% (Robins, 2007; Letada *et al.*, 2007).

2. LITERATURE REVIEW

The genetic conditions that affect human hair loss or growth loss might be isolated or may be connected with associated abnormalities. The prominent abnormalities reported with partial or total absences of hair either alone or in combinations are Cataracts, Mental Retardation, Nail dystrophy, Dwarfism, Epilepsy, Immunodeficiency, Impaired Sweating, and Retinitis Pigmentosa (Pinheiru and Freire, 1985; Feinstein *et al.*, 1987). The syndromic alopecia pattern of inheritance is both recessive and dominant type. The syndromic alopecia patterns include alopecia with mental retardation syndrome, congenital hypotrichosis with macular dystrophy, Netherton syndrome and Human Nude Phenotype. The isolated form of congenital alopecia has been reported in familial and sporadic cases. The inheritance is normally autosomal recessive, but autosomal dominant families have also been recognized. Isolated forms of congenital alopecia include monilethrix, congenital atrichia, hereditary hypotrichosis, hypotrichosis simplex, Androgenetic Alopecia and Alopecia areata.

2.1 Netherton Syndrome (NS)

This skin disorder having severe autosomal recessive pattern of inheritance that is characterized by atopic manifestations, ichthyosiform erythroderma and hair shaft defect (trichorrhexis invaginata) (Netherton, 1958; Wilkinson *et al.*, 1964). Minors having NS normally have life-threatening problems that result in high postnatal mortality. Patients with this disease having brittle and sparse hair on the scalp and through microscopic examination it show that the hairs have nodes (bamboo hairs or trichorrhexis invaginata).

Lately, netherton syndrome was mapped to 5q32 chromosome on *Serine Protease inhibitor kazal type-5 (SPINK5)* gene's mutational defect on the same chromosome results in the loss of reduction of expression of a *serine protease inhibitor* (*SPI*) and in the (*SPI*) *(LEKTI) lymphoepithelial Kazal-type 5* (Chavanas *et al.*, 2000; Magert *et al.*, 1999; Sprecher *et al.*, 2001). This *LEKTI/SPINK5* gene encodes a pre-protein inhibitor, serine protease of 125 kDa having 15 potential inhibitory domains (Chavanas *et al.*, 2000). Its protein, *SPINK5* protein considered to

be sliced by furin to give up 14 separately operational serine protease inhibitor domains (Mitsudo *et al.*, 2003; Komatsu *et al.*, 2002) of which *13 exhibit kazal-type 4 cystein* residue that signify a new module of protein. In 13 families, 11 distinct mutations have been recognized by Chavanas *et al.*, 2000) in which nine families created premature termination codons of translation and guessed mRNA instability. These mutations were mononucleotide insertions, splice-site mutations, di-nucleotide deletions and a nonsense mutation. Moreover, in *SPINK5* gene, six coding polymorphism were recognized and the glu20lys variant illustrate momentous connection with atopy and atopic dermatitis in two independent panels of families (Walley *et al.*, 2001). So far, in *SPINK5* gene 45 mutations recognized of which eight were intronic and 36 were exonic (Zhao *et al.*, 2007; Mizuno *et al.*, 2006; Sprecher *et al.*, 2004).

2.2 Alopecia with Mental Retardation

APMR is a type of syndromic alopecia, where the affected individuals have alopecia in combination with mental retardation. The mental retardation feature of the patients is varying among the different members of the same family or with other family members. This mental retardation can be counted as from mild to severe. In recent times, three distinct genetic loci have been mapped for alopecia with mental retardation syndrome. John *et al.*, (2006a) observed the localization of *APMR1* locus on chromosome 3q26.33-q27.3 in a seven-generation Pakistani family, in which four males and three females were affected with alopecia and having severe mental retardation. In another five-generation large consanguineous Pakistani family, Wali *et al.*, (2006b) mapped second alopecia with mental retardation locus on 3q26.2-q26.31 chromosome. Family having 3-males and 2-females had total alopecia and mild to moderate mental retardation. Nails, hearing, teeth and sweating were all normal. Analysis demonstrated that *APMR2* locus is different but close by *APMR1* locus on 3q26.33-q27.3. Newly, Wali *et al.*, (2007b) have identified a third locus for alopecia with mental retardation syndrome *(APMR3)*, on 18q11.2-q12.2 chromosome. Mapping of the above three distinct loci gave the confirmation of heterogeneity underlying autosomal recessive form of alopecia with mental retardation syndrome.

2.3 Human Nude Phenotype (HNP)

The human nude phenotype is a syndromic alopecia type having the recessive phenotype distinguished by severe immunodeficiency and congenital absence of hair. Mutation at the 'nude' locus of rats and mice interrupt thymus development and normal hair growth. Nehls *et al.*, (1994) observed that the mutation in *WHN* (winged-helix-nude) gene located on chromosome 11 known to contain nude locus. Segre *et al.*, (1995) observed that mutation in *WHN* gene produce the nude phenotype in mice, which produce both athymia and hairlessness.

Schorpp *et al.*, (1997) allocated the human *WHN* gene on chromosome 17q11-q12. *Foxn1* gene is expressed in the differentiating cells of the hair follicle in human. Frank *et al.*, (1999) scrutinized a nonsense homozygous mutation in the *Foxn1* gene in two sisters with T-cell immunodeficiency, nail dystrophy and congenital alopecia. Adriani *et al.*, (2004) recognized the same nonsense mutation in the *Foxn1* gene in 55 individuals having congenital alopecia and rigorous immunodeficiency.

2.4 Hypotrichosis with Juvenile Macular Dystrophy (HJMD)

HJMD is a rare disorder noticeable at birth having sparse and short hair. The disease is caused by mutation in *cadherin 3 (CDH3)* gene spans 55kb on 16q22.1 chromosome comprising 16 exons (Sprecher *et al.*, 2001). *CDH3* encodes *P-cadherin* (prime component of adherens junctions), which is greatly expressed in the hair matrix and retinal pigment epithelium, and is the only classical cadherin that are expressed at certain developmental stages (Perez-Moreno *et al.*, 2003; Xu *et al.*, 2002). Kjaer *et al.*, (2005) scrutinized that macular dystrophy, ectrodyctyly, and abnormal expression of *P-cadherin* leading to ectodermal dysplasia are due to mutation in *CDH3* gene. Numerous mutations like deletion, nonsense and missense mutations have been identified in *CDH3* gene (Jelani *et al.*, 2008; Indelman *et al.*, 2006; 2007; Kjaer *et al.*, 2004, Sprecher *et al.*, 2001).

2.5 Congenital Atrichia

It is a rare type of isolated alopecia having autosomal recessive pattern of inheritance. Atrichia phenotype has been mapped to chromosome 8p21-p22, a locus that is synthetic to area on mouse chromosome 14, which has the *murine hairless*

gene (Nothen *et al.*, 1998; Ahmad *et al.*, 1998a). The phenotypic resemblances of patients with atrichia and *hr/hr* mice encouraged researchers to seek a human homologue of the *murine hr* gene, leads to mutation in *HR* gene, which results in atrichia (Ahmad *et al.*, 1998a; Cichon *et al.*, 1998). The hairless gene encodes a putative transcription factor with a single zinc-finger domain and largely expressed in the brain and skin. It come out to purpose in the cellular transition to the first adult hair cycle and in its lack hair growth wholly close down, a fresh hair is never stimulated, and the result is a complete form of inherited alopecia (Ahmad *et al.*, 1998b; Zlotogorski *et al.*, 1998).

Irvine and Christiano, (2001) hypothesized that *HR* protein has as vital role in the prolongation of the balance between differentiation, proliferation and apoptosis in the cycling of the hair follicle. So far, 30 distinct pathogenic mutations for the human hairless *HR* gene, having 19 exons span over 14kb on 8p21 chromosome; have been bulletined in the patients. That comprised of 2-insertion mutation, 5-splice sites, 6-nonsense mutation, 7-missense mutation and 10-deletions (Ahmad *et al.*, 1998a; Ashoor *et al.*, 2005; Betz *et al.*, 2007; Cichon *et al.*, 2006; Nothen *et al.*, 1998; Paradisi *et al.*, 2003; Sprecher *et al.*, 1999; Wali *et al.*, 2006a).

So far, 17 distinct background families having APL, that are German, Israeli, Italian, Japanese, Korean, Mediterranean population, Mexican, Pakistani, Palestinian and Polish. Greater than 30 mutations, in human hairless *(HR)* gene are known to date, since the first discovery of the patho-genetic mutations basic for APL (Wali *et al.*, 2006a; Ahsoor *et al.*, 2005; John *et al.*, 2005; Ahmed *et al.*, 1998a).

2.6 Monilethrix

It is hereditary disorder of hairs where at birth the affected individuals have normal hairs and after 2-3 months of life. The inheritance pattern of monilethrix is autosomal dominant observed, and in few cases, recessive transmission has observed (Salamon and Schnyder, 1962).

It has been mapped to chromosome 12, having *type II keratin gene cluster* (Birch-Machin *et al.*, 1997; Stevens *et al.*, 1996). Mutation *(E413K) in type II, hair cortex keratin* identified known as *hBb6* (Winter *et al.*, 1997a). This mutation was the

direct proof for the involvement of hair keratins in hair loss. At the helix termination motif of the gene *hHb6*, Horve *et al.*, (2003) recognized missense mutations (*E402K* and *E413K*). Missense mutation from the monilethrix family was recognized by Winter *et al.*, (1997b) in type II hair keratin, *hHbl*. Van Steelsel *et al.*, (2005) in *type II hair keratin hHb3* gene recognized missense mutation that causes substitution of a glutamic acid by lysine at position 407.

Recently, from the Zlotogorski *et al.*, (2006) study it came under consideration where autosomal recessive hypotrichosis overlap with autosomal recessive form of monilethrix, in which he studied 12 Jewish monilethrix families and identified 4 distinct mutation in *desmoglein 4* gene (*DSG4; MIM 607892*). In the Japanese family, patient-having monilethrix like congenital hypotrichosis, Shimomura *et al.*, (2006) recognized a novel heterozygous mutation in the *DSG4* gene. The mentioned *DSG4* gene, belonging to the *desmosomal cadherin* family and is expressed in the cortex of the hair follicle.

2.7 Marie Unna Hereditary Hypotrichosis (MUHH)

MUHH was first recognized by Marie Unna (Unna, 1925), a German Dermatologist. It is autosomal rare type of isolated alopecia, whose inheritance pattern is dominant. The genome-wide scan mapped the MUHH gene to 8p21 chromosome in several families (Lefevre *et al.*, 2000; Sreekumar *et al.*, 2000; Cichon *et al.*, 2000). This interval contains *HR* gene, which is mutated in autosomal recessive congenital atrichia. He *et al.*, (2004) mapped the MUHH gene to a 1.1cM region flanked by *D8S282* and *D8S1839* which present confirmation that the existence of a gene is distinct from *HR* gene in chromosomal region 8p21 playing an immense role in the hair follicle biology. In a Chinese family, Yang *et al.*, (2005) observed the second locus for MUHH to a 17.5 cM interval at 1p21.1-1q21.3 chromosome. These signified that genetically MUHH is heterogeneous disease. Wen *et al.*, (2009) scrutinized codon mutation in *U2HR* and made a spectrum of imperfection in *U2HR* from distinct ancestral group having confirmed clinical diagnosis of MUHH. In the 5′ URF, *U2HR* is an inhibitory upstream (*uORF*) open reading frame region of human hairless *(HR)* gene. Moreover, this *U2HR* is predicted to be encoding 34-amino acid polypeptide, which is greatly preserved in mammals.

2.8 Hypotrichosis Simplex

Hereditary simplex is divided into main three types generalized hypotrichosis where the defects cover the full body hairs; hypotrichosis simplex of the scalp where the loss of hairs is only restricted to scalp; and localized hypotrichosis where the hair loss is only restricted to specific area of the body (Just *et al.*, 1998; Cambiaghi and Barbareschi, 1999). The patient show short and sparse thin hair, in few cases long and less sparse hair been enunciated. This sparse hair only observed for the scalp and body and no abnormalities enunciated for the beard, eyebrows and eyelashes.

The pattern of inheritance of localized hypotrichosis simplex of the scalp is dominant and autosomal. It has been mapped to 6p21.33 chromosome and the conjoined haplotypic data recognized the critical interval of 14.9cM having *corneodesmosin* gene (*CDSN; MIM 602593*). Levy-Nissenbaum *et al.*, (2003) recognized a non-sense mutation in the coding area of *CDSN* that results in the pre-mature aging stop codons *Q200X* and *Q215X*. Three other novel mutations have been recognized in *CDSN* gene (Davalos *et al.*, 2005).

The inheritance pattern of generalized hereditary hypotrichosis simplex recognized to be different in different families. Some families come out with autosomal recessive (MIM241900) and some with autosomal dominant (MIM 144520; MIM 146550) pattern although the differences in the clinical features like severity, eyelashes and eyebrow involvement, and age of onset among the families were observed (Winter *et al.*, 1997; Toribio *et al.*, 1974).

2.9 Localized Autosomal Recessive Hypotrichosis (LAH)

LAH is also a rare form of alopecia, where hypotrichosis is limited to the arms, legs, chest and scalp. So far, for the autosomal recessive hypotrichosis four genes have been reported and are observed to link with 18q12 chromosome on a small region of 700 kb (Rafique *et al.*, 2003; Kljuic *et al.*, 2003). This small area has cluster of *desmocollin (DSC1, DSC2, & DSC3)* and *desmoglein (DSG1, DSG2, DSG3, & DSG4)* genes. The *desmocollins* and *desmoglein,* both are glycoproteins of desmosomes, type of inter-cellular junctions mediating cell-to-cell adhesion, of the epithelial cells in vertebrates (Townes and Behringer, 1990). In two un-related Pakistani origin families, in *desmoglein-4* gene an intragenic deletion mutation (*EX5-*

8del) has been identified (Kljuic *et al.*, 2003). This is a large deletion mutation began 35 bp upstream of 5-exon and ended on 289 bp downstream of 8-exon generated and in-frame deletion that creates missing amino acids of 125-335 (Kljuic *et al.*, 2003). The similar deletion mutation was observed in other Pakistani family that showed an inherited mutation, which has been widely dispersed (John *et al.*, 2006; Rafiq *et al.*, 2004; Moss *et al.*, 2004). Besides this, Wajid *et al.*, (2007) observed deletion mutations in *DSG4* gene. Moreover, in patients having monilethrix hairs, few other mutations have been observed as element of their phenotypic presentation, which stated to widen our knowledge of the phenotypic-genotypic connection within LAH (Shimomura *et al.*, 2006; Schweizer, 2006; Zlotogorski *et al.*, 2006; Schaffer *et al.*, 2006). Ayub *et al.*, (2009) identified a novel mutation a large family having skin vesicles with hypotrichosis in the same chromosomal region in *DSC3* gene.

In a Pakistani family, second locus on chromosome 3q27 has been mapped to 7.59 cM for LAH (Aslam *et al.*, 2004). In this family, the affected individuals were nearly devoid of normal eyelashes, eyebrows, and body hairs. The male affected individuals were almost devoid of legs and arms hairs although they have normal beard and the nails and teeth of all affected members were normal.

In the AH region of exon 4 of *Lipase H (LIPH)* gene, homozygous deletion mutation was observed in the affected families of Volga-Ural region of Russia (Kazantseva *et al.*, 2006). Deletion of exon 4 in LIPH gene doesn't change its reading frame although it eliminates the conserved domain in the predicted protein. So far, in the *LIPH* gene different disease causing mutation have been recognized (Naz *et al.*, 2009; Ali *et al.*, 2007). In the Pakistani consanguineous family, Naz *et al.*, (2010) mapped fourth locus in human chromosome 10q11.23-22.3 for autosomal recessive hypotrichosis.

2.10 Androgenetic Alopecia (AGA)

Androgenetic alopecia is androgen-dependant and a hereditary disease. In this, the patients get the progressive thinning of the hairs and in men results in M-shape hairs on the scalp. In men the ordinary type of the early baldness has been thought to be autosomal dominant and in females autosomal recessive, who transmit the trait if heterozygous but are bald only if homozygous (Osborn, 1916; Snyder and Yingling, 1935). The prevalence of AGA is 50% in men by the age of 50 years and in the later

stages, it leads up to 70 % in all men (Norwood, 1975). The prevalence of androgenetic alopecia is assumed to be as 39.6% in women in one of the study in Iran, which is higher to world, reported data i.e. 20% (Fatemi *et al.*, 2010).

