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Atrichia with Papular Lesions in Five Consanguineous Palestinian Families Caused by a Single Base Pair Deletion Mutation within the Human Hairless Gene

By

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The undersigned hereby certify that they have read and recommend to the Faculty of Scientific Research and Higher Studies at the Palestine Polytechnic University and the Faculty of Science at Bethlehem University for acceptance a thesis entitled:

Atrichia with Papular Lesions in Five Consanguineous Palestinian Families Caused by a Single Base Pair Deletion Mutation within the Human Hairless Gene

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Atrichia with Papular Lesions in Five Consanguineous Palestinian Families Caused by a Single Base Pair Deletion Mutation within the Human Hairless Gene

Abstract

Background:

Hair loss has significant psychological and social impacts on individuals affected with the disease and can cause considerable anxiety among family members. Atrichia with Papular Lesions (APL; Mendelian Inheritance in Man no. 209500) is an example of rare genetic hair loss disease that is inherited in an autosomal recessive pattern. It is characterized by complete irreversible hair loss on the scalp and entire body during the first months of life with widespread papules (keratin-filled cysts) on the skin that appear within the first years of life. This disease spreads in consanguineous families with high fertility rate especially residents in small geographically isolated regions. Molecular analysis revealed mutations in the hairless gene *(HR)* on chromosome 8p12 which are considered to be the main cause of this genetic disorder. This gene encodes a putative transcription factor with a single zinc-finger domain of 1189 amino acids and is highly expressed in brain and skin. The purpose of our study is to discover the genetic causes of APL in a number of Palestinian families suffering from this disease.

Methods:

To find the causative mutation for APL, five Palestinian families with the disease were recruited for our study. Linkage Exclusion Analysis was used at the beginning of the study to exclude this locus as the cause of the disease. HR gene could not be excluded in our families thus we performed Sanger Sequencing to identify the causative mutation in this gene. Sanger sequencing was also used to test the genotype phenotype segregation within the families and to determine the carrier frequency of this mutation in 100 healthy controls.

Results:

Sanger sequencing results revealed a single base pair deletion mutation at position 2147 (2147delC), which leads to a frameshift and premature termination codon. This stop codon is 544 bps downstream in exon 12. This deletion of C causes a frameshift mutation that changes the reading frame (P716Q fS*186). Also, Sanger sequencing results showed that this mutation in HR gene segregates perfectly in a recessive mode of inheritance in all families. This mutation was not detected among 100 healthy Palestinian controls.

Conclusion:

Our study reports a single base pair deletion in exon 9 of HR gene in a homozygous form in all affected members of five Palestinian families with Congenital Atrichia. Mutations in this gene were also discovered to cause APL in people from different ethnic backgrounds.

Key words:

Atrichia with Papular Lesions (APL), Linkage Exclusion Analysis, Sanger Sequencing, Alopecia, HR gene.

ملخص الدراسة:الشعر هو زوائد بروتينية تنمو على اجسام الثديات. يعتبر الشعر من الاجزاء المهمة جدا في الجسم فهو متعدد الوظائف ويعتبر الطابع الجمالي الذي يضفيه على جسم الكائن الحي هو من اهم وظائفه. تساقط الشعر وفقدانه يؤثر بشكل كبيير على الانسان وعلى مسار حياته وخصوصا من النواحي النفسية والاجتماعية.

(**Atrichia with Papular Lesions**) هو مرض فقدان الشعر الدائم الوراثي, وهو مرض نادر متنحي يصيب الذكور والاناث بلا استثناء ويتميز بتساقط الشعر بعد الولادة وعدم نموه مرة اخرى مع انتشار لطفح جلدي على معظم اجزاء الجسم خلال السنوات الاولى من حياة المصاب. اظهرت الدراسات على ان هذا المرض ناتج عن طفرات في جين يسمى (HR). وهذا المرض يظهر بشكل كبير في المناطق الجغرافية الصغيرة التي ينتشر فيها زواج الاقارب. والغرض من دراستنا هو اكتشاف الأسباب الجينية في الأسر الفلسطينية التي تعاني من هذا المرض.

الطرق والأساليب المستخدمة في الدراسة:

أولا تم استخدام تقنية (**Linkage exclusion approach**) لتحديد ان **HR** هو الجين المسؤول عن المرض في خمس عائلات فلسطينية يعاني بعض أفرادها من هذا المرض. بعد ذلك تم استخدام (**Sanger sequencing**) للكشف عن الطفرة المسببة لهذا المرض. ثم تم فحص امكانية و كيفية انتقال الطفرة المكتشفة من جيل الى آخر, وما علاقتها بالاعراض الموجودة لدى الأفراد المصابين بالعائلة. بالإضافة إلى اكتشاف نسبة غير المصابين الحاملين لهذه الطَّفرات في100 فرد من المجتمع الفلسطينيّ.