Ellis *et al.*, (2001) recognized for the first time significant confirmation of a genetic relationship in the androgenetic receptor *(AR)* gene located on *Xq12* chromosome, between baldness and a synonymous coding SNP (rs6152). Androgen receptor, a member of the nuclear receptor super family, functions as a ligand-dependent transcription factor and is essential for androgen action (Gelmann *et al.*, 2002). The *AR* gene, located at chromosome *X q12*, is highly conserved, with germline loss-of-function mutations resulting in androgen insensitivity syndrome, also known as testicular feminization (Gottlieb *et al.*, 2004). Levy-Nissenbaum *et al.*, (2005) scrutinized a silent polymorphism in the *AR* in a group of 39 non-bald males and 41 bald males and he discover the association between *AR* gene and male pattern baldness (MPB). For the development of early-onset of androgenetic alopecia, Hillmer *et al.*, (2005) demonstrated that genetic discrepancy in the *AR* gene is the prime perquisite. Ellis *et al.*, (2007) verified that whilst the non-functional rs6152, *AR SNP* is strongly linked with male pattern baldness (AGA).

2.11 Alopecia Areata

Alopecia areata is an autoimmune, non-scaring and inflammatory hair loss of the scalp. It is one of the most common human autoimmune diseases. Welsh *et al.*, (1994) recognized the significant connection between the *AA* and *DRB1*1104* allele. Family based study that has 46% controlled and 85% of AA affected patients has exposed the inclusion of *DQB1*03* alleles (De-Andrade *et al.*, 1999). Xiao *et al.*, (2005) scrutinized that *HLA-A*02, 03, HLA-B*18, 27, 52* and *HLA-Cw*0704* alleles were strikingly higher in affected individuals with AA than in controls. Mutations in the *filaggrin* (*FLG*) gene were observed to be promising candidates in AA (Bettina, 2007). Bettina, (2007) scrutinized the participation of *FCRL3* gene in vulnerability to alopecia areata. In other study scientist confirmed, that *Sox21* gene could be responsible for hair loss conditions in humans (Lyn, 2009). Recently scientists identified eight genes that are probably linked with alopecia areata. One gene in particular fixed the eye of study known as *ULBP3* on chromosome 6q25.1, this gene is normally not present in hair follicles, but *ULBP3* proteins were found in high

concentrations in hair follicles affected by alopecia areata. From their study, they identified 139 single nucleotide polymorphisms that are significantly associated with AA (Petukhova *et al.*, 2010; Christiano, 2010).

2.11.1 Alopecia Totalis

It is a severe type of alopecia areata, in which the hairs from the full scalp removed and the eyelashes, eyebrows and all other body hairs like axillary hairs and public hairs all are normal. The gene encoding the lymphoid protein tyrosine phosphatase has been shown to be associated with severe forms of AA (Kemp *et al.*, 2006).

2.11.2 Alopecia Universalis

It is the most severe type of alopecia areata were the patients having no hairs on the full body. Ahmed *et al.*, (1998a) studied different forms of alopecias whose molecular basis is totally unknown. A specific rare form of alopecia, alopecia universalis was observed for the detection of locus by homozygosity maping and in a 6-centimorgan interval on chromosome 8p12, linkage was established. Through the radiation hybrid mapping, the human homolog of a *murine* gene was localized in this interval and missense mutation was observed in affected individuals. The study also observed that human hairless encode putative single zinc finger transcription factor protein, whose expression is in skin and brain. Nothen *et al.*, (1998) mapped that congenital alopecia universalis to a locus on 8p21-22 chromosome.

Potter *et al.*, (2001) investigated that the mammalian hairless *(hr)* gene plays significant role in the maintenance of hair growth. They observed that the two independent regions of *Hr* mediate, *TR* binding and their interaction needs a bunch of hydrophobic residues parallel to the binding motifs proposed for nuclear receptor co-repressors (*N-CoR* and *SMRT*). They also scrutinized that *Hr* binds to specific region of *TR* as recognized co-repressors, and *Hr* interacts with histone deacetylases (*HDACs*) and is restricted to matrix-associated deacetylase (*MAD*) bodies, representing that the mechanism of Hr-mediated repression is likely through linked *HDAC* activity. Their discovery that *Hr* is the co-repressor machinery provides a molecular basis for specific hair loss syndromes in both mice and humans.

3. MATERIALS AND METHODS

3.1 Ascertainment of Families

Three families of alopecia universalis were ascertain in the present study from the different areas of district Swabi, Khyber Pukhtunkhwa province of Pakistan. Beforehand the study, an approval was obtained from the Board of Study (BOS), International Islamic University. Written consent was obtained from the families who agreed to participate in our research. The affected individuals were examined by the Skin specialist and dermatologist, Dr. Abdul Hakeem in the Khyber Teaching Hospital, Peshawar. The clinical examination of nails, sweat glands, scalp hairs, eyelashes, eyebrows, and body hairs was performed meticulously.

The elders and relatives of the families were interviewed at their residency to take the information regarding hair loss and related matters of the affected members. The number of generation involved in the disease, affected individuals, onset of the hair loss and the associated defects were vigilantly noted. The affected individual in the families were also photographed.

3.2 Construction of Pedigrees

To describe the genetic relationship among the normal individuals and patients, pedigrees were constructed from the information collected from the elder members of families and their relatives. Circle and square shapes were used as symbols for individuals as females and males respectively. The affected individuals were represented by filled symbols and the normal individuals with unfiled symbols. The deceased individuals in the families were represented by the crossed shapes and to represent every generation in the pedigree, Roman numerals were used and all members within a single generation by an Arabic numerals. Horizontal line joining two individuals represents marriage. Consanguineous marriage was represented by a double line between partners.

3.3 Collection and Storage of blood

After obtaining a written consent from the head of the family, using disposable syringes (10ml), venous blood was collected from all the individuals (normal and

affected) of the family in the BD vacutainer tubes having anticoagulant ethylenediaminetetraacetic acid (EDTA). The collected blood samples were then kept at 4 °C before being processed for the genomic DNA extraction.

3.4 Genomic DNA isolation from human blood

High molecular weight genomic DNA was extracted from the white blood cells of venous blood samples through the organic method or phenol-chloroform method as defined by Sambrook and Russel, (2001). About 750 µl of blood and 750 µl of solution A [5 mM $MgCl_2$, 0.32 M Sucrose, 1 % Triton X-100, 10 mM Tris pH 7.5] were mixed in an 1.5 ml eppendorf tube (Micro centrifuge tube) and then kept for about 5-10 min. Vortex the mixture and centrifuged on Centrifuge 5424 (Eppendorf, Germany) at 13000 rpm for 1 min. After discarding the supernatant, nuclear pellet was re-suspended in 400 µl of solution A and again vortex it and kept for 5 min. The mixture was centrifuged for 1 min at 13000 rpm, discards the supernatant. After discarding the supernatant 400 µl of solution B [400 mM NaCl, 2mM EDTA, 10 mM Tris] along with 12 µl of SDS and 25 µl of proteinase-K (100µl/ml) were added to re-suspend the pellet. Samples were incubated at 65 °C for about 3 hours. Before adding Sol-C [Phenol] + Sol-D [Chloroform and Isoamyl-alcohol, 24:1], mixture of solution C+D was formed and were kept for 30 min so that a bubble be formed, when bubble formed then 500 µl of Sol C+D were poured in the samples, vertex here and centrifuged for 10 minutes at 13000 rpm. The supernatant/aqueous phase in the sample tubes were transferred in the new tubes. This aqueous phase was mixed with 55 µl of sodium acetate (pH 6, 3M) and equal amount, almost 500 µl of chilled iso-propanol and for complete separation of DNA pellet, tubes were inverted many times and again centrifuged at 8000 rpm for 5 minutes, DNA was settled down. Supernatant was discarded and the settled DNA was washed with 70% ethanol, discard the ethanol and the tubes were kept in the incubator (Memmert, Germany) for 5 min at 37 °C to dry the DNA pellet. Finally, the DNA was dissolved in the 100 µl of TE/Tris-EDTA buffer [pH 8, 0.1mM EDTA, 10mM Tris] and was kept in the refrigerator at 4 °C.

3.5 Extracted DNA analysis

The analysis of the extracted DNA was performed by using 1% of agarose gel. Almost 100 ml of 1-X-TBE buffer [0.89 M Tris-Borate, 0.025 M EDTA] and 1 g of

agarose was added in the conical flask and were heated in the microwave oven for 3 minutes. To stain the DNA, 5-µl of ethidium bromide was added in the conical flask having 100 ml of 1-X-TBE in combination to agarose. Combs were inserted in the UV-transparent gel tray to make wells in the gel upon solidifying the gel. The liquid gel were poured in the UV-transparent tray to solidify, after some time the gel along with the UV-transparent gel tray was put in the gel caster/buffer chamber. After that the 3-µl of loading dye [0.25 % bromophenol blue and sucrose] mixed with 3-µl of DNA on paraffin sheet and poured in the gel's well, each sample in different wells. Electrophoresis was run at 120 volts (80 mA) for 20 minutes. To visualize the genomic DNA, UV transilluminator T12 (Wealtec, Taiwan) was used. Concentrated genomic DNA was diluted for PCR reaction by mixing 80-µl of PCR water with 20-µl of concentrated DNA.

3.6 Amplification and Sequencing of the Human Hairless (*HR*) and Purinergic receptor P2Y, G-protein coupled, 5 (*P2RY5*/LPAR6) gene

Human Hairless (*HR*) is the causative gene for the Congenital Alopecia Universalis. *HR* gene has 19-exons. All the coding exons and splice junctions of *HR* gene were amplified and sequenced. *P2RY5/LPAR6* is recognized to be the causative gene for Localized Autosomal recessive Hypotrichosis. *P2RY5/LPAR6* gene has 5-exons. The hotspots and splice junctions of P2RY5/LPAR6 gene were amplified in three overlapping sets of primers for sequencing. The new name of *P2RY5* gene is lysophosphatidic acid receptor 6, (*LPAR6*) gene.

3.6.1 Polymerase chain reaction

Primers were designed for the amplification of exons of *HR* gene from the intronic sequences of exons (Table 3.2). Primers were designed for the amplification of exon 2 considered being the hotspot of *P2RY5/LPAR6* gene (Table 3.3).

For every PCR amplification reaction, big reaction of 50-µl reaction mix containing 5-µl of DNA, 5-µl of forward primer, 5-µl of reverse primer, 10-µl of PCR water and 25-µl of GoTaq® green master mix (Promega, USA) were prepared according to the concentration given in Table 3.1. Amplification of the exons were

carried out in 40 cycles of PCR, in which initial 5 min was for denaturation of template DNA at 95 °C, followed by amplification. Every amplification cycle having three steps DNA denaturation, annealing of primers and extension at 95 °C, 50-65 °C (different for different exons) (Table 3.2), 72 °C and finally 10 min at 72 °C. The reactions were carried out in the thermocyclers (Palm-Cycler Gradient, Corbett Life Sciences, Australia and Biometra, Germany.

3.6.2 Amplicon Analysis

To analyse the amplicon 2% of agarose gel was used. In about 100 ml of 1X TBE in the conical flask, 2 g of agarose were added and heated for 3 min in the microwave oven. For staining the amplified exons 5-µl of ethidium bromide was used. Combs were inserted in the UV-transparent gel tray to make wells in the gel upon solidifying the gel. The liquid gel were poured in the UV-transparent tray to solidify, after some time the gel along with the UV-transparent gel tray was put in the buffer chamber/gel caster. Then 3-µl of loading dye [0.25 % bromophenol blue and sucrose] mixed with 3-µl of amplified exon on paraffin sheet and poured in the gel's well. Electrophoresis was run at 120 volts (80 mA) for 20 minutes. To visualize the amplified exons, UV transilluminator T12 (Wealtec, Taiwan) was used.

3.6.3 PCR Product Purification

For the purification of amplicon, gel extraction kit (Fermentas, Life Sciences) was used. The gel having amplified exons, of the specific length was weighted in the pre-weighted eppendorf tube (mg) adding half volume of TBE conversion buffer and 4.5 volume of binding buffer. Tube was incubated in the incubator to dissolve the gel completely for 5 min. Powder silica suspension of 5-µl were added to the gel solution in the eppendorf tube, vortex and again incubated at 55 °C so that the silica be fully dissolved in the gel solution. Mixture was centrifuged at 13000 revolutions for 1 min and the supernatant was discarded, 500-µl of ice cold washing buffer was added to the tube and centrifuged for 1 min and then supernatant was discarded again. The above washing process was repeated three times. Then pellet was dried in open led eppendorf for 10-15 minutes. DNA pellet was re-suspended in 12-µl of de-ionized water/PCR water and incubated for 5 minutes at 55 °C. Lastly, the purified PCR

product after centrifugation, 10-µl supernatant was collected in new eppendorf tube and this supernatant having the amplicon (amplified exons).

3.6.4 Sequencing Reaction

Purified amplicon were subjected to 2nd PCR or sequencing PCR for 30 cycles of 10-µl reaction mixture having 1.5-µl Big Dye Terminator version 3.1 ready reaction mixture, 1-µl forward primer, 1-µl of 5X sequencing buffer (PE Applied Bio-systems, Foster City, CA, USA), 5.5-µl of PCR water and 1-µl amplicon. PCR reaction was employed as, initially 1 min of denaturation at 95 °C of template DNA/exons followed by 28 cycles of 30 sec at 95 °C, 48-62 °C for 30 seconds, and for 4 min at 72 °C, lastly a final extension stage for 10 min at 72 °C.

3.6.5 Purification of Sequencing Product

The second amplicon were subjected to second purification (Ethanol Purification). The purified products were re-suspended in 20-µl Hi-Di Formamide (HDF) (PE Applied Bio-systems, Foster City, CA, USA), and were placed in 0.5 ml septa tubes for directly sequencing in and ABI Prism 310 Genetic Analyzer (PE Applied Bio-systems, Foster City, CA, USA).

3.6.6 Sequencing Data Analysis

For the identification of sequence variations in the exons of *HR* gene and *P2RY5/LPAR6* gene, the exons sequencing results from the healthy and affected individuals were aligned with reference sequence obtained from Ensemble database (www.ensemble.org/Homo_sapiens/Info/Index) to recognize the sequence variant. The variations were identified with BioEdit Sequence Alignment Editor, Version 7.1.3 (Tom Hall, Isis Pharmaceuticals Inc.).

3.6.7 Insilco Tools

Sequence for *HR* and *P2RY5/LPAR6* was retrieved from NCBI. Reported mutations were retrieved from Literature and HGMD (Human Gene Mutation Database). STRING (Known and Predicted Protein-Protein Interaction) Database was used to find the human hairless protein and *P2RY/LPAR6* gene's protein interactions with associated functional partners. The sequence was retrieved in FASTA format

from Uniprot Knowledge base. The automated protein modelling program MODELLER 9v10 was used to generate models. It predicted the 3D structure of the protein by satisfying spatial restraints. Normal and mutated protein structure of *P2RY5/LPAR6* gene was found out through this MODELLER 9v10.