النتائج:

أظهرت تقنية(**Linkage exclusion approach**) ان **(HR)** هو الجين المسؤول عن المرض. حيث ان هذا الجين مسؤول عن ترجمة بروتين يسمى (a putative transcription factor with a single zinc-finger domain). ثم كشفت تقنية(**Sanger sequencing**) عن وجود طفرة متنحية في الاكسون التاسع لهذا الجين, تسبب حذف قاعدة نيتروجينية واحدة, والتي ادت الى كودون وقف مبكر. وهذا الحذف يؤثر بشكل مباشر على شكل البروتين مما يؤدي الي عدم قدرته على القيام بوظائفه الطبيعية وبالتالي تظهر الاعراض السابقة على المصابين. وتبين ان هذه الطفرة المتنحية تنتقل من الآباء الى الأبناء في هذه العائلات الفلسطينية و تسبب هذا المرض. وكذلك لم نجد أيًّا من ال100 فرد غير المصابين من المجتمع الفلسطيني يحملون هذه الطَّفرات.

وقد تم الكشف في دراسات اخرى ان هذا الجين مسوؤل عن ظهور هذا المرض لدى اشخاص في عدة مناطق في العالم من اصول عرقية مختلفة.

DECLARATION

I declare that the Master Thesis entitled "**Atrichia with Papular Lesions in Five Consanguineous Palestinian Families Caused by a Single Base Pair Deletion Mutation within the Human Hairless Gene**" is my own original work, and hereby certify that unless stated, all work contained within this thesis is my own independent research and has not been submitted for the award of any other degree at any institution, except where due acknowledgment is made in the text.

Name and signature: Roa'a M. Thawabtih.

Date: Jan. 2017.

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Dedication

I would to dedicate this thesis to my father, the one who encouraged me to achieve my goals in this life. Who gave me all he could to attend the university and complete the education. Also I dedicate my thesis to my mother, sister and brother who have supported me all the way during my studies. Special thanks to my supportive husband 'Dr. Amin Thawabteh' who never loses faith in every step I take, the one who gave me his time and efforts to reach this stage.

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List of Abbreviations

APL	Atrichia with Papular Lesions
AU	Alopecia Universalis
HR	Hairless gene
TH	Thyroid Hormone
TR	Thyroid Receptor
HF	Hair Follicle
DP	Dermal Papilla
HS	Hair Shaft
ORS	Outer Root Sheath
IRS	Inner Root Sheath
VDDR IIA	Vitamin D–dependent rickets type IIA
PCR	Polymerase chain reaction
DNA	Deoxyribonucleic acid
TR-ID1	Thyroid receptor interacting domains 1
TR-ID2	Thyroid receptor interacting domains 2
ROR	Retinoic acid receptor-related Orphan Receptor
RD	Repression Domain
EDTA	Ethylenediaminetetraacetic acid
Tm	Melting temperature

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CHAPTER ONE

1.1 Introduction

Hair loss has several forms, one of these forms known as alopecia. Patients with alopecia differ in the period of symptoms appearance, strength of the disease, and in presence or absence of ectodermal abnormalities (Ahmad, Irvine et al. 1998). Atrichia with Papular Lesions (APL; Mendelian Inheritance in Man no. 209500) or Congenital Atrichia is an example of inherited hair loss. It is a rare autosomal recessive form of total hair loss characterized by complete irreversible hair loss on the scalp and entire body during the first months of life, with a widespread papules (keratin-filled cysts) on the skin that appear within the first years of life (Ahmad, Zlotogorski et al. 1999). Histologically, mature hair follicles are completely absence in APL patients (Zlotogorski, Panteleyev et al. 2002). Often patients with APL are born with normal hairs, but these neonatal hairs usually shed completely within the first few weeks or months of life and never grows again (Ahmad, Zlotogorski et al. 1999). Hair is always absent from the scalp, axillary, pubic region and other parts of the body, whereas eyebrows and eyelashes are sparse. Otherwise these patients show normal nails, teeth, hearing and sweating. They have normal growth and development. Heterozygous carrier individuals have normal hair, which are indistinguishable from normal individuals (Thomas and Daniel 2011). Males and females are equally affected by this genetic disease (Ahmad, Irvine et al. 1998).

Congenital Atrichia has been reported and published in medical literature at the beginning of the 20th century (Wang, Tu et al. 2013). It is universally misdiagnosed with phenocopy Alopecia Universalis (AU) (MIM 203655) (Yip, Horev et al. 2008). In 1998, this genetic disease was linked to chromosome 8p12 (Ahmad, Irvine et al. 1998, Nothen, Cichon et al. 1998, Sprecher, Bergman et al. 1998). Molecular analysis of families affected with the disease lead to the discovery of mutations in the hairless gene *(HR)* on chromosome 8p12 (Ahmad, Faiyaz ul Haque et al. 1998). This gene consists of 19 exons ranging from 70 to 793 bps in size which spans more than 14 kb. Human Hairless gene has high homology with the mouse and rat HR genes (84% and 83% respectively). This high homology indicates high conservation in the HR gene among different mammalian taxon (Ahmad, Zlotogorski et al. 1999).

The HR gene encodes 127 kDa Hairless (HR) protein of 1189 amino acids. It is a putative transcription factor with a single zinc-finger domain or Lysine-specific demethylase hairless protein. It is highly expressed in brain and skin. This protein has two structural motifs; zinc-finger domain and JmjC domain. In skin, apparently it works in the cellular transition to the first adult hair cycle. It appears to be an important regulator for apoptosis during the remodeling of the catagen phase. After the hair follicle regress and the hair are shed, the telogen-stage follicle never reenters the anagen stage, and hair growth completely stops. New hair never grows, and the result of that is a complete form of inherited alopecia (Panteleyev, Botchkareva et al. 1999). Also, hairless protein plays an important role in the regulation of thyroid hormone in the developed rat brain (Thompson 1996). HR is one of the transcriptional corepresser for thyroid receptor (TR). It interacts with TR in the absence of thyroid hormone (TH). Moreover, HR interacts with other nuclear receptors, including retinoic acid receptor-related orphan receptor and vitamin D receptor (Potter, Beaudoin et al. 2001, Hsieh, Sisk et al. 2003).

1.1.1 A short overview about hair development

Hair is a filamentous structure composed of a strong structural protein called keratin. Its formation usually starts in the third month of fetal life. It has many useful biologic functions; for example, it plays a large role in maintaining body temperature and dryness. It provides a protection from ultra-violet radiation exposure and other elements. Also, it has psychosocial importance in our culture (Paus and Cotsarelis 1999). Each hair consists of two main parts; hair shaft and hair root. The hair root located under the skin surface includes a hair follicle (HF), a hair bulb, a dermal papilla (DP) and inner root sheath (three types; Henley, Huxley and cuticle layers). The hair shaft (HS) consists of three layers the cuticle, cortex and medulla. The medulla is the inner most layer in the hair shaft which present only in thick and large hair. While, the cortex is the middle layer in HS that provides color, strength and texture to the hair. It is surrounded and protected by the cuticle which is the outer most layer in HS (Figure 1.2). The DP is located at the base of the hair follicle and has a very important role in regulation of hair growth (Hardy 1992).

Each hair grows in a follicle (HF) which is a dynamic structure that generates hair through a complex and regulated cycles of growth and remodeling (Panteleyev,

Botchkareva et al. 1999). Stages of hair follicle development constitute a complex process that it requires a series of epithelial-mesenchymal signals to start these multistep processes. Two important cells that control hair follicle cycling, the follicular epithelial stem cells and the specialized mesenchymal cells that form the follicular papilla (Lavker, Sun et al. 2003). In uterus, the epithelial stem cells and underlying mesenchymal cells interact to form hair follicles (Hardy 1992). During this time, the accurate distribution of hair follicles over the surface of the body is formed and the future phenotype of each hair (e.g., long scalp hair and short eyebrow hair) is determined. Many of the molecular signals that control these events were first discovered in drosophila (fruit flies). For example, the mammalian counterparts of the patched, hedgehog, disheveled, notch, wnt, engrailed, armadillo, engrailed, and other signaling pathways, needed for the normal development of drosophila, are critical for the normal formation of hair follicles as well (St-Jacques, Dassule et al. 1998). About 5 million hair follicles cover the human body at birth. There is no additional formation of follicles after birth, although the size of the follicles and hairs can change with time. Several genes are expressed very early in the morphogenesis of the follicles which play a role in the determination of the exact distribution and spacing of the follicles within the body (Paus and Cotsarelis 1999).

1.1.1.1 Hair-follicle morphogenesis

To start follicle development, there are a series of epithelial-mesenchymal signals and reciprocal interactions between the dermis and epidermis. There are three main signals that control the hair follicle growing (Figure 1.1). The initial message (First dermal message) is derived from the dermis and directs the epidermis to thicken, forming a placode and then grow toward dermis, to form hair plug. After that, the second signal comes from epidermis (the epidermal message from the hair bulg) that instructs the dermis to form the dermal papilla (DP). Then, the dermal papilla transmitted the second dermal message to the neighboring epithelial cells of the hair plug which are then known as the 'hair matrix cells' to stimulate them to divide rapidly. These cells then are differentiated into either inner root sheath cells (three types; Henley, Huxley and cuticle layers) or hair shaft cells (three types; the cuticle, cortex and medulla layers of the HS), depending on their position relative to the longitudinal axis of the hair follicle (Hardy 1992).

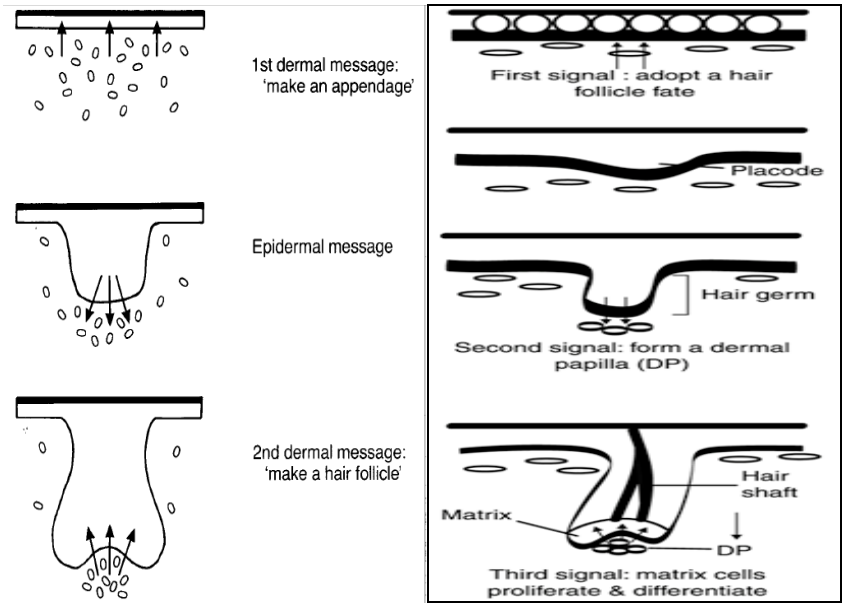


Figure 1.1: Epithelial-mesenchymal interactions during hair follicle development in mammals (Hardy 1992).