Table 3.1 Concentration of master mix used in 50 µl of reaction mix

For a 50µl reaction volume:

Components	Volume	Final Concentration
GoTaq® Green Master Mix,2X	25µl	1X
Upstream Primer, 10µM	5.0µl	1.0µM
Downstream Primer, 10µM	5.0µl	1.0µM
DNA Template	5.0µl	50ng
Nuclease-Free Water	10µl	NA

Table 3.2 Primer's sequences used for the screening of *HR* gene

EXON	PRIMERS TYPE	PRIMERS (5′-3′) (FORWARD+REVERSE)	SIZE	ANEALING TEMP (°C)
2a	FORWARD	GCCTTACTGGTTTGAGCTGC	549	50.4
	REVERSE	TGAGATGGCCACCACTATGC		
2b	FORWARD	TCCTGAGCACCCCAGACTCC	614	65
	REVERSE	CTTGGGGTTGACTGTGGGGC		
3a	FORWARD	GAGGGCTTCAGTATTCTCCC	494	52
	REVERSE	AGTGGGTGGGTAGGATGAAC		
3b	FORWARD	GAATCCTTGCCCGCTCTTCC	777	53
	REVERSE	CTGAGGAACTCCCAGAGAGC		
4-5	FORWARD	CATCCTCAGACTCCCTGCTC	724	62.9
	REVERSE	CCTTAGGTCTAGGAGCTGGC		
6	FORWARD	CTCTCCATGGAAGCTGCTCC	361	57.7
	REVERSE	GCCAACGAATGACCACAGGC		
7	FORWARD	GCTGTGTCTCTATGTGACCC	393	56
	REVERSE	GGTGGTGAGTCTAGACCAAC		
8	FORWARD	AGCTTCCCGTCTGATTGTCC	595	60
	REVERSE	CCATTTGCAGGCACGATACC		
9	FORWARD	GGTAGAAGTCCATGAGCAAC	422	53
	REVERSE	AAGGTGTTTGGAGGCATGTC		
10	FORWARD	TGCAGGAAAAGCAGTAGAGC	462	52.1/52
	REVERSE	ATGTTGGTGATGCGGTCATC		
11	FORWARD	AGCGAATACACATGGCCTTC	529	55
	REVERSE	TAAGGGCAGTAGAACAGCTC		
12	FORWARD	TCCCCGAGCTGTTCTACTGC	430	60
	REVERSE	ACAGGAGGAGACAGAACGGC		
13	FORWARD	AGCGTAAGTGTCCCCAACAC	358	57
	REVERSE	ACATGAGAGTACCAGGGACC		
14	FORWARD	CCTGGTACTCTCATGTTTGC	358	55
	REVERSE	TGGAATCAGAGAAGCGCTTC		
15	FORWARD	ACTCCTGACCTCAGGTGATC	357	56
	REVERSE	TCCAGGCCTGAAAGGAAGTC		

16	FORWARD	TCAGCATCCTGGTGCATGCC	400	**61**
	REVERSE	TTGGGTCTGTGCAGCTCACC		
17	FORWARD	CTGCCCTTCAAGACTTGACC	465	**56**
	REVERSE	CTCAGTGACTTCAAGGCCTC		
18	FORWARD	GAATCTGCTCTCTGAGAGCC	348	**55**
	REVERSE	AGGGTGGGATCTGCTATGTC		
19	FORWARD	CTGGGATTACAGGTGTGAGC	1107	**64**
	REVERSE	GCTGCCCTACTCCATTTGTC		

Exon 2 was amplified by two sets of overlapping primers

Exon 3 was amplified by two sets of overlapping primers

Table 3.3 Primer's sequences used for the screening of *P2RY5/LPAR6* gene

EXON	**PRIMERS TYPE**	**PRIMERS (5′-3′) (FORWARD+REVERSE)**	**SIZE (bp)**	**ANEALING TEMP (°C)**
2a	FORWARD	CTGTTGAAGAACCCAGCGG	690	**53**
	REVERSE	CCCTGAGAGTGGGTAGACTG		
2b	FORWARD	CTTCACAACACGGAATTGGC	595	**55**
	REVERSE	TGGGTACATTGTCCTTACTGC		
2c	FORWARD	GGGTAACAATGCCTCAGAAG	847	**52**
	REVERSE	GCACCACATAATTTAAGTCAG		

Exon 2 was amplified by three sets of overlapping primers

4. RESULTS

4.1 Human Subjects and Clinical Features

4.1.1 Family A

This family is affected with congenital alopecia universalis, belongs to Pashtu speaking family of district Swabi, Village Mani, Khyber Pukhtunkhwa, Pakistan. This family rarely marry outside the family that is why consanguineous unions are very common. The five generations family comprised of four affected individuals (V:1, V:2, V:3, and V:5) in 15 total individuals (Figure 4.1). The normal parents of affected children are cousins, disorder is transmitted in an autosomal recessive manner as both males, and females are affected. Blood samples were taken from four affected individuals including two males and two females (V:1, V:2, V:3 and V:5) and three normal individuals having two males (III:3 and V:4) and one female (IV:1).

The affected individuals of the congenital alopecia universalis do not have physical or mental problem. Patient V:5 has no hairs on her full body, two to three hairs representing eyelashes and eyebrows (Figure 4.2, A & B). Skin and nails are normal, no skin rashers and nail dystrophy were reported in the affected individuals. The patient V-2 has pimples and the eyebrows were somewhat prominent (Figure 4.2, C). All the affected individuals have normal sweating. Patient V-1 has few hairs on his cheeks.

4.1.2 Family B

This family belongs to Zaida city of District Swabi in the Khyber Pukhtunkhwa province of Pakistan. This family have consanguineous marriages due to which the family got the congenital alopecia universalis in their descendants. The four-generation pedigree comprised of 13 members having one affected female (IV:4) and one affected male (IV:5) (Figure 4.3). The normal parents of affected children are cousins, disorder is transmitted in an autosomal recessive manner as both males, and females are affected. The affected individuals are siblings and are present in fourth

generations. The blood samples were collected from both affected (IV:4, IV:5) and three normal individuals (III:1, III:2, and IV:1) (Figure 4.3).

All the affected individuals of this disease are mentally and physically all right. They were having no hairs on their full body. The hair loss in the affected individuals occurs within two weeks after birth. Hair was present on the scalp but never re-grew after ritual shave. The affected individuals (IV:4, IV:5) were almost completely devoid of eyelashes, eyebrows, axillary and public hairs (Figure 4.4, A & B). The nails were normal and no nail dystrophy was recorded. Sweating was normal in both the affected individuals. Figure 4.4, C shows the clinical features of siblings who are devoid of complete body hairs.

4.1.3 Family C

The members of this family C normally prefer to marry within their tribe and thus consanguineous marriages are very common. The family comprised of eight individuals, including six affected with autosomal recessive hypotrichosis, two of which are males (IV:1, IV:2) and four are females (III:6, IV:4, IV:5, IV:6) (Figure 4.5). One affected female (III:6) is deceased. The normal parents of affected children are cousins, disorder is transmitted in an autosomal recessive manner as both males, and females are affected (Figure 4.5). Peripheral blood samples were collected from eight individuals, three normal (III:1, III:2, IV:3) and five affected (IV:1, IV:2, IV:4, IV:5, IV:6).

The affected individuals shared the common phenotype of hereditary autosomal recessive hypotrichosis disorder. Affected individuals were showing loss of hair from scalp, face and other parts of body. Ages of the affected individuals range from 6-20 years. The affected persons are present in generation III and IV of the pedigree. Photograph of two of the affected individuals (IV-6) is shown in the Figure 4.6, A she has a squint in her eyes and (IV-5) shown in Figure 4.6, B.

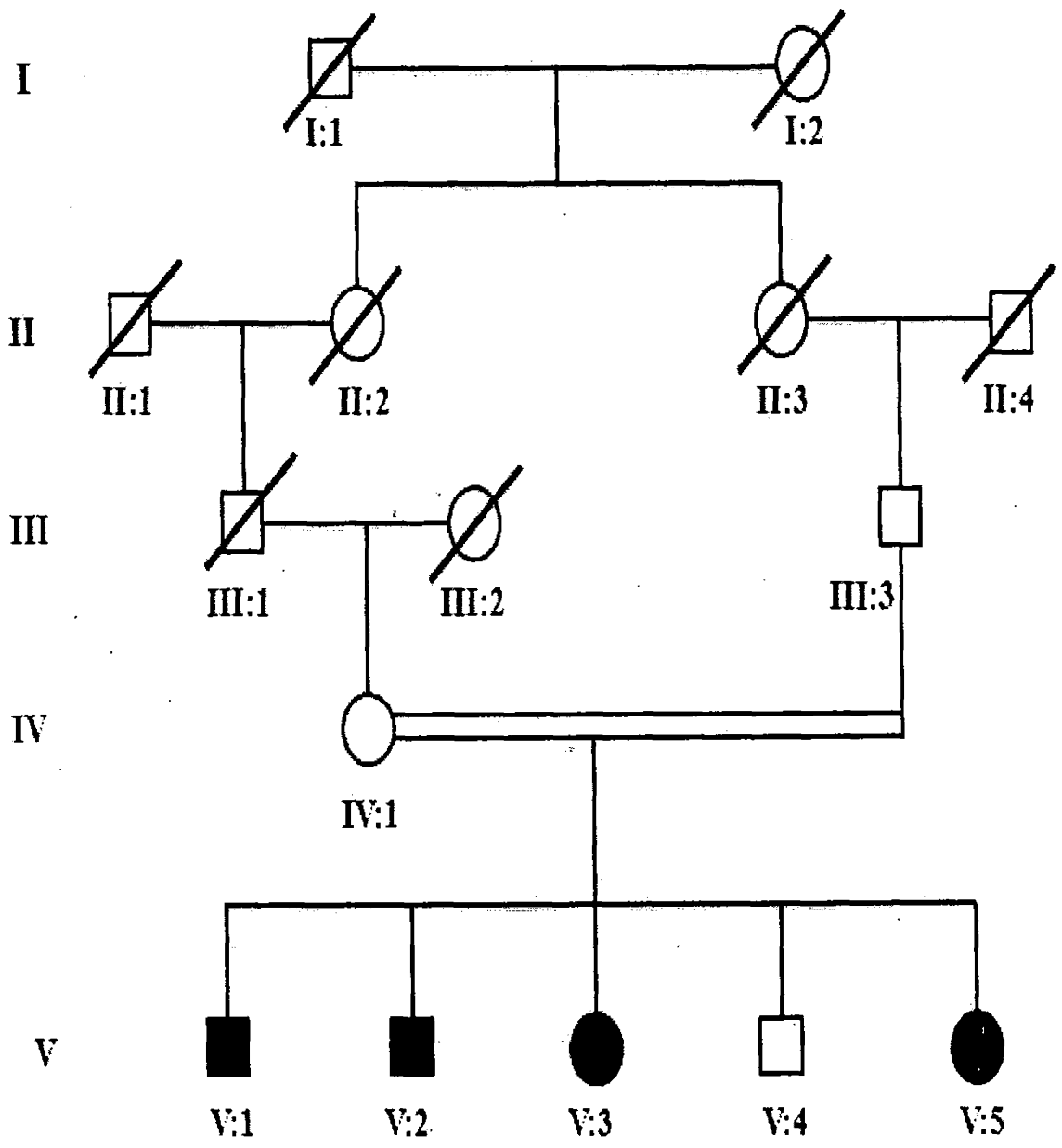

Figure 4.1 Pedigree of Family A having Congenital Alopecia Universalis. Squares represent male individuals and circles represent female individuals. The filled squares and circles represent affected individuals. The crossed lines on the squares and circles represent deceased individuals and the double lines between the individuals represent the consanguineous marriage

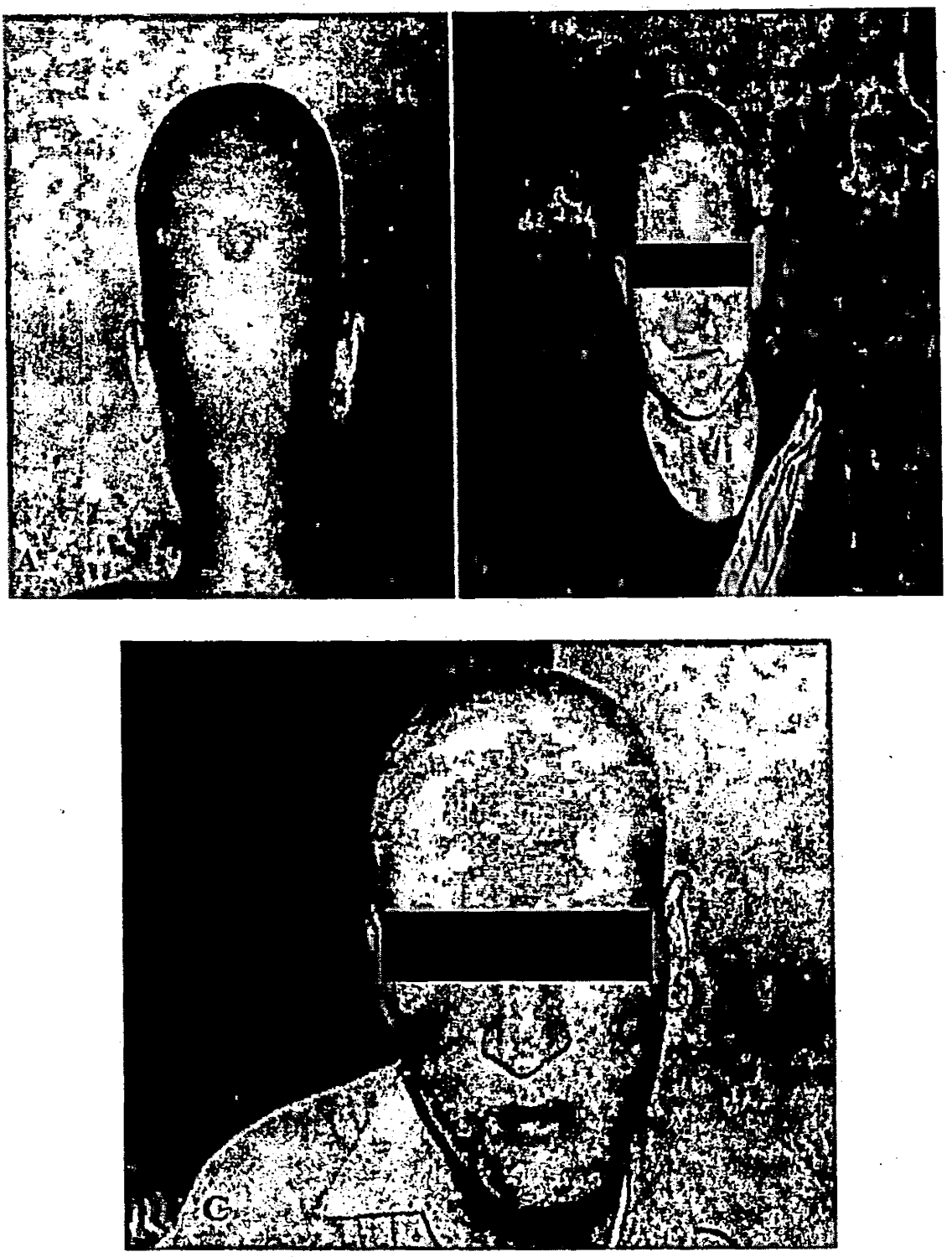

Figure 4.2 Clinical features of congenital alopecia universalis in Family A. A & B represent the phenotypic appearance of affected individual (V:5), 2-3 hairs represent her eyebrows and eyelashes and a complete absence of the scalp hairs. C represents the physical features of affected individual V:2, few hairs represent the eyebrows

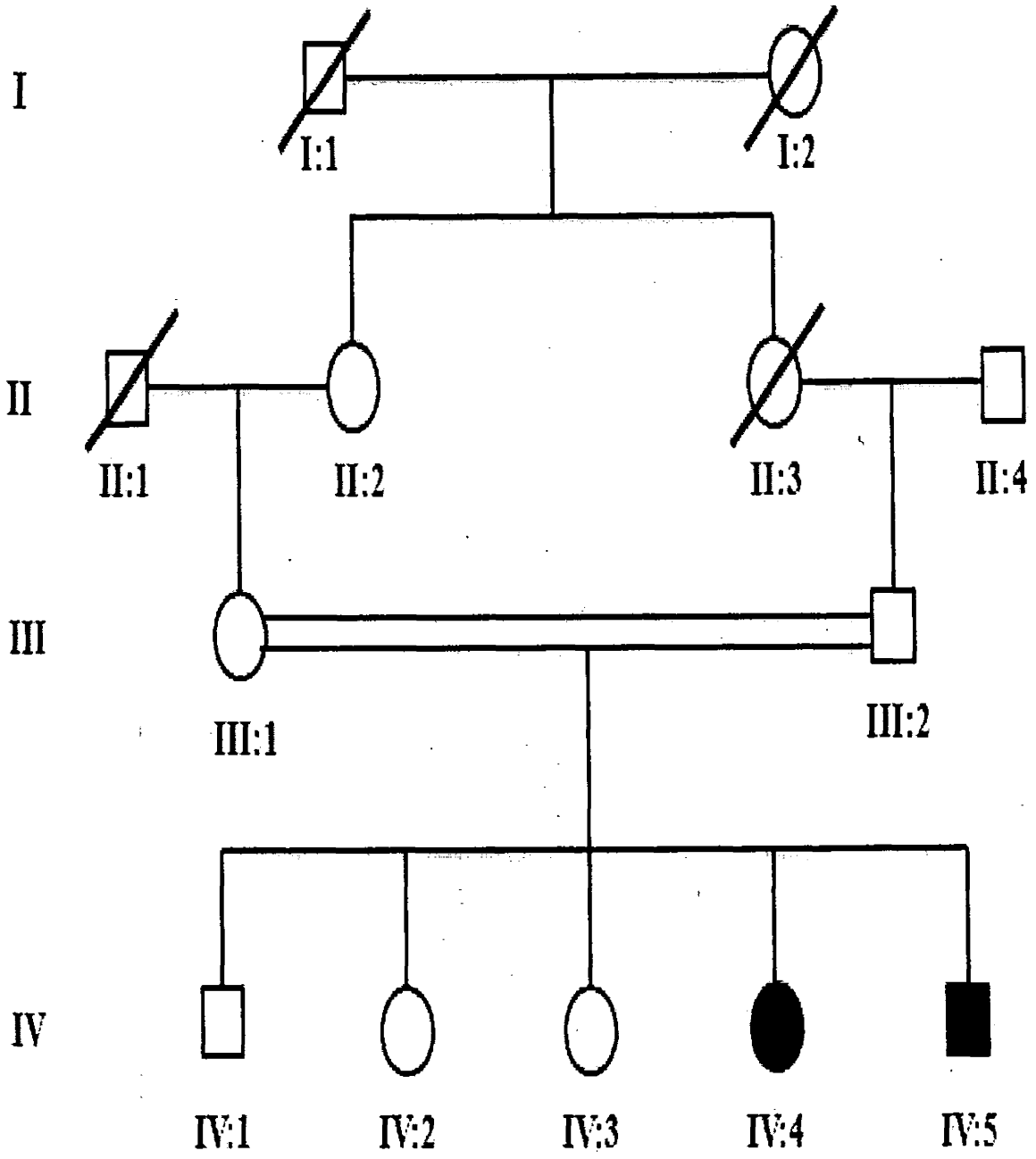

Figure 4.3 Pedigree of Family B, affected with Congenital Alopecia Universalis. Squares in the pedigree represent male individuals and circles represent female individuals. The filled squares and circles represent affected individuals. The crossed lines on the squares and circles represent deceased individuals and the double lines between the two individuals represent the consanguineous marriage