1.1.1.2 Hair-Follicle cycling

Each hair follicle always goes through three defined stages: growth (anagen), involution (catagen), and rest (telogen) (Hardy 1992). Most of molecular basis of hair follicle development cycling in human was derived from clinical studies in mice. Through each anagen stage, hair follicle grow and the entire hair shaft or new hair produced from root to tip but during catagen and telogen, follicles enter a rested phase and prepare their stem cells to start new hair growing cycle again. So they can get the signal to begin the next growth phase and make the new hair shaft. The next growth phase begins when telogen transit to anagen phase again. In this stage, epithelial stem cells start to activate. These cells are located in the follicle outer root sheath (ORS) that known as the bulge. The signals of activation come from the dermal papilla when it migrate upward toward these cells and recruit them to start differentiation rapidly to form a new hair matrix from which the new hair emerge; the inner root sheath cells and the hair shaft cells (Alonso and Fuchs 2006) (Figure 1.2).

1.1.1.2.1 Anagen Phase.

It is an active or growing phase during which hair growth occurs, it lasts two to seven years and determines the length of our hair (Philpott, Green et al. 1990). In this stage, epithelial 'hair matrix cells' are stimulated to divide rapidly by the dermal papilla message to differentiate into either inner root sheath cells (Henley, Huxley and cuticle layers) or hair shaft (HS) cells (the cuticle, cortex and medulla). After the HS layers are produced, they are packed tightly with 10-nm cysteine-rich hair keratins filaments, this will give the hair shaft more strength and flexibility (Alonso and Fuchs 2006). There are two molecules that play important roles in hair-follicle cycling and development during this stage which are Fibroblast growth factor 7 and Insulin like growth factor 1. The two molecules are produced by the dermal papilla, and their receptors are mainly found in the matrix cells (Danilenko, Ring et al. 1996). Mice that don't have fibroblast growth factor 7 usually have normal hair follicle, but the receptors are affected for fibroblast growth factor 7 and for fibroblast growth factor 2. The disruption of the receptor causes reduced in the hair-follicle formation (Guo, Degenstein et al. 1996). In addition, the other molecule, Insulin-like growth factor 1 increases and maintains the follicle growth in vitro. So, mice that lack it or its receptor have badly developed hair follicles (Stenn, Combates et al. 1996).

The duration of the anagen cycle play a role in the determining of the length of hair in different areas of the body. For example, scalp hair follicles stay in the anagen stage for two to eight years and produce long hairs, whereas eyebrow hair follicles stay only for two to three months and produce short hairs (Alonso and Fuchs 2006). Fibroblast growth factor 5 is another molecule that controlled the cessation of the anagen stage. Without this growth factor mice have an extended anagen stage, and this causes "angora" phenotype, with hair is 50% longer than normal hair (Hebert, Rosenquist et al. 1994).

1.1.1.2.2Catagen Stage

It is a stage of hair follicles involution during which the majority of follicular keratinocytes and some follicular melanocytes go to die by a programmed cell death (apoptosis) (Lindner, Botchkarev et al. 1997). At the end of this stage, the dermal papilla condenses and moves upward until it reaches and rests underneath the hair-follicle Bulge (Figure 1.2). If the dermal papilla cannot access or fail to reach the bulge, the follicle stops cycling and the hair is lost, as discovered in both humans and mice with mutations of the hairless gene. This gene is responsible to translate a

transcription factor whose disruption prevents the dermal papilla from reaching and interacting with the stem cells of the bulge, causing permanent alopecia (Ahmad, Faiyaz ul Haque et al. 1998, Panteleyev, van der Veen et al. 1998).

1.1.1.2.3 Telogen Stage.

Here in the telogen stage, the hair shaft matures into a club hair. Then it is eventually shed from the follicle, usually through washing or combing. Most people lose 50 to 150 scalp hairs per day. The duration of this stage is often from two to three months before the scalp follicles reenter the anagen stage and the cycle is repeated (Paus and Cotsarelis 1999).