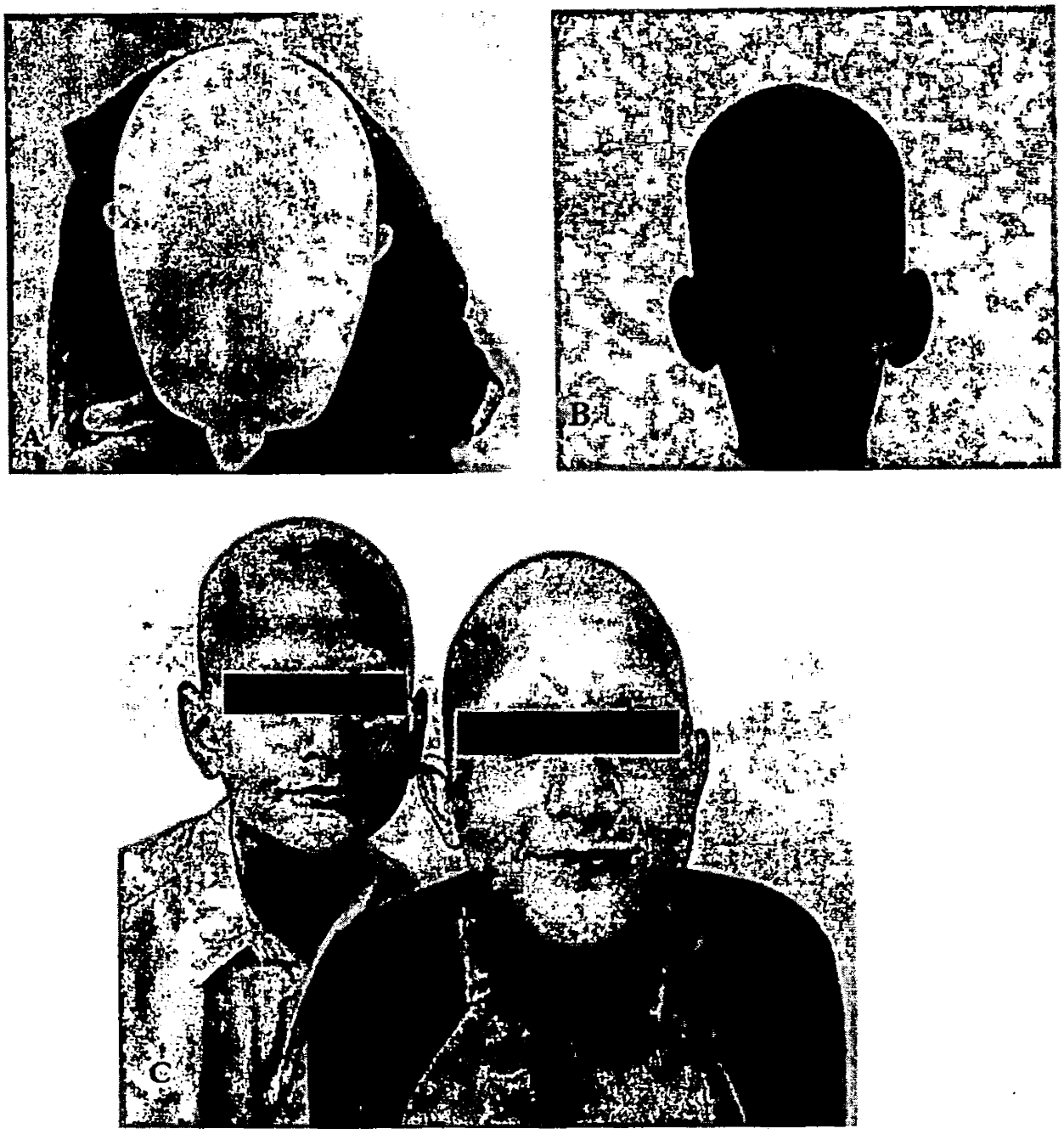

Figure 4.4 A: represent the clinical features of congenital alopecia universalis in Family B representing patient (IV:4) that are completely devoid of full body hairs. B: represent the phenotypic appearance of affected individual (IV:5), no hairs on full body and has complete absence of the scalp hairs. C: represents clinical features of both the siblings that are almost completely devoid of full body hairs, and eyelashes and 1-2 hairs represent the eyebrows

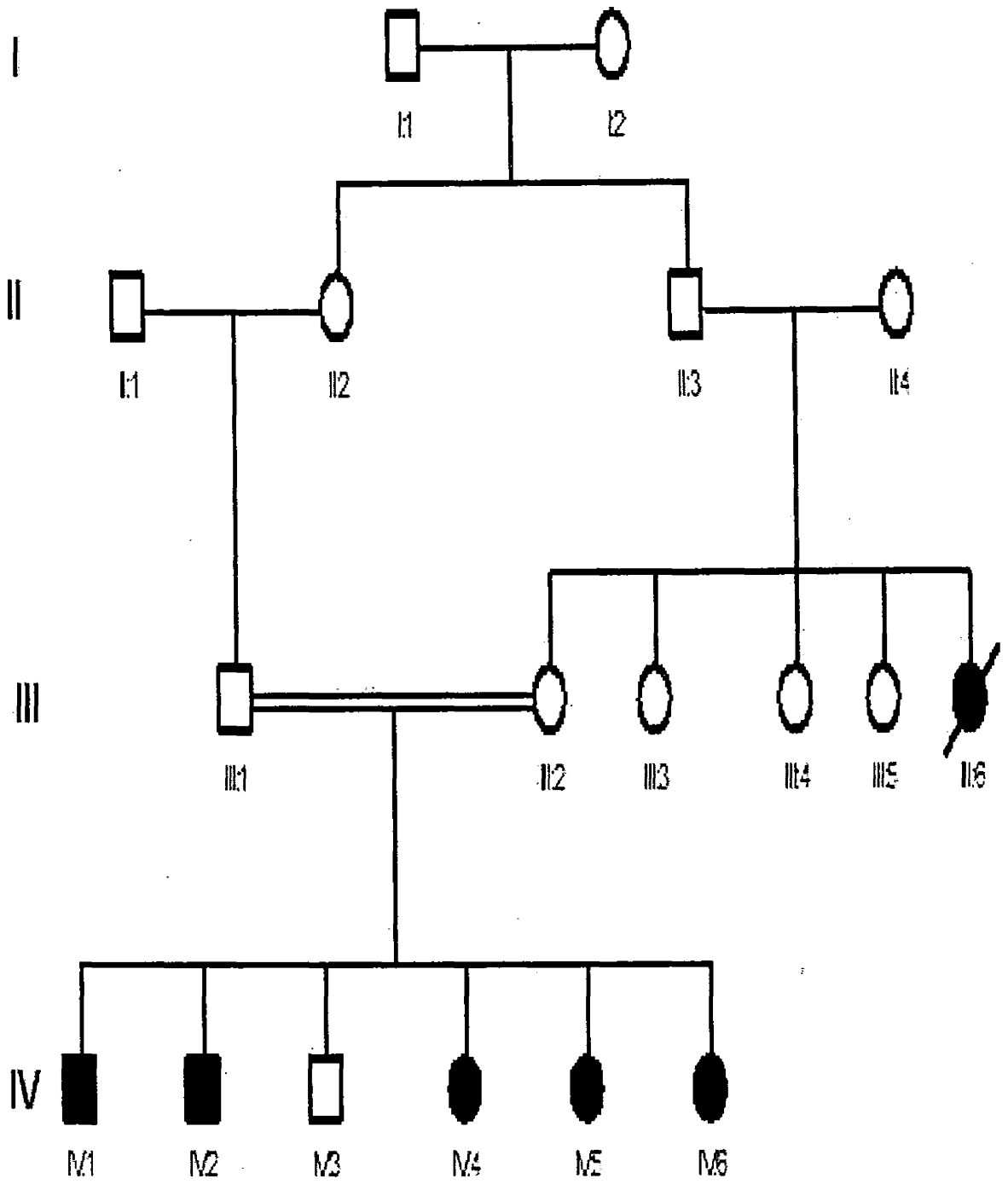

Figure 4.5 Pedigree of family C showing autosomal recessive mode of inheritance of AP. Circles represent females and squares show males. Filled squares and circles represent affected individuals, whereas double lines indicate consanguineous marriage.

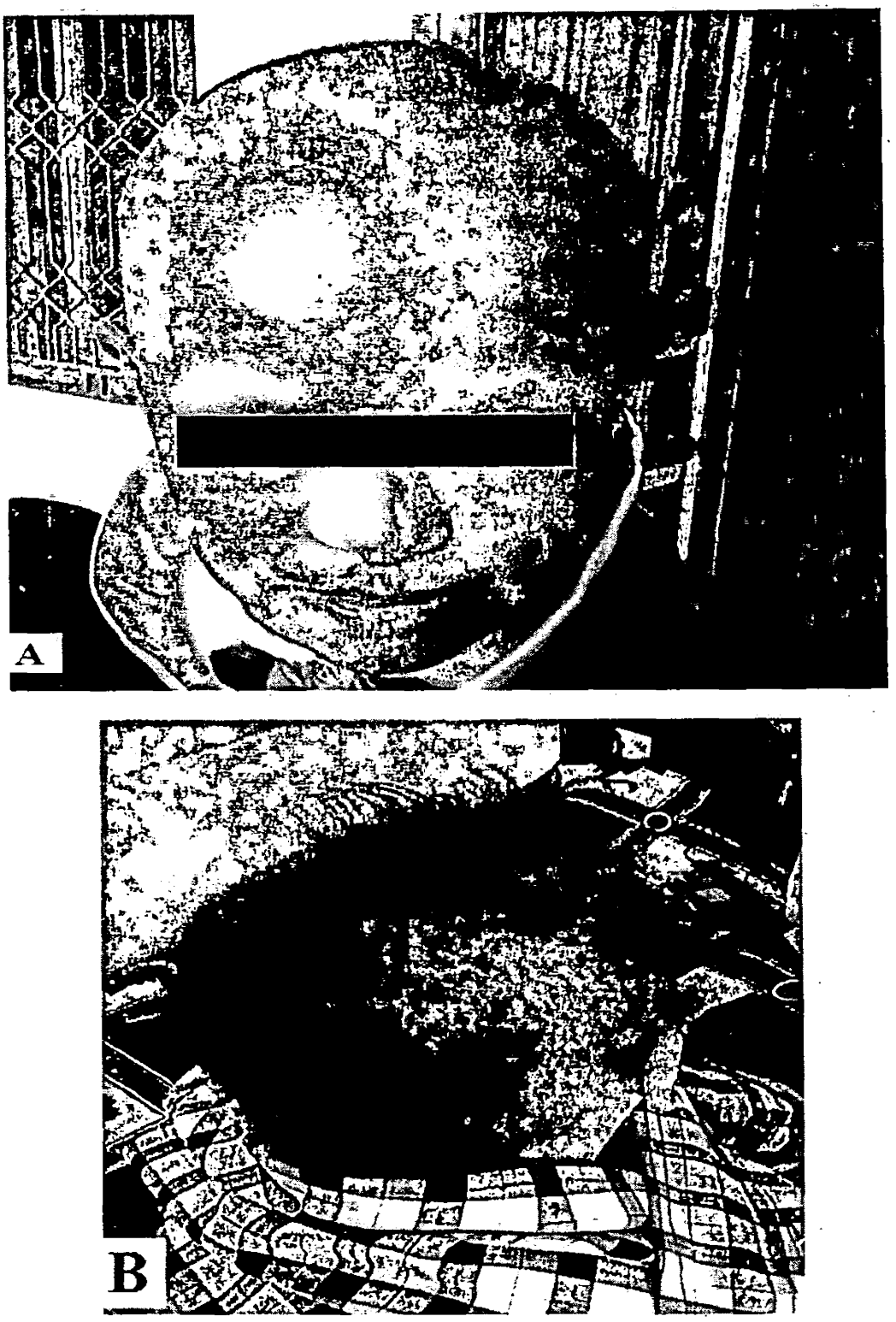

Figure 4.6 A: shows the clinical features of affected individual (IV-6) in family C showing autosomal recessive hypotrichosis phenotype. B: represents the clinical presentation of affected individual (IV-5) showing Autosomal recessive hypotrichosis phenotype

4.2 Mutational Screening

4.2.1 Mutational Screening of Hairless Gene (*HR*) in Family A & B

All the exons and their splice junctions of human hairless (*HR*) gene were amplified (Table 3.2) using Polymerase chain reaction (PCR) and were sequenced directly in ABI Prism 310 DNA Sequencer (PE Applied Bio-systems, Foster City, CA, USA). The mentioned coding portion and the exon-intron borders of the hairless gene (*HR*) were sequenced in normal and affected individuals of the two families.

In family A, the sequence analyses of the *HR* gene show us no mutation in the any amplified exons of the *HR* gene. We therefore suggest that may be the mutation is present is somewhat that exons of the *HR* gene which we were not sequenced or it may predicted that the mutation may lie in the *HR*'s regulatory region.

In family B, the sequence analyses of the *HR* gene show us the substitution mutation in exon 3a, at position 285 (GsubstA). The substitution mutation changes the glycine (hydrophobic) to serine (polar). Sequence electropherograms of the affected and normal individuals for the mutation is shown in the Figure 4.7.

4.2.2 Mutational Screening of *P2RY5/LPAR6* gene in the Family C

The bigger exon 2 and their splice junctions in total 5 exons of *P2RY5/LPAR6* gene were amplified (Table 3.3) using Polymerase chain reaction (PCR) and were sequenced directly in ABI Prism 310 DNA Sequencer (PE Applied Bio-systems, Foster City, CA, USA). Exon 2 was amplified by three sets of overlapping primers. The mentioned coding portion and the exon-intron borders of the *P2RY5/LPAR6* gene were sequenced in normal and affected individuals of the family.

In family C, the sequence analyses of the *P2RY5/LPAR6* gene show us the substitution known mutation in exon 2b, at position 565 (GsubstA). Sequence electropherograms of the affected and the normal individuals are shown in the Figure 4.9. This specific missense mutation was also recognized by Shimmomura *et al.*, (2008) and Azeem *et al.*, (2008). Thus, our results for this family are matching with their study.

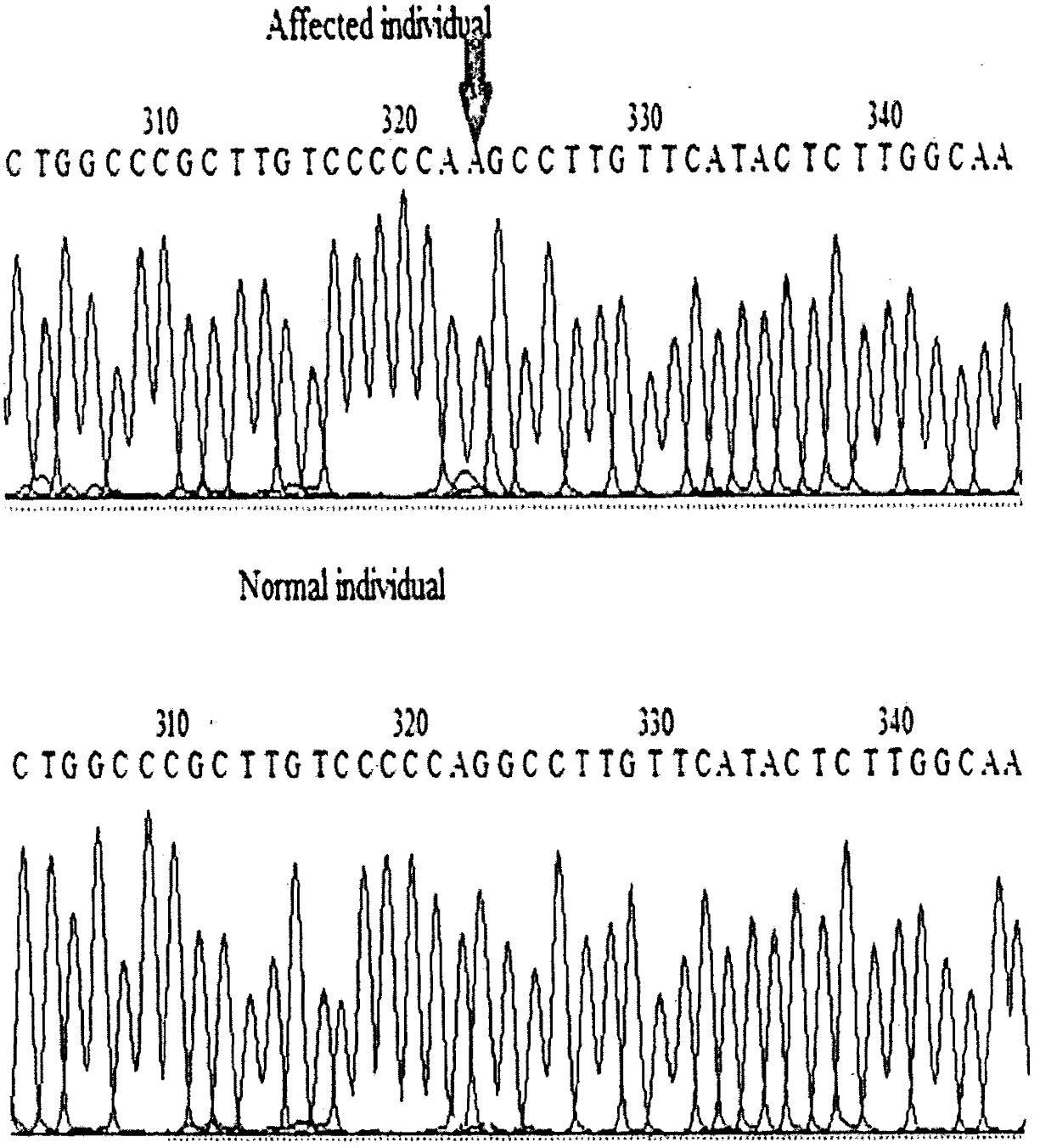

Figure 4.7 Mutational analyses in the Family B of the *HR* gene. Sequence analysis of exon 3a of hairless gene in both affected and normal individual, the substitution mutation of the nucleotide G with A at position 285 is shown in figure.