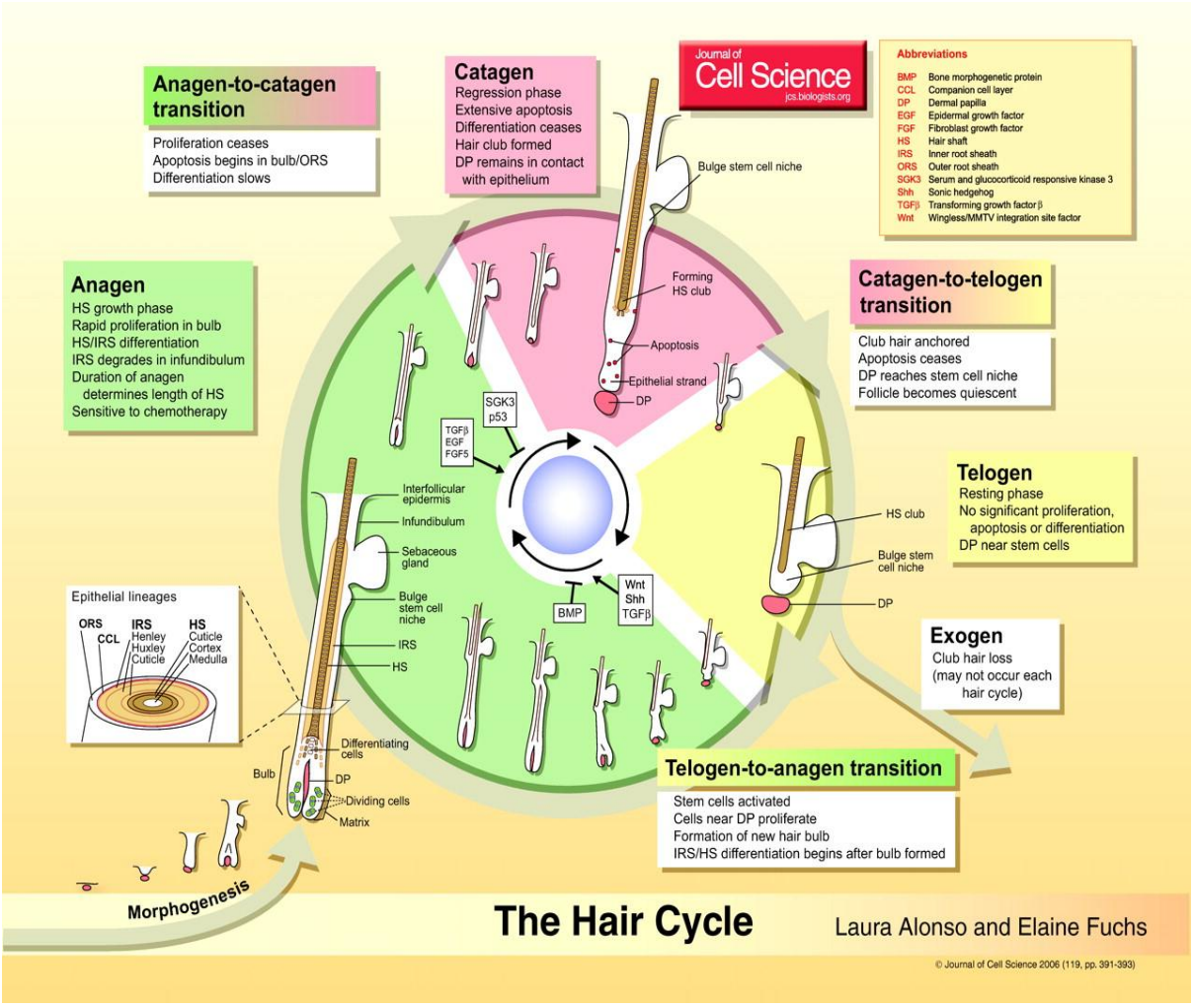


Figure 1.2: Development and Cycling of Hair Follicles. Stages of the morphogenesis of hair follicles and the three stages of follicular cycling (anagen, catagen, and telogen) are shown (Alonso and Fuchs 2006).

1.2 Problem statement and objectives

Atrichia with papular lesions (APL) is an autosomal recessive genetic skin disease with total loss of body hair, followed by eruptions of papular lesions on the skin that appears within the first years of life. Hairs are always lost from the scalp, axillary, pubic region and other parts of the body, and sparse eyebrows and eyelashes (Ahmad, Zlotogorski et al. 1999).

As we all know, hair is important for males and females. It has many useful biologic functions. Usually it keeps most mammals warm, dry and gives protection from the elements and dispersion of sweat-gland products (e.g., pheromones). Also, it has psychosocial importance in our culture (Paus and Cotsarelis 1999). In APL disorder, males and females are always equally affected which exacerbates the disease, because this disorder prevents the body hairs growth in both genders which is one of the most important parts in the human body. Human needs hair to show beauty and to increase individual's self-confidence.

APL is a rare disorder spreads in consanguineous families with high fertility rate especially residents in small geographical regions. The exact prevalence for this disease is unknown or very low and most cases are Arab Palestinians, Pakistani, Caucasians and Australians. However, the number of cases that occur in offspring of unrelated parents has been increased. This increase led us to hypothesize that APL is much more common than previously considered. In the past and until now, many cases of APL were misdiagnosed with Alopecia Universalis (AU). APL patients fail to respond to AU manufactured therapies, and sometimes these drugs cause side effects for APL patients. This misdiagnosis resulted from lack of awareness, lack of known diagnostic criteria and the belief that patients with APL are found only in consanguineous families and rarely (Indelman, Bergman et al. 2003).

Genetic tests like PCR and sequencing can be performed for members of APL families to identify mutations that are responsible for the disorder and to understand its molecular basics. Also, the most important reason for doing these tests is to identify carriers and normal members among families that have patients with APL. Complete understandings of the etiology of this condition will facilitate its prevention. Detection of the mutations can also help to reduce the incidence of this condition, especially in societies which are known to suffer from high rate of consanguineous marriages, such as the Palestinian community.