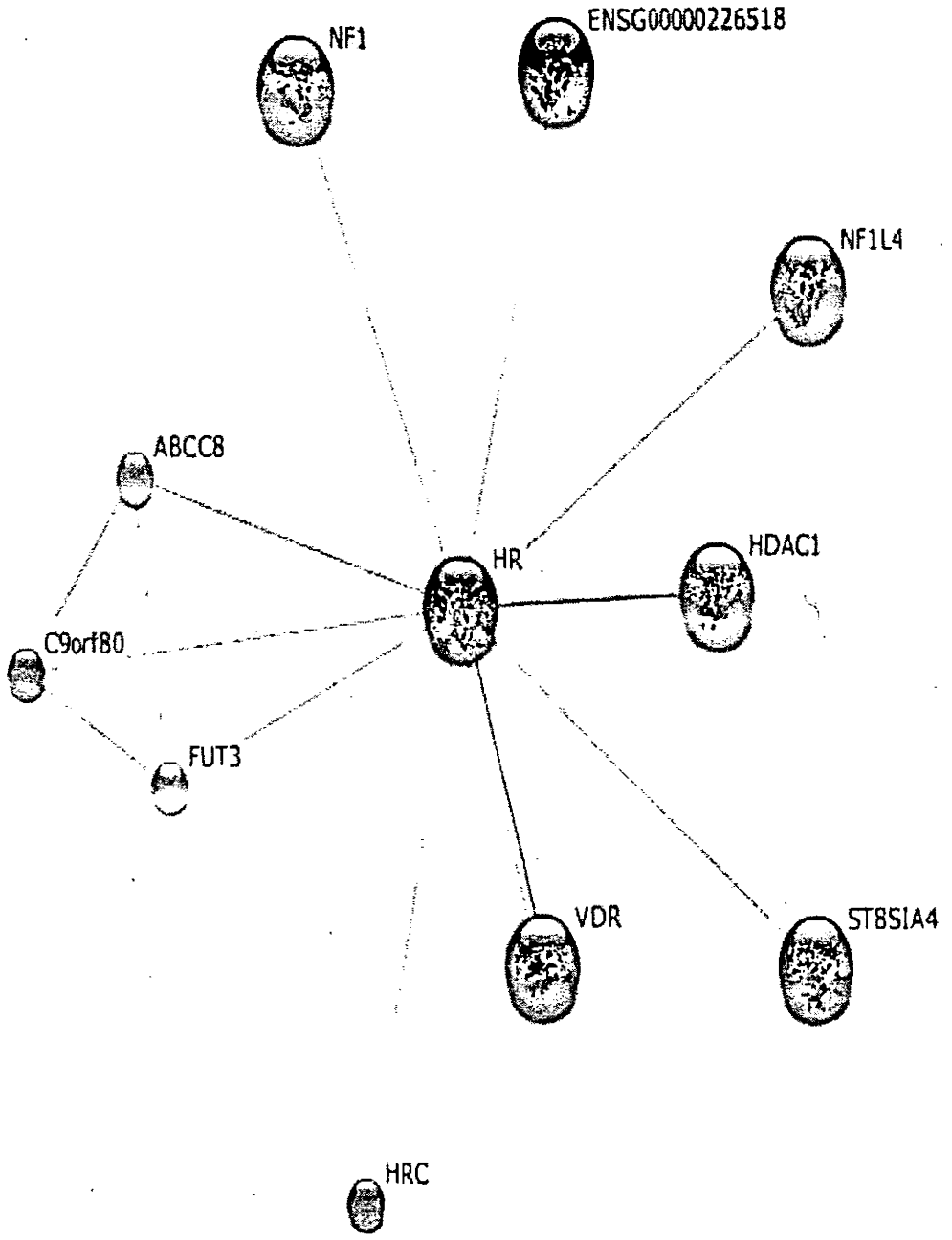

Figure 4.8 Human Hairless protein interaction which may act as a transcription factor that could act on to regulate one of the phases of hair growth (STRING 9.0). Human hairless protein have close interaction with HDAC1 having the confidence score of 0.824.

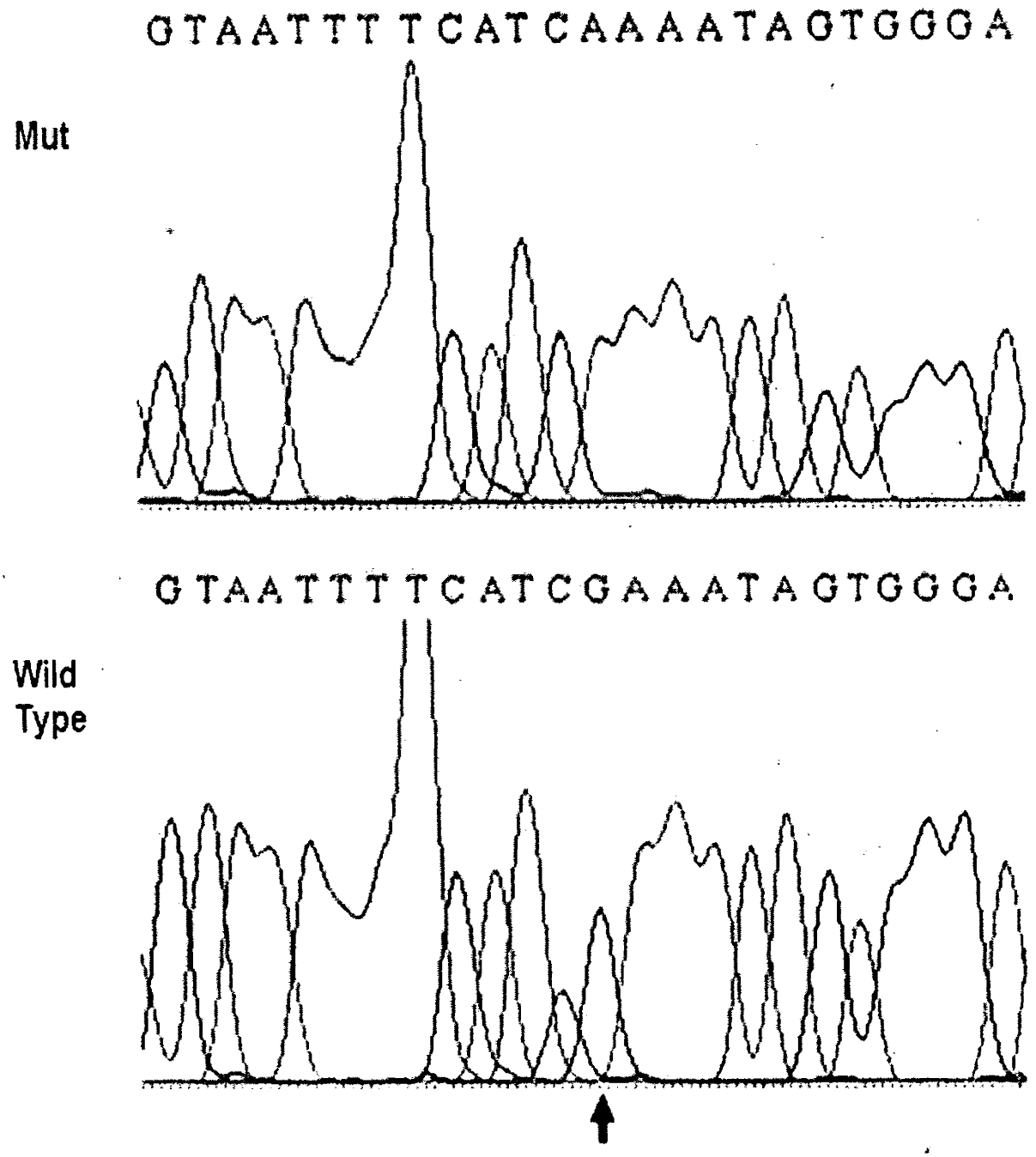

Figure 4.9 Mutational analyses in the Family C of the *P2RY5* gene. Sequence analysis of exon 2b of hairless gene in both affected and normal individual, the substitution mutation of the nucleotide G with A at position 565 is shown in figure

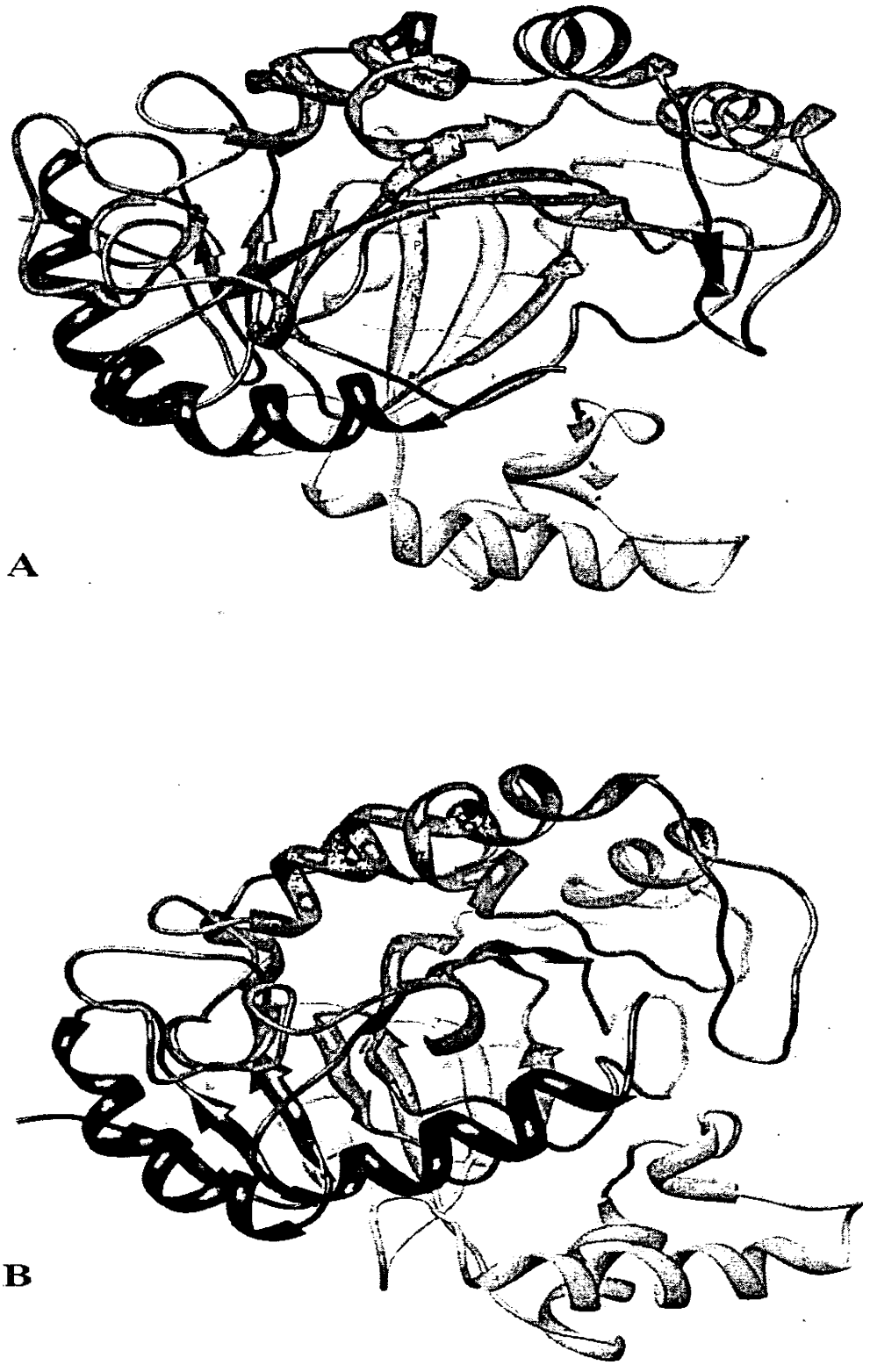

Figure 4.10 Predicted structures of Normal (A) and mutated (B) proteins of *P2RY5/LPAR6* gene

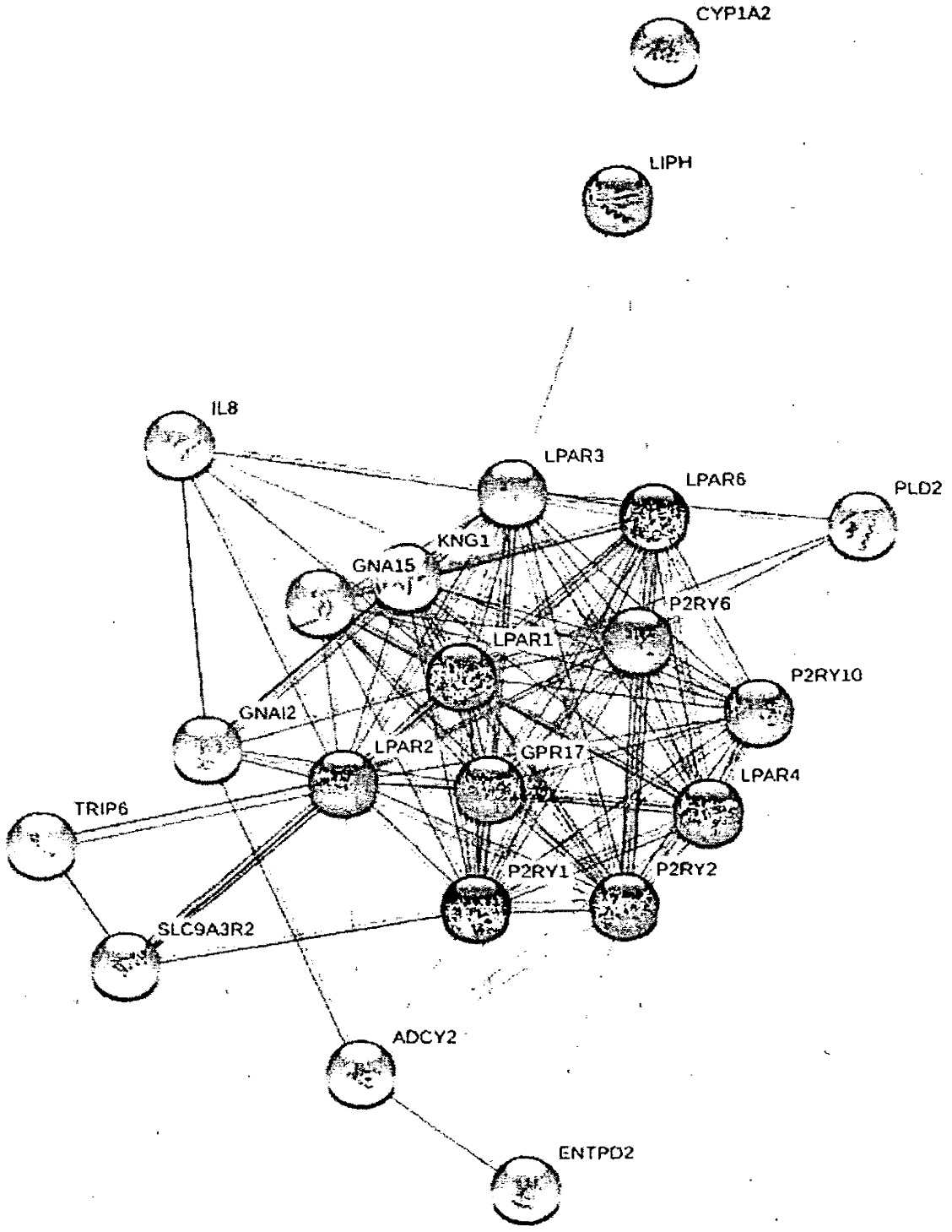

Figure 4.11 *P2RY5/LPAR6* (*Lysophosphatidic acid receptor 6*) binds to *LIPH*, *LPAR* Important of the maintenance of hair growth and texture. LPAR6 protein has close interaction with LIPH having the confidence score of 0.959. (STRING 9.0)

4.3 Insilco Analysis:

A comparative modeling technique (MODELER 9v10) was adopted to predict the three dimensional structure of the hairless protein encoded by the *HR* gene. The protein data bank (PDB) was checked for the 3D structure of the selected protein, and it was confirmed that no 3D structure had been predicted to date. Hairless protein interacts with its predicted partner proteins are *HDAC1, NF1, NF1L4, ST8SIA4, VDR, HRC, FUT3*, and *ABCC8* (Figure 4.8). Human hairless protein has close interaction with HDAC1 having the confidence score of 0.824. The second close interaction of human hairless is with NF1 having the confidence score of 0.786. *HR* may act as a transcription factor that could act on to regulate one of the phases of hair growth. *HDAC1* is the histone deacetylase 1, which is responsible for the de-acetylation of lysine residues at the N-terminal of core histones; this de-acetylation gives a tag for the epigenetic repression and plays a prime role in cell cycle progression, developmental events and transcriptional regulation. *NF1* is a neurofibromin 1, which stimulates the GTPase activity of Ras. *FUT3* is a fucosyltransferase 3, which may catalyze alpha-1, 3 and alpha-1, 4 glycosidic linkages. *VRD* is the vitamin D receptor, which mediates the action of vitamin D3 by controlling the expression of hormone sensitive gene. *HRC* is a histidine rich calcium binding protein, may play a role in the regulation of calcium withdrawal (STRING 9.0).

Homology modeling was implemented to generate the 3D structure of the encoded protein encoded by LPAR6/P2RY5 gene. MODELER 9v10 was used to construct the protein model. The amino acid sequence of P2RY5 in FASTA format was retrieved from Uniprot. The structure predicted by MODELLER 9v10 with the alpha helices and beta-pleated sheets visualized by Chimera 1.6 is illustrated in Figure 4.10. *P2RY5/LPAR6* protein interacts with its predicted partner proteins that are *LIPH, LPAR1, LPAR2, LPAR3, LPAR4, P2RY1, P2RY2, P2RY6,* and *P2RY10* (Figure 4.11). *LPAR6 protein has* close interaction with LIPH having the confidence score of 0.959. The second close interaction of LPAR6 protein is with LPAR2 having the confidence score of 0.957.The confidence score of LPAR6 protein with LPAR3 protein is 0.930 *P2RY5/LPAR6* is a lysophosphatidic acid receptor 6, binds to oleoyl-L-alpha-lysophosphatidic acid (*LPA*). Intracellular *cAMP* is involved in the receptor activation that is important for the maintenance of hair growth and texture (344aa). *LIPH* is a

lipase, member H that hydrolyzes specifically phosphatidic acid (*PA*) to produce lysophosphatidic acid (*LPA*). *LPAR1* is a lysophosphatidic acid receptor 1, receptor for lysophosphatidic acid (*LPA*). *LPAR2* is a lysophosphatidic acid receptor 2, it is a receptor for lysophosphatidic acid (*LPA*) a mediator of diverse cellular activities plays a prime role in phospholipase C-beta signalling pathway (STRING 9.0).

5. DISCUSSION

All hair disorders are normally hair follicle disorders, as hair formation and growth takes place in hair follicle structure. Genetic conditions that affect hair structure or other hair growth cycle may be isolated or they may occur as part of complex syndromes with associated abnormalities of other ectodermal appendages. Alopecias are of different types based on related disorders; syndromic alopecias and isolated alopecias or non-syndromic alopecias. Syndromic forms of alopecia shows hair loss condition in association with various clinical conditions i.e. nail dystrophy, mental retardation, epilepsy, impaired sweating, immunodeficiency, cataracts and retinas pigmentation. The inheritance pattern of these syndromic autosomal alopecia syndromes are both recessive and dominant type. In isolated alopecia, the genetic studies of several forms of hypotrichosis or alopecia in inherited families led to the identification of novel genes/loci controlling hair growth. Several genetic defects recognized for the isolated alopecias. The inheritance pattern observed as both autosomal recessive and dominant in these isolated forms of alopecias.

All the exons and their splice junctions of human hairless (*HR*) gene were amplified and were sequenced of both normal and affected individuals of the two families. In family A, the sequence analyses of the PCR product show us no mutation in the any amplified exons of the *HR* gene. In family B, the sequence analyses of the amplicon showed us the substitution mutation in exon 3a, at position 285 (GsubstA). This substitution mutation changes the glycine (hydrophobic) to serine (polar).

In the *HR* gene, glycine formation participates in the formation of normal phenotype, but due to mutation at position 285 (GsubstA), glycine changed to serine, which is predicted to be the cause of phenotype. Hairless protein interacts with its predicted partner proteins are *NF1, NF1L4, HDAC1, ST8SIA4, VDR, HRC, FUT3*, and *ABCC8*. Human hairless protein has close interaction with HDAC1 having the confidence score of 0.824. *HR* may act as a transcription factor that could act on to regulate one of the phases of hair growth.

So far, 43 different mutation of *HR* gene have been recognized (Zlotogorski *et al.*, 2002, Ahsoor *et al.*, 2005, Sprecher *et al.*, 1999, Ashoor *et al.*, 2005, Ahmad *et*

al., 1999, Cichon *et al.*, 1998, Indelman *et al.*, 2002, 2003). It has been cleared that *HR* gene plays a main role in alopecia disease.

The exon 2 and splice junctions of *P2RY5/LPAR6* gene were amplified. The mentioned coding region of *P2RY5/LPAR6* gene was sequenced in both affected and normal of family C. The sequence analysis of amplicon of this family showed the substitution mutation in exon 2b at position 565 (GsubstA). This specific missense mutation was also recognized by Shimmomura *et al.*, (2008) and Azeem *et al.*, (2008). Therefore, our results for this family is matching with the study took by Azeem *et al.*, (2008) and Shimmomura *et al.*, 2008.

MODELLER 9v10 was employed for comparative modelling. This mutated structure might have reduced binding with Guanine binding proteins, which leads to reduction in transduced extra cellular signals. *P2RY5/LPAR6* protein interacts with its predicted partner proteins that are *LIPH, LPAR1, LPAR2, LPAR3, LPAR4, P2RY1, P2RY2, P2RY6,* and *P2RY10. LPAR6 protein has* close interaction with LIPH having the confidence score of 0.959.

So far, 13 mutations are recognized in the *P2RY5/LPAR6* gene, in which seven are missense mutation, four are frameshaft mutation, one is non-sense and one is complex deletion mutation (Shimmomura et al. 2008; Azeem et al. 2008).

FUTURE PROSPECTS

Identification of novel genes in family B and role of novel mutation in family B and subsequent characterization of proteins they encode will further increase our knowledge of molecular mechanisms underlying the disease. This and further studies in this field will help getting rid of such genetic anomalies and securing human beings.

6. REFERENCES

Adriani M., Martinez-Mir A., Fusco F., Busiello R., Frank J., Telese S., Matrecano E., Ursini M.V., Christiano A.M., and Pignata C., (2004) Ancestral founder mutation of the nude (FOXN1) gene in the congenital severe combined immunodeficiency associated with alopecia in southern Italy population, Annals of Human Genetics, 68, p. 256-268

Ahmad M., Abbas H., and Haque S., (1993) Alopecia Universalis as a single abnormality in an inbred Pakistani kindred, American Journal of Medical Genetics, 46, p. 369-371

Ahmad W., Haque M.F.U., Brancolini V., Tsou H.C., Ul-Haque S., Lam H.M., Aita V.M., Owen J., Blaquiere M., Frank J., Peter B., Friedman C., Ahmad M., Ott J., and Christiano A.M., (1998a) Alopecia universalis associated with a mutation in the human hairless gene, Science, 297, p. 720-724

Ahmad W., Irvine A.D., Lam H., Buckley C., Bingham E.A., Panteleyev A.A., Ahmad M., McGrath J.A., and Christiano A.M., (1998b) A missense mutation in the zinc-finger domain of the human hairless gene underlies congenital atrichia in a family of Irish travellers, American Journal of Human Genetics, 63, p. 984-991

Ahmad W., Zlotogorski, A., Panteleyev, A.A., Lam, H., Ahmad, M., ul Haque, M.F., Abdallah, H.M., Dragan, L., and Christiano, A.M., (1999) Genomic organization of the human hairless gene (HR) and identification of a mutation underlying congenital atrichia in an Arab Palestinian family, Genomics, 56, p. 141–148

Ahsoor G.G., Greenstein R.M., and Lam H., (2005) Novel compound heterozygous nonsense mutations in the hairless gene causing atrichia with papular lesions, Journal of Dermatological Science, 40, p. 29–33

Aita V.W., Ahmad W., and Panteleyev A.A., (2000) A novel missense mutation (C622G) in the zinc-finger domain of the human hairless gene associated

with congenital atrichia with papular lesions, Experimental Dermatology, 9, p. 157–162

Ali G., Chishti M.S., Raza S.I., John P., and Ahmad W., (2007) A mutation in the Lipase H (LIPH) gene underlie autosomal recessive hypotrichosis, Human Genetics, 121, p. 319-325

Anzari H., Shimizu H., and Nishikawa T., (1996) X- linked recessive inheritance in a family with isolated congenital alopecia, The Lancet, 347, p. 337-338

Ashoor G.G., Greenstein R.M., Lam H., Martinez-Mir A., Zlotogorski A., and Christiano A.M., (2005) Novel compound heterozygous nonsense mutation in the hairless gene causing atrichia with papular lesions, Journal of Dermatological Science, 40, p. 29-33

Aslam M., Chahrour M.H., Razzaq A., Haque S., Yan K., Leal S.M., and Ahmad W., (2004) A novel locus for autosomal recessive form of hypotricosis maps to chromosome 3q26.33-q27.3, Journal of Medical Genetics, 41, p. 849-852

Ayub M., Basit S., Jelani M., Ur-Rehman F., Iqbal M., Yasinzai M., and Ahmad W., (2009) A homozygous nonsense mutation in human desmocollin-3 (DSC3) gene underlies hereditary hypotrichosis and recurrent skin vesicles, American Journal of Human Genetics, 85, p. 515–520

Azeem Z., Jelani M., Naz G., Tariq M., Wasif N., Kamran-Ul-Hassan., Naqvi S., Ayub M., Yasinzai M., Amin-Ud-Din M., Wali A., Ali G., Chishti M.S., and Ahmad W., (2008) Novel mutations in G proteincoupled receptor gene (P2RY5) in families with autosomal recessive hypotrichosis (LAH3), Human Genetics, 123, p. 515–519

Balazs L., Okolicany J., Ferrebbe M., Tolley B., and Tigyi G., (2001) Topical application of the phospholipids growth factor lysophosphatidic acid promotes wound healing in vivo, American Journal of Physiology, Regulatory, Integrative and Comparative Physiology, 280, p. R466-R472

Baumer A., Belli S., Trueb R.M., and Schinzel A., (2000) An autosomal dominant form of hereditary hypotrichosis simplex maps to 18pl1.32-pl 1.23 in an Italian family, European Journal of Human Genetics, 8, p. 443-448

Bettina B., (2007) Genetic factors in alopecia areata, Publisher Universiteit Antwerpen (Belgium), page 160

Betz R.C., Indelman M., Pforr J., Schreiner F., Bauer R., Berman R., Lentze M.J., Nothen M.M., Cichon S., and Sprecher E., (2007) Identification of mutations in the human hairless gene in two new families with congenital atrichia, Archives of Dermatological Research, 299, p. 157-161

Betz R.C., Lee Y.A., Bygum A., Brandrup F., Bernal A.l., Toribio J., Alvarez J.I., Kukuk G.M., Ibsen H.H.W., Rasmussen H.B., Wienker T.F., Reis A., Propping P., Krüse R., Cichon S., Nothen M.M., (2000) A gene for hypotrichosis simplex of the scalp maps to chromosome 6p21.3, American Journal of Human Genetics, 66, p. 1979-1983

Birch-Machin M.A., Healy E., Turner R., Haldane F., Belgaid C.E., Darlington S., Stephenson A.M., Munro C., Messenger A.G., and Rees J.L., (1997) Mapping of monilethrix to the type II keratin gene cluster at chromosome 12q13 in three new families, including one with variable expressivity, British Journal Dermatology, 137, p. 339-343

Chavanas S., Garner C., Bodemer C., Ali M., Hamel-Teillac D., Wilkinson J., Bonafe J.L., Paradisi M., Kelsell D.P., Ansai S., Mitsuhashi Y., Larregue M., Leigh I.M., Harper J.I., Taieb A., Prost Y., Cardon L.R., and Hovnanian A., (2000) Localization of the Netherton syndrome gene to chromosome 5q32 by linkage analysis and homozygosity mapping, American Journal of Human Genetics, 66, p. 914-921

Christiano A.M., (2010) Scientists Find Possible Genetic Roots of Type of Hair Loss: Genetic Finding Could Lead to New Targets for Treatment of Alopecia Areata, WebMD Health News, June 30 Available online on this link http://www.webmd.com/skin-problems-and-treatments/hair-loss/news/20100630/sci entists-find-possible-genetic-roots-of-alopecia-areata

Cichon S., Anker M., Vogt 1.R., Rohleder H., Putzstuck M., Hillmer A., Farooq S.A., Al-Dhafri K.S., Ahmad M., Ul-Haque S., Rietschel M., Propping P., Kruse R., and Nothen M.M., (1998) Cloning, genomic organization, alternative transcripts and mutational analysis of the gene responsible for autosomal recessive universal congenital alopecia, Human Molecular Genetics, 7, p. 1671-1679

Cichon S., Hillmer A.M., Betz R.C., Kruse R., and Nothen M.M., (2006) Congenital atrichia. In: Sprecher E (ed) Progress in monogenic hair disorders, Nova Science, New York, p. 77-90

Cichon S., Kruse R., Hillmer A.M., Kukuk G., Anker M., Altland K., Knapp M., Propping P., and Nothen M.M., (2000) A distinct gene close to the hairless locus on chromosome 8p21 underlies hereditary Marie Unna type hypotrichosis in a German family, British Journal of Dermatology, 143, p. 811-814

Davalos N.O., Garcia-Vargas A., Pforr J., Davalos I.P., Picos-Cardenas V.J., Garcia-Cruz D., Kruse R., Figuera L.E., Nothen M.M., Betz R.C., (2005) A non-sense mutation in the corneodesmosin gene in a Mexican family with hypotrichosis simplex of the scalp, British Journal of Dermatology, 153, p. 1216-1219

De-Andrade M., Jackow C.M., Dahm N., Hordinsky M., Reveille J.D., and Duvic M., (1999) Alopecia areata in families: association with the HLA locus, Journal of Investigative Dermatology Symposium Proceedings, 4, p. 220-223

Djabali K., Zlotogorski A., and Metzker A., (2004) Interaction of hairless and thyroid hormone receptor is not involved in the pathogenesis of atrichia with papular lesions, Experimental Dermatology, 13, p. 251–256

Ellis J.A., Scurrah K.J., Cobb J.E., Zaloumis S.G., Duncan A.E., and Harrap S.B., (2007) Baldness and the androgen receptor: the AR polyglycine repeat polymorphism does not confer susceptibility to androgenetic alopecia, Human Genetics, 121, 451-457

Ellis J.A., Stebbing M., and Harrap S.B., (2001) Polymorphism of the androgen receptor gene is associated with male pattern baldness. Journal of Investigative Dermatology, 116, p. 452-455

Ellis J.A., Stebbing M., Harrap S.B., (1998) Genetic analysis of male pattern baldness and the 5alpha-reductase genes. Journal of Investigative Dermatology, 110, p. 849–853

Fatemi F., Rahmaniyan N., Vetankhah M., and Hashemi F., (2010) Prevalence of androgenic alopecia in women of 20-70 years in Isfahan during 2008-2009, Journal of Pakistan Association of Dermatologists, 20, p.75-78