Since the disease is found among Palestinian families which have high percentage of consanguineous marriages, and due to the special care that is required for patients; we decided to study the genetic causes of APL in consanguineous Palestinian families. The purpose of this study is to determine the mutations responsible for the APL phenotype in five Palestinian families with congenital alopecia.

1.2.1 Specific objectives

- Carry out linkage exclusion analysis to indicate that HR gene mutations are responsible for this phenotype among APL Families before inter in deep in this gene.
- Identify the mutation/s using Sanger sequencing and bioinformatics tools
- Confirm the mutation/s by determining the segregation of the mutation with APL in all families.
- Determine the carrier frequency of each mutation discovered by genotyping at least 100 healthy, unaffected Palestinian controls.

CHAPTER TWO

Review of literature

There are many terms that are usually used to express hair loss, such as alopecia, hypotrichosis, and atrichia. These three terms are different from each other in definition. Alopecia is a non-congenital permanent or reversible hair loss. It could be partial or complete. While hypotrichosis is a diffuse form of baldness congenital or acquired leading to lack of hair. Whereas atrichia is a more severe type of congenital hair loss, rare and characterized by an absence of hair follicles with early-onset of hair loss. Two such conditions of atrichia with papules have been described; Atrichia with Papular lesions (APL; OMIM 209500) and atrichia in patients of vitamin D–dependent rickets type IIA (VDDR IIA; OMIM 277440). VDDR IIA has the same symptoms of vitamin D deficiency such as hypocalcemia, rickets, and osteomalacia. It occurs as a result of mutations in the vitamin D receptor gene (VDR) on chromosome 12q12-q14. (Zlotogorski, Hochberg et al. 2003)

Therefore several types of hereditary hair loss occur as a result of dysregulation of hair follicle cycling (Hardy 1992), Atrichia with Papular Lesions (APL) (OMIM 209500) is a rare genetic disorder inherited in autosomal recessive fashion. It has been reported in medical literature at the beginning of the 20th century. In 1950, scientists released the name of "Atrichia with Papular lesion" on this rare human disease. Patients with APL are usually characterized by normal hair formation at birth, but then this hair was shed. In addition, papular keratin cysts were formed over the body (Zlotogorski, Ahmad et al. 1998). So, in 1954, Damste and Prakken were the first that diagnosed three unrelated women with Congenital Atrichia and diffuse follicular cysts (Damste and Prakken 1954). In Congenital Atrichia, the malformed follicle appears to develop into a follicular cyst instead of mature hair follicle. So, mature hair follicles are absent and keratin-filled cysts are established. Later, in 1989, hairless mouse was suggested as a model for studying hair loss problems in human such as Papular Atrichia disease. Also, in 1989, this disease was first proposed as a homologue to the hairless mouse gene with mutations that mapped to mouse chromosome 14 (Sundberg 1989).

Hair loss occurrence is controlled by several factors; among these reasons are genetics and consanguinity. Inherited types of total hair loss without any skin or internal anomalies are rare diseases. Two main types of this condition have been

described: Alopecia Universalis (AU) (OMIM 203655) (Ahmad, Abbas et al. 1993) and Atrichia with Papular lesions (APL) (OMIM 209500) (Damste and Prakken 1954). AU is an autoimmune disease that occurs as a result of a mutation in the human hairless gene (Ahmad, Irvine et al. 1998). The mode of inheritance of these disorders is the same, which is autosomal recessive. Different mutations in HR gene cause these genetic diseases. Clinical reports of patients with these two inherited diseases typically described, as early hair loss after birth and never regrow again. However, differentiation between AU and APL is of extreme importance but rather difficult. The only difference between these two diseases is that skin in AU patients is normal whereas APL patients develop -between the age of 2 and 26 y- a striking papular rash covering most parts of their skin, particularly on the face, scalp, neck and extremities or may be spars. Papular lesions and follicular cysts filled with cornified material represent a unique skin abnormality among inherited alopecia (Zlotogorski, Panteleyev et al. 2002). The report by Dameste' and Prakken was the first to describe the papules as the unique clinical feature of Congenital Atrichia (Damste and Prakken 1954).

In the past and until now, many cases of APL are misdiagnosed with AU. Mostly APL patients fail to respond to AU manufactured therapies, and sometimes these drugs cause side effects for APL patients. This misdiagnosis occurred as a result of lack of awareness, lack of known diagnostic criteria and the belief that patients with APL are found only in consanguineous families and are usually rare in their prevalence (Indelman, Bergman et al. 2003). Zlotogorski et al.(2002), described 12 clinical and laboratory criteria for diagnosing APL but later Yip et al. (2008), revised them which included family history of consanguinity with autosomal recessive inheritance, atrichia at birth or permanent and complete absence of normal scalp hair by the first few months after birth with failure to regrow; few to widespread smooth, pale, or milia like papules (most commonly on the face, scalp, arms, elbows, thighs or knees) within the first year of life from infancy or childhood, sparse eyebrows and eyelashes; absence of secondary axillary, pubic, or body hair growth, normal growth and development including normal bones, nails, teeth, and sweating, replacement of mature hair follicle structure by follicular cyst filled with cornified material in scalp history, mutation(s) in the human Hairless gene through genetic testing, whitish hypopigmented streaks on the scalp and lack of response to any treatment modality to

restore hair growth (Zlotogorski, Panteleyev et al. 2002), (Yip, Horev et al. 2008), (Loewenthal and Prakken 1961).