Feinstein A., Engelberg S., and Goodman R.M., (1987) Genetic disorders associated with severe alopecia in children: a report of two unusual cases and a review, Journal of Craniofacial Genetics and Developmental Biology, 7, p. 301-310

Frank J., Cserhalmi-Friedman P.B., and Aluiiad W., (2001) Characterization of the desmosomal cadherin gene family, Experimental Dermatology, 10, p. 90-94

Frank J., Pignata C., Panteleyev A.A., Prowse D.M., Baden H., Weiner L., Gaetaniello L., Ahmad W., Pozzi N., Caserhalmi-Friedman P.B., Aita V.M., Uyttendaele H., Gordon D., Qtt J., Brissette J.L., and Christian A.M., (1999) Exposing the human nude phenotype, Nature, 398, p. 473-474

Gilhar A., and Kalish R.S., (2006) Alopecia areata: a tissue specific autoimmune disease of hair follicle, Autoimmunity Reviews, 5, p. 64-69

Green J., and Sinclair R.D., (2000) Genetics of alopecia areata, Australasian Journal of Dermatology, 41, p. 213–218

Green J., Fitzpatrick E., and de-Berker D., (2003) Progressive patterned scalp hypotrichosis, with wiry hair, onycholysis, and intermittently associated cleft lip and palate: clinical and genetic distinction from Marie Unna, Journal of Investigative Dermatology, 8, p. 121–125

Hardy M.H., (1992) The secret life of the hair follicle, Trends Genetics, 8, p.55-60

He P.P., Zhang X.J., Yang Q., Li M., Liang Y.H., Yang S., Yan K.L., Cui Y., Shen Y.Y., Wang H.Y., Sun L.D., Du W.H., Shen Y.J., Xu S.J., and Huang W., (2004) Refinement of a locus for Marie Unna hereditary hypotrichosis to a 1.1-cM interval at 8p21.3. British Journal of Dermatology, 150, p. 837-842

Herzog H., Darby K., Hort Y.J., and Shine J., (1996) Intron 17 of the human retinoblastoma susceptibility gene encodes an actively transcribed G protein-coupled receptor gene. Genome Res, 6, p. 858–861

Hillmer A.M., Hanneken S., Ritzmann S., Becker T., Freudenberg J., Brockschmidt F.F., Flaquer A., Freudenberg-Hua Y., Jamra R.A., Metzen C., Heyn U., Schweiger N., Betz R.C., Blaumeiser B., Hampe J., Schreiber S., Schulze T.G., Hennies H.C., Schumacher J., Propping P., Ruzicka T., Cichon S., Wienker T.F., Kruse R., and Nothen M.M., (2005) Genetic variation in the human androgen receptor gene is the major determinant of common early-onset androgenetic alopecia American Journal of Human Genetics, 77, p. 140-148

Hiramatsu T., Sonoda H., Takanezawa Y., Morikawa R., Ishida M., Kasahara K., Sanai Y., Taguchi R., Aoki J., and Arai H., (2003) Biochemical and molecular characterization of two phosphatidic acid-selective phospholipase A1s, mPA-PLA1alpha and mPA-PLA1beta, The Journal of Biological Chemistry, 278, p. 49438–49447

Hiraoka M., Abe A., and Shayman J.A., (2005) Structure and function of lysosomal phospholipase A2: identiWcation of the catalytic triad and the role of cysteine residues, The Journal of Lipid Research, 46, p. 2441–2447

Horev L., Djabali K., Green J., Sinclair R., Martinez-Mir A., Ingber A., Christiano A.M., and Zlotogorski A., (2003) De novo mutation in monilethrix, Experimental Dermatology, 12, p. 882-885

Hunt D.M., Rickman L., Whittock N.V., Eady R.J., Simrak D., Dopping J.C., Stevens H.P., Armstrong D.B., Henries H.C., Kuster W., Hughes A.E., Arnemann J., Leigh I.M., McGrath J.A., Kelsell D.P., and Buxton R.S., (2001) Spectrum of dominant mutations in the desmosomal cadherin

desmoglein 1, causing the skin disease striate palmoplantar keratoderma. European Journal of Human Genetics, 9, p. 197-203

Indelman M., Bergman R., and Lestringant G.G., (2003) Compound heterozygosity for mutations in the hairless gene causes atrichia with papular lesions, British Journal of Dermatology, 148, p. 553–557

Indelman M., Eason J., Hummel M., Loza O., Suri M., Leys M.J., Bayne M., Schwartz F.L., Sprecher E., (2007) Novel CDH3 mutation in Hypotrichosis with juvenile macular dystrophy, Clinical Experimental Dermatology, 32, p. 191-196

Indelman M., Leibu R., Jammal A., Bergman R., and Sprecher E., (2005) Molecular basis of hypotrichosis with juvenile macular dystrophy in two siblings, British Journal of Dermatology, 153, p. 635-638

International Human Genome Sequence Consortium., (2001) Initial sequence and analysis of the human genome, Nature, 409, 860–921

Irvine A.D., and Christiano A.M., (2001) Hair on a gene string: recent advances in understanding the molecular genetics of hairloss, Clinical Experimental Dermatology 26, p. 59-71

Irvine A.D., and McLean W.H., (1999) Human keratin diseases: the increasing spectrum of disease and subtlety of the phenotype–genotype correlation, British Journal of Dermatology, 140, p. 815–828

Jelani M., Chishti M.S., and Ahmad W., (2008) A novel splice-site mutation in the CDH3 gene in hypotrichosis with juvenile macular dystrophy, Clinical and Experimental Dermatology, 34, p. 68–73

John P, Tariq M, Rafiq MA, Amin-ud-din M, Muhammad D, Waheed I, Ansar M, Ahmad W (2006) Recurrent intragenic deletion mutation in desmoglein 4 gene underlies autosomal recessive hypotrichosis in two Pakistani families of Balochi and Sindhi origins. Archives of Dermatological Research 298:135-137.

John P., Ali G., Chishti M.S., Naqvi S.M.S., Leal S.M., and Ahmad W., (2006a) Localization of a novel locus for alopecia with mental retardation syndrome to chromosome 3g26.33-g27.3, Human Genetics, 118, p. 665-667

Just M., Ribera M., Fuente M.J., Bielsa I., and Ferrándiz C., (1998) Hereditary Hypotrichosis simplex, Dermatology, 196, p. 339-342

Kazantseva A., Goltsov A., Zinchenko R., Grigorenko A.P., Abrukova A.V., Moliaka Y.K., Kirillov A.G., Guo Z., Lyle S., Ginter E.K., Rogaev E.I., (2006) Human hair growth deficiency is linked to a genetic defect in the phospholipase gene LIPH, Science, 314, 982-985

Kemp E.H., McDonagh A.J., Wengraf D.A., Messenger A.G., Messenger A.G., Gawkrodger D.J., Cork M.J., Tazi-Ahnini R., (2006) The non-synonymous C1858T substitution in the PTPN22 gene is associated with susceptibility to the severe forms of alopecia areata, Human Immunology, 67, 535-539

Kjaer K.W., Hansen L., Schwabe G.C., Marques-de-Faria A.P., Eiberg H., Mundlos S., Tommerup N., Rosenberg T, (2005) Distinct CDH3 mutations cause ectodermal dysplasia, ectrodactyly, macular dystrophy (EEM syndrome), Journal of Medical Genetics, 42, p. 292–298

Kljuic A., Bazzi H., Sundberg J.P., Martinez M.A., O'Shaughnessy R., Mahoney M.G., Levy M., Montagutelli X., Ahmad W., Aita V.M., Gordon D., Uitto J., Whiting D., Ott J., Fischer S., Gilliam T.C., Jahoda C.A.B., Morris JR, Panteleyev AA, Nguyen VT, Clu•istiano AM (2003a) Desmoglein 4 in hair follicle differentiation and epidermal adhesion: evidence from inherited hypotrichosis and acquired pemphigus vulgaris, Cell, 113, p. 249-260

Koepf-Maier P., Mboneko V., and Merker H., (1990) Nude mice are not hairless, Acta Anatomica, 138, p. 178-190

Kohn G., Metzker A., (1987) Hereditary hypotrichosis simplex of the scalp, Clingy Genetics, 32, p. 120-124

Komatsu N., Takata M., Otsuki N., Ohka R., Amano O., Takehara K., and Saijoh K., (2002) Elevated stratum corneum hydrolytic activity in Netherton syndrome

suggests an inhibitory regulation of desquamation by SPINK5-derived peptides, Journal of Investigative Dermatology, 118, p. 436–443

Kong X., Murphy K., Raj T., He C., White P.S., and Matise T.C., (2004) A combined linkage-physical map of the human genome, American Journal Human Genetics, 76, p. 373

Kruse K., Cichon S., and Anker M., (1999) Novel hairless mutations in two kindreds with autosomal recessive papular atrichia, Journal of Investigative Dermatology, 113, p. 954–959

Kubiak R.J., Yue X., Hondal R.J., Mihai C., Tsai M.D., and Bruzik K.S., (2001) Involvement of the Arg-Asp-His catalytic triad in enzymatic cleavage of the phosphodiester bond, Biochemistry, 40, p. 5422–5432

Lefevre P., Rochat A., Bodemer C., Vabres P., Barrandon Y., de-Prost Y., Garner C., and Hovnanian A., (2000) Linkage of Marie-Unna hypotrichosis locus to chromosome 8p21 and exclusion of 10 genes including the hairless gene by mutation analysis, European Journal of Human Genetics, 8, p. 273-279

Letada P.R., Sparling J.D., and Norwood C., (2007) "Imiquimod in the treatment of alopecia universalis", Cutis; Cutaneous medicine for the practitioner, 79, p. 138–40

Levy-Nissenbaum E., Bar-Natan M., Frydman M., and Pras E., (2005) Confirmation of the association between male pattern baldness and the androgen receptor gene, European Journal of Dermatology, 15, p. 339-340

Levy-Nissenbaum E., Betz R.C., and Frydman, M., (2003) Hypotrichosis simplex of the scalp is associated with nonsense mutations in CDSN encoding corneodesmosin. Nature Genetics, 34, p. 151–153

Indelman M., Bergman R., Lurie R., Richard G., Miller B., Petronius D., Ciubutaro D., Leibu R., and Sprecher E., (2002) A missense mutation in CDH3, encoding P-cadherin, causes hypotrichosis with juvenile macular dystrophy, Journal investigative Dermatology 119, p. 1210-1213

Indelman M., Hamel C.P., Bergman R., Nischal K.K., Thompson D., Surget M.O., Ramon M., Ganthos H., Miller B., Richard G., Lurie R., Leibu R., Russell-Eggitt I., and Sprecher E., (2003) Phenotypic diversity and mutation spectrum in hypotrichosis with juvenile macular dystrophy, Journal investigative Dermatology, 121, p.1217-1220

Lyle S., ChristoWdou-Solomidou M., Liu Y., Elder D.E., Albelda S., and Cotsarelis G., (1998) The C8/144B monoclonal antibody recognizes cytokeratin 15 and deWnes the location of human hair follicle stem cells, Journal of Cell Science, 111, p. 3179–3188.

Lyn T.E., (2009) Researchers in Japan have identified a gene that appears to determine cyclical hair loss in mice and believe it may also be responsible for hair loss, or alopecia, in people, Routers, also available on http://uk.reuters.com/article/2009/05/25/science-us-hair-loss idUKTRE54O4D020090525

MacNeil S., (2007) Progress and opportunities for tissue-engineered skin, Nature, 445, 874-880

Madison K.C., (2003) Barrier function of the skin: "la raison d'etre" of the epidermis, Journal of Investigative Dermatology, 121, p. 231-241

Magert H.J., Standker L., Kreutzmann P., Zucht H.D., Reinecke M., Sommerhoff C.P., Fritz H., and Forssmann W.G., (1999) LEKT1, a novel 15-domain type of human serine proteinase inhibitor, The Journal of Biological Chemistry, 274, p. 21499-21502

Marshall R.C., and Orwin D.F., (1991) Structure and biochemistry of mammalian hard keratin, Electron Microscopy Reviews, 4, 47-83

Masse M., Martinez-Mir A., and Lam H., (2005) Identification of a recurrent mutation in the human hairless gene underlying atrichia with popular lesions, Clinical Experimental Dermatology, 30, p. 363–365

Matoltsy A.G., (1958) Keratinization of embryonic skin, Journal of Investigative Dermatology, 31, p. 343-346

McDonagh A.J., and Tazi-Ahnini R., (2002) Epidemiology and genetics of alopecia areata, Clinical Experimental Dermatology, 27, 405-409

McKusick VA., (2000) OMIM (On-line Mendelian Inheritance in Man) In: 2000 Release Edn. Baltimore: Johns Hopkins University, (www3.ncbi.nlm.nih/gov/omim/)

Messenger A.G., Bazzi H., Parslew R., Shapiro L., Christiano A.M., (2005) A missense mutation in the cadherin interaction site of the desmoglein 4 gene underlies localized autosomal recessive hypotrichosis, Journal of Investigative Dermatology, 125, p. 1077-1079

Millar S.E., (2002) Molecular mechanisms regulating hair follicle development, Journal of Investigative Dermatology, 118, p. 216–225

Milner Y., Sudnik J., Filippi M., Kizoulis M., Kashgarian M., Stenn K., (2002) Exogen, shedding phase of the hair growth cycle: characterization of a mouse model, Journal of Investigative Dermatology, 119, p. 639–644

Mitsudo K., Jayakumar A., Henderson Y., Frederick M.J., Kang Y., Wang M., El Naggar A.K., and Clayman G.L., (2003) Inhibition of serine proteinases plasmin, trypsin, subtilisin A, cathepsin G, and elastase by LEKTI: A kinetic analysis, Biochemistry, 42, p. 3874–3881

Mizuno Y., Suga Y., Haruna K.H., Muramatsu S., Hasegawa T., Kohroh K., Shimizu T., Komatsu N., Ogawa H., and Ikeda S., (2006) A case of a Japanese neonate with congenital ichthyosiform erythroderma diagnosed as Netherton syndrome, Clinical Experimental Dermatology, 31, p. 677-680

Moolenaar W.H., van Meeteren L.A., and Giepmans B.N., (2004) The ins and outs of lysophosphatidic acid signalling, Bioessays, 26, p. 870–881

Moore K.A., Lemischka I.R., (2006) Stem Cells and Their Niches, Science, 311, p. 1880-1885

Morling N., Frentz G., Fugger L., Georgsen J., Jakobsen B., Odum N., and Svejgaard A., (1991) DNA polymorphism of HLA class II gene in alopecia areata, Dis Marker, 9, p. 35-42

Moss C., Martinez-Mir A., Lam H., Tadin-Strapps M., Kljuic A., Christiano A.M., (2004) A recurrent intragenic deletion in the desmoglein 4 gene underlies localized autosomal recessive hypotrichosis, Journal of Investigative Dermatology, 123, p. 607–610

Naz G., Ali G., Naqvi S.K., Azeem Z., Ahmad W., (2010) Mapping of a novel autosomal recessive hypotrichosis locus on chromosome 10q11.23–22.3, Human Genetics, Online.