2.1 Mapping of the gene locus for Congenital Atrichia.

Scientists identified the gene that causes Congenital Atrichia by using positional candidate approach. The first step in this approach was to use linkage analysis with microsatellite markers to determine the gene locus to an exact chromosome region. The linked region is expected to present a cluster of homozygous marker alleles in affected individuals (homozygosity-mapping). When homozygosity mapping is done and the chromosome locus was determined, the Scientists moved to the second step which is searching for mutations in known candidate gene to identify the responsible mutation(s) in affected individuals.

To map the gene locus to an exact chromosome region for Congenital Atrichia disorder, three independent groups worked on large consanguineous families with APL in different geographical regions. All of them found genetic linkage between APL locus and markers located on Chromosome 8 in a region containing the human HR gene. In 1998, Nöthen, et al, performed an intensive search in large inbred Pakistani family that had been originally described by Ahmad et al in 1993 by using more than 175 highly informative microsatellite markers of the human genome. Haplotype analysis of recombination events localized the gene to a 15-centimorgan region between marker loci D8S261 and D8S1771 on chromosome 8p21-22 (Ahmad, Abbas et al. 1993, Nothen, Cichon et al. 1998). In addition, Ahmed and his colleagues in 1998 worked on another large Pakistani family to search for a locus of the gene by using homozygosity mapping and the linkage analysis as Nöthen, etal worked. They found that the disease gene localized to a 6-cM interval on chromosome 8p12 (Ahmad, Faiyaz ul Haque et al. 1998). Sprecher et al also used linkage analysis method to find the gene locus; they worked on the APL affected Arab-Israeli consanguineous kindred by using 6 polymorphic markers, which are spanning the human hairless gene region. They found that the APL locus maps to chromosome region 8p12 in a 5-cM interval between marker D8S560 and marker D8S1739 (Sprecher, Bergman et al. 1998).

2.2 Identification of the human hairless gene.

The phenotypic similarities between patients with Papular Atrichia and mutant hairless mouse are high. For this reason, scientists used this mouse as a model for studying hair loss problems in human. Brooke in 1924 was the first one who described the relationship between the mouse phenotype and murine HR gene. He identified a mutation in this gene in the mouse 75 years ago (Potter, Beaudoin et al. 2001). The molecular basics for the hairless mouse mutations were determined by Stoy and his colleagues. They discovered the result of the hairless mutation in mice was due to insertion of a murine leukemia virus in intron 6 of the hairless gene (Stoye, Fenner et al. 1988). Hairless (HR) and rhino are two groups of autosomal recessive mouse mutations. Both caused the same phenotypes of total hairless within about 21 days postnatal. At birth, hairless (HR) mice and rhino mice developed normal hair until the second hair cycle is started. At 3-4 weeks of age hairs are completely shed from head to tail and never regrow (Ahmad, Irvine et al. 1998).

HR mice gene was located on chromosome 14 and contained 19 exons (Cachon-Gonzalez et al., 1994). Due to the phenotypic similarity between hairless mice and hairless human, as I mentioned before, the HR murine gene was used as a query to find the human homologue gene. The human HR gene was highly homologous to the murine hairless gene and it had the same genomic structure of the HR gene in mouse; spanning 14 kb of genomic sequence and including 19 exons. The sequence of the human HR gene showed high identity with mouse HR gene sequence at the DNA level and protein level (81% and 80% respectively) (Ahmad, Faiyaz ul Haque et al. 1998). Also, there was large homology between human hairless gene and mouse and rat hairless genes (84% and 83% respectively), suggesting high conservation and thus functional significance of the HR gene among different mammalian taxon's [Ahmad et al., 1999].

2.3 Expression of hairless mRNA.

The expression of the hairless gene was restricted to brain and skin with lower levels of expression in other tissues in our body. This conclusion was reported by many scientists from different geographical regions. Cachon-Gonzalez et al. 1994, Thompos 1996 and Ahmad et al. 1998, were the first researchers that described the expression pattern of HR gene. By using northern blot analysis, they found that this gene was expressed in the brain and skin (Cachon-Gonzalez, Fenner et al. 1994, Thompson

1996, Ahmad, Faiyaz ul Haque et al. 1998). Cachon-Gonzalez et al. 1994 also performed in situ hybridization experiments, and showed that this gene was expressed in hair follicles

Eventually, many studies were done to determine the expression of this gene in other tissues of the human body. In 1998, Cichon and his colleagues defined the expression pattern for this gene in different tissues. They used multiple-tissue cDNA (MTC) panels and showed strongest expression was in skin and small intestine with weaker signals in brain, testis and colon. While a trace expression for this gene was detected in liver, kidney, pancreas, spleen and thymus (Cichon, Anker et al. 1998). In addition, in 1999, Cachon-Gonzalez and his callaboraters identified a more widespread tissue distribution. They reported the HR expression in novel tissues such as cartilage, developing tooth, inner ear, retina, and colon as well as in skin and brain (Cachon-Gonzalez, San-Jose et al. 1999). For more understanding about the expression pattern of HR gene and to determine more sites for the expression, Ahmad et al at 1999 have done another experiment by using a dot blot analysis that containing poly (A)RNA from several human tissues at different developmental stages. They found that high levels of this gene were expressed in several parts of the brain in adult and fetal. Whereas, low levels were seen in colon, stomach, pituitary gland, salivary gland, small intestine, and appendix (Ahmad, Zlotogorski et al. 1999). So, these finding shown that this gene was expressed in a widespread tissues with a temporally regulated. Also, humans, mice and rats share the same tissue expression pattern for this gene (Thompson 1996, Ahmad, Faiyaz ul Haque et al. 1998).