Nehls M., Pfeifer D., Schorpp M., Hedrich H., Boehm T., (1994) New member of the winged-helix protein family disrupted in mouse and rat nude mutations, Nature, 372, p. 103-107

Netherton E.W., (1958) A unique case of trichorrhexis nodosa: 'bamboo hairs, Archives of Dermatological Research, 78, p. 483-487

Norwood O.T., (1975) Male-pattern baldness; Classification and incidence, South Medical Journal, 68, p. 1359-1370

Nothen M.M., Cichon S., Vogt I.R., Hemmer S., Kruse R., Knapp M., Holler T., Faiyaz ul Haque M., Haque S., Propping P., Ahmad M., and Rietschel M., (1998) A gene for universal congenital alopecia maps to 8p21-22, American Journal of human Genetics, 62, p. 386-390

Oliver R.F., Jahoda C.A., (1988) Dermal-epidermal interactions, Clinical Dermatology, 6, p. 74–82

Osborn D., (1916) Inheritance of baldness, Various pattern due to heredity and sometimes present at birth a sex-linked character-dominant in man and woman women not bald unless they inherit tendency from both parents, Journal of Hereditary, 7, p. 347-355

Oshima H., Rochat A., Kedzia C., Kobayashi K., Barrandon Y., (2001) Morphogenesis and renewal of hair follicles from adult multipotent stem cells, Cell, 104, p. 233–245

Paller A.S., Varigos G., and Metzker A., (2003) Compound heterozygous mutations in the hairless gene in atrichia with papular lesions, Journal of Investigative Dermatology, 121, p. 430–432

Panteleyev A.A., Ahmad W., Malashenko A.M., Ignatieva E.L., Paus R., Sundberg J.P., and Christiano A.M., (1998) Molecular basis for the rhino Yurlovo (hr(rhY)) phenotype: severe skin abnormalities and female reproductive defects associated with an insertion in the hairless gene, Experimental Dermatology, 7, p. 281-288

Panteleyev A.A., Botchkareva N.V., Sundberg J.P., Christiano A.M., and Paus R., (1999) The role of the hairless (hr) gene in the regulation of hair follicle catagen transformation, American Journal of Pathology, 155, p. 159–171

Panteleyev A.A., vander-Veen C., Rosenbach T., Sokolov V.E., Müller-Röver S., and Paus R., (1998) Towards defining the pathogenesis of the hairless phenotype, Journal of Investigative Dermatology, 110, p. 902-907

Paradisi M., Chuang G.S., Angelo C., Pedicelli C., Martinez M.A., and Christian A.M., (2003) Atrichia with papular lesions resulting from a novel homozygous missense mutation in the hairless gene, Clinical Experimental Dermatology, 28, p. 535-538

Pasternack S.M., von-Kugelgen I., Al-Aboud K., Lee Y.A., Rüschendorf F., and Voss K., (2008) G protein-coupled receptor P2Y5 and its ligand LPA are involved in maintenance of human hair growth, Nature Genetics, 40, p. 329–334

Pecoraro V., Astore I.P.L., (1990) Measurement of hair growth under physiological conditions, In: Orphanos C.E., Happle R., editors., Hair and hair disease, Heidelberg, Germany: Springer Verlag Berlin, p. 237–254

Petukhova L., Duvic M., Hordinsky M., Norris D., Price V., Shimomura Y., Kim H., Singh P., Lee A., Wei V. Chen, Katja C. Meyer, Paus R., Jahoda C.A.B, Amos C.I, Gregersen P.K., & Christiano A.M., (2010) Genome-wide association study in alopecia areata implicates both innate and adaptive immunity, Nature, 466, p. 113–117

Pinheiro M., Freire M.N., (1985) Atrichias and Hypotrichiosis: a brief review with description of a recessive atrichia in two brothers, Human Hereditary, 35, p. 53-55

Powell B.C., Rogers G.E., (1997) The role of keratin proteins and their genes in the growth structure and properties of hair, Experimental Supplement, 178, p. 59-148

Pragst F., Balikova M.A., (2006) State of the art in hair analysis for detection of drug and alcohol abuse, Clinica Chimica Acta, 370, p. 17-49

Pragst F., Rothe M., Spiegel K., and Sporkert F., (1998) Illegal and therapeutic drug concentrations in hair segments—a timetable of drug exposure, Forensic Science Reviews, 10, p. 81–111

Proksch E., Brandner J.M., and Jensen J.M., (2008) The skin: an indispensable barrier, Experimental Dermatology, 17, p. 1063-1072

Rafiq M.A., Ansar M., and Mahmood S., (2004) A recurrent intragenic deletion mutation in DSG4 gene in three Pakistani families with autosomal recessive hypotrichosis, Journal of Investigative Dermatology, 123, p. 247–248

Rafique M.A., Ansar M., Jamal S.M., Malik S., Sohail M., Ul-Haque M.F., Ul-Haque S., Leal S.M., and Ahmad W., (2003) A locus for hereditary hypotrichosis localized to human chromosome 18g21.1, European Journal of Human Genetics, 11, p. 623-628

Raison-Peyron N., Duval P.A., Barneon G., Durand L., Arnaud B., Meynadier J., Hamel C., (2000) A syndrome combining severe hypotrichosis and macular dystrophy: absence of mutations in TIMP genes, British Journal of Dermatology, 143, 902-904

Robins D.N., (2007) "Case reports: alopecia universalis: hair growth following initiation of simvastatin and ezetimibe therapy", Journal of drugs in dermatology, 6, p. 946–947

Rogers G.E., and Powell B.C., (1993) Organization and expression of hair follicle genes, Journal of Investigative Dermatology, 101, p. 50S±5S

Salamon T., Schnyder U.W., (1962) Ueber die Monilethrix, Archiv für klinische und experimentelle Dermatologie, 215, 105-136

Sambrook J., and Russel D.W., (2001) Molecular Cloning, 3rd edition, 3 vol, Cold Spring Harbor Laboratory Press, New York

Schaffer J.V., Bazzi H., Vitebsky A., Witkiewicz A., Kovich O.I., Kamino H., Shapiro L.S., Amin S.P., Orlow S.J., and Christiano A.M., (2006) Mutations in the desmoglein 4 gene underlie localized autosomal recessive hypotrichosis with monilethrix hairs and congenital scalp erosions, Journal of Investigative Dermatology, 126, p. 1286–1291.

Schmidt-Ullrich R., and Paus R., (2005) Molecular principles of hair follicle induction and morphogenesis, Bioessays, 27, p. 247–261

Schneider M.R., Schmidt-Ullrich R., and Paus R., (2009) The hair follicle as a dynamic miniorgan, Current Biology, 19, p. 132-142

Schorpp M., Hofmann M., Dear T.N., and Boehm T., (1997) Characterization of mouse and human nude genes, Immunogenetics, 46, p. 509-515

Schweizer, J., (2006) More than one gene involved in monilethrix: intracellular but also extracellular players, Journal of Investigative Dermatology, 126, p. 1216–1219

Segre J.A., Nemhauser J.L., Taylor B.A., Nadeau J.H., and Lander ES (1995) Positional cloning of the nude locus: genetic, physical, and transcription maps of the region and mutations in the mouse and rat, Genomics, 28, p. 549-559

Shimomura Y., and Christiano A.M., (2010) Biology and genetics of hair, Annual Review of Genomics Human Genetics 11, p. 109-132

Shimomura Y., Wajid M., Ishii Y., Shapiro L., Petukhova L., Gordon D., Christiano A.M., (2008) Disruption of P2RY5, an orphan G protein-coupled receptor, underlies autosomal recessive woolly hair, Nature Genetics, 40, p. 335-339

Shimomura Y., Sakamoto F., Kariya N., (2006) Mutations in the desmoglein 4 gene are associated with monilethrix-like congenital hypotrichosis, Journal of Investigative Dermatology, 126, p. 1281–1285

Shimomura Y., Wajid M., Ishii Y., Shapiro L., Petukhova L., Gordon D., Christiano A.M., (2008) Disruption of P2RY5, an orphan G protein-coupled receptor, underlies autosomal recessive woolly hair, Nature Genetics, 40, p. 335–339

Snyder L.H., Yingling H.C., (1935) The application of the gene-frequency method of analysis to sex-influenced factors, with special reference to baldness, Human Biology, 7, p. 608-615

Sonoda H., Aoki J., Hiramatsu T., Ishida M., Bandoh K., Nagai Y., (2002) A novel phosphatidic acid-selective phospholipase A1 that produces lysophosphatidic acid, Journal of Biological Chemistry, 277, p. 34254–34263

Sperling L.C., (1991) Hair anatomy for the clinician, Journal of American Academy of Dermatology, 25, p. 11-17

Sprecher E., Bergman R., Richard G., Lurie R., Shalev S., Petronius D., Shalata A., Anbinder Y., Leibu R., Perlman I., Cohen N., and Szargel R., (2001) Hypotrichosis with juvenile macular dystrophy is caused by a mutation in CDH3, encoding P-cadherin, Nature Genetics, 29, p. 134-136

Sprecher E., Bergman R., Szargel R., Friedman-Birnbaum R., and Cohen N., (1999) Identification of a genetic defect in the hairless gene in atrichia with papular lesions: evidence for phenotypic heterogeneity among inherited atrichias, American Journal of Human Genetics, 64, p. 1323-1329

Sprecher E., Molho-Pessach V., Ingber A., Sagi E., Indelman M., Bergman R., (2004) Homozygous splice site mutations in PKP1 result in loss of epidermal plakophilin 1 expression and underlie ectodermal dysplasia/skin fragility syndrome in two consanguineous families, Journal of Investigative Dermatology, 122, p. 647–651

Sreekumar G.P., Roberts J.L., Wong C.Q., Stenn K.S., Parimoo S., (2000) Marie Unna hereditary hypotrichosis gene maps to human chromosome 8p21 near hairless, Journal of Investigative Dermatology, 114, p. 595-597

Stevens H.P., Kelsell D.P., Bryant S.P., Bishop D.T., Dawber R.P., Spurr N.K., Leigh I.M., (1996) Linkage of monilethrix to the trichocyte and epithelial keratin gene cluster on 12q11-q13, Journal of Investigative Dermatology, 106, 795-797

Stucker M., Struk A., Altmeyer P., Herde M., Baumgartl H., and Lubbers D.W., (2002) The cutaneous uptake of atmospheric oxygen contributes significantly to the oxygen supply of human dermis and epidermis, Journal of Physiology 538, p. 985-994

Tariq M., Ayub M., Jelani M., Basit S., Naz G., Wasif N., Raza S.I., Naveed A.K., ullah-Khan A., Azeem Z., Yasinzai M., Wali A., Ali G., Chishti M.S., Ahmad W., (2009) Mutations in the P2RY5 gene underlie autosomal recessive hypotrichosis in 13 Pakistani families, British Journal Dermatology, 160, p.1006–1010

Toribio J., Quinones P.A., (1974) Hereditary hypotrichosis simplex of the scalp–evidence for autosomal dominant inheritance, British Journal of Dermatology, 91, 687–696

Tosti A., Bellavista S., & Lorizzo M., (2006), Alopecia areata; A long term follow up study of 191 patients, Journal of the American Academy of Dermatology, 55, p. 438-441

Townes T.M., Behringer R.R., (1990) Human globin locus activation region (LAR): role in temporal control, Trends in Genetics, 6, p. 219–229

Trembath R.C., (1997) Identification of amajor susceptibility locus on chromosome 6p and evidence for further disease loci revealed by a two stage genome-wide search in psoriasis, Human Molecular Genetics, 6, p. 813–820

Unna M., (1925) Ueber Hypotrichosis congenita hereditaria, Dermatology, 81, p. 1167-1178

Urlaub G., Mitchell P.J., Ciudad C.J., Chasin L.A., (1989) Nonsense mutations in the dihydrofolate reductase gene affects RNA processing, Molecular Cell Biology, 7, p. 2868-2680

Van-Neste D., Leroy T., Conil S., (2007) Exogen hair characterization in human scalp. Skin Research Technology, 13, p. 436-443

Van-Steelsel M.A.M., Steijlen P.M., Bladergroen R.S, Vermeer M., van-Geel M., (2005) A missense mutation in the type II hair keratin hHb3 is associated with monilethrix, Journal of Medical genetics, 42, p. 19

Van-Steensel M., Smith F.J., Steijlen P.M., Kluijt I., Stevens H.P., Messenger A., Kremer H., Dunnill M.G., Kennedy C., Munro C.S., Doherty V.R., McGrath J.A., Covello S.P., Coleman C.M., Uitto J., and McLean W.H., (1999) The gene for hypotrichosis of Marie Unna maps between D8S258 and D8S298; exclusion of the hr gene by cDNA and genomic sequencing, American Journal of Human Genetics, 65, p. 413–419

Wagner H., (1935) Maculaaffektion, vergesellschaftet mit Haarabnormitat von Lanugotypus, beide vielleicht angeboren bei zwei Geschwistem, Graefe's Archive for Clinical and Experimental Ophthalmology, 134, p. 74-81

Wajid M., Bazzi H., Rockey J., Lubetkin J., Zlotogorski A., and Christiano A.M., (2007) Localized autosomal recessive hypotrichosis due to a frameshift mutation in the desmoglein 4 gene exhibits extensive phenotypic variability within a Pakistani family, Journal of Investigative Dermatology, 127, 1779-1782

Wali A., Ansar M., Khan M.N., and Ahmad W., (2006a) Atrichia with papular lesions resulting from a novel insertion mutation in the human hairless gene, Clinical Experimental Dermatology, 31, p. 695-698

Wali A., Chishti M.S., Ayub M., Yasinzai M., Kafaitullah., Ali G., John P., and Ahmad W., (2007) Localization of a novel autosomal recessive hypotrichosis locus (LAH3) to chromosome 13814.11-q21.32, Clinical Genetics, 72, p. 23-29

Wali A., John P., Gul A., Lee K., Chishti M.S., Ali G., Hassan M.J., Leal S.M., Ahmad W., (2006b) A novel locus for alopecia with mental retardation syndrome (APMR2) maps to chromosome 3g26.2-g26.31, Clinical Genetics, 70, p. 233-239

Walley A.J., Chavanas S., Moffatt M.F., Esnouf R.M., Ubhi B., Lawrence R., Wong K., Abecasis G.R., Jones E.Y., and Harper J.I., (2001) Gene polymorphism in Netherton and common atopic disease, Nature Genetics, 29, p.175–178

Weiner J.A., Fukushima N., Contos J.J., Scherer S.S., and Chun J., (2001) Regulation of schwann cells morphology and adhesion by receptor-mediated lysophosphatidic acid signalling, Journal of Neuroscience, 21, p.7069–7078

Welsh E.A., Clark H.H., Epstein S.Z., Reveille J.D., Duvic M., (1994) Human leukocyte antigen-DQB1*03 alleles are associated with alopecia areata, Journal of Investigative Dermatology, 103, p. 758-763

Welsh N., and Guy A., (2009) The lived experience of alopecia areata: A qualitative study, Body Image, 6, p. 194–200

Wen Y., Liu Y., Xu Y., Zhao Y., Hua R., Wang K., (2009) Loss-of-function mutations of an inhibitory upstream ORF in the human hairless transcript cause Marie Unna hereditary hypotrichosis, Nature Genetics, 41, p. 228–233

Whiting D.A., (1993) Diagnostic and predictive value of horizontal sections of scalp biopsy specimens in male pattern androgenetic alopecia, Journal of American Academy of Dermatology, 28, p. 755–763

Whittock N.V., and Bower C., (2003) Genetic evidence for a novel human desmosomal cadherin, desmoglein 4, Journal of Investigative Dermatology, 120, p. 523-530

Wilkinson R.D., Curtis G.H., and Hawk W.A., (1964) Netherton's disease: trichorrhexis invaginata (bamboo hair), congenital ichthyosiform erythroderma and the atopic diathesis; A istopathologic study, Archives of Dermatological Research, 89, p. 46-54

Wilson C., Cotsarelis G., Wei Z.G., Fryer E., Margolis-Fryer J., Ostead M., Tokarek R., Sun T.T., and Lavker R.M., (1994) Cells within the bulge region of mouse hair follicle transiently proliferate during early anagen: heterogeneity and functional diVerences of various hair cycles. DiVerentiation, 55, p. 127–136

Winter H., Rogers M.A., Gebhardt M., Wollina U., Boxall L., Chitayat D., Babul-Hirji R., Stevens H.P., Zlotogorski A., and Schweizer J., (1997) A new mutation in the type II hair cortex keratin hHb1 involved in the inherited hair disorder monilethrix, Nature Genetics, 101, p. 165–169

Winter H., Rogers M.A., and Langbein L., (1997b) Mutations in the hair cortex keratin hHb6 cause the inherited hair disease monilethrix, Nature Genetics, 16, p.372–374

Xiao F.L., Zhou F.S., Liu J.B., Yan K.L., Cui Y., Gao M., Liang Y.H., Sun L.D., Zhou S.M., Zhu Y.G., Zhang X.J., and Yang S., (2005) Association of HLA-DQA1 and DQB1 alleles with alopecia areata in Chinese Hans, Archives of Dermatology Research, 297, p. 201-209

Yang S., Gao M., Cui Y., Yan K.L., Ren Y.Q., and Zhang G.L., (2005) Identification of a novel locus for Marie Unna hereditary hypotrichosis to a 17.5 cM interval at 1p21.1– 1q21.3, Journal of Investigative Dermatology, 125, p. 711–714

Zhao Y., Ma Z.H., Yang Y., Yang S.X., Wu L.S., Ding B.L., Lin Z.M., Wang A.P., Bu D.F., and Tu P., (2007) SPINK5 gene mutation and decreased LEKTI

activity in three Chinese patients with Netherton's syndrome, Clinical Experimental Dermatology, 32, p. 564-567

Zlotogorski A., Ahmad W., and Christiano A.M., (1998) Congenital atrichia in five Arab Palestinian families resulting from a deletion mutation in the human hairless gene, Human Genetics, 103, p. 400-404

Zlotogorski A., Marek D., and Horev L., (2006) An autosomal recessive form of monilethrix is caused by mutations in DSG4: clinical overlap with localized autosomal recessive hypotrichosis. Journal of Investigative Dermatology, 126, p. 1292–1296

Zlotogorski A., Martinez-Mir A., and Green J., (2002) Evidence for pseudodominant inheritance of atrichia with papular lesions, Journal of Investigative Dermatology, 118, p. 881–886

Electronic Data Base Used

String: http://string-db.org/

Ensemble Human Genome Browser: http://www.ensembl.org/index.html

UCSC Genome Browser: http://genome.ucsc.edu/

National Centre for Biotechnology Information: http://www.ncbi.nlm.nih.gov/