2.4 Tissue specificity in HR gene

At least two isoforms are generated by alternative splicing in the human HR gene. These two isoforms differ from each other by alternative usage of exon 17 in human tissues. Isoform 1, the longest isoform that includes exon 17. It is the most abundant in all the human tissues except the skin. Skin is the site where the shorter isoform; isoform 2, is only expressed. Whereas, kidney and testis only express isoform1. In all other tissues, both isoforms are expressed with the high intensity of the long one (Cichon, Anker et al. 1998).

2.5 Structure of the human hairless gene.

Previously, the murine hairless gene was shown to be organized into 19 exons extending over 19 kb of genomic DNA [Cachon-Gonzalez et al., 1994], with a long open reading frame starting from exon 2 and ending 93 nucleotides before the site of polyadenylation. Translation begins from AUG codon that is located from 378 bps to 380 bps in exon 2. After translation, the HR gene encodes a protein called a putative single zinc-finger transcription factor. The length of this protein is 1182 amino acids and a molecular weight is 130 kDa (Benavides, Oberyszyn et al. 2009)**.**

In mice and human, many different studies showed that the HR gene was highly conserved at DNA and protein levels. In human, the HR gene had the same genomic structure of the HR gene in mouse. It spans more than 14 kb on chromosome 8 at minus strand from 22,114,415 bps to 22,133,384 bps and including 19 exons ranging from 70 to 793 bps in size. Exon 1 includes the 5′ UTR. The sequence from 5′ to exon 1 has a lot of transcription factor-binding sites like GATA-1, GATA-2. While exon 2 contains the methionine starting codon, exon 19 includes the termination codon, 60 bps of the open reading frame and 655 bps of 3′ untranslated region. The size of introns is also the same between human and mice hairless gene (Ahmad, Zlotogorski et al. 1999) (figure 2.1).

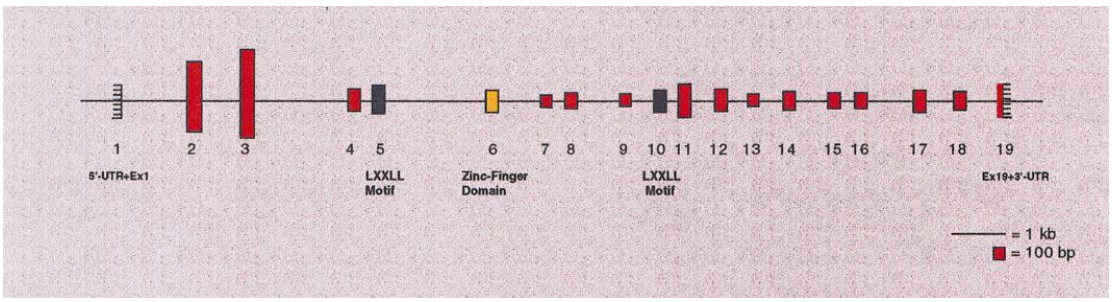


Figure 2.1: Genomic organization of the human HR gene. This gene contains 19 exons spanning 14 kb on Chr8. Exons are represented by bars, while introns by the horizontal line. Exon 1 contains the 5′ UTR, exon 2 contains the starting codon (methionine, AUG), and exon 19 contains the 3′ end of the coding sequence, the termination codon, and the 3′ UTR. The highlighted bars represent the functional domain in this gene. Exon 6 is highlighted with yellow color that represents the zinc-finger domain. As well as exons 5 and 10 is colored with black which is the LXXLL motifs (Ahmad, Zlotogorski et al. 1999).

2.6 The function of the hairless protein.

HR gene encodes approximately 127 kDa putative zinc-finger transcription factor or Lysine-specific demethylase hairless protein of 1189 amino acids. This protein has five functional domains. These domains play an important role to determine the function of this gene. Figure 2.4 shows the organization of these functional domains along the human HR protein (zinc finger, JmjC, TR-IDs, ROR-IDs, and RDs) and their relative orthologs in mouse, dog, opossum and platypus. The first one is a zinc-finger domain containing six cysteines with high evolutionary conservation that is conserved among mouse, rat and human (figure 2.2). This domain is located from amino acid 600 to amino acid 625 (Wali, Ansar et al. 2006). The presence of this domain gives a signal to scientists about the function of this protein which is transcriptional regulation with high evolutionary conservation (Cachon-Gonzalez, Fenner et al. 1994). The second domain is JmjC domain which starts from amino acid 946 to amino acid 1157. It belongs to members of the Jumonji family of eukaryotic transcription factors. This domain was identified in a number of proteins with putative transcriptional function. It is shown to act in histone demethylation mechanism that is conserved from yeast to human (Abbasi 2011). Also, it is predicted to be a candidate enzyme that regulates chromatin remodeling (Clissold and Ponting 2001)

C S R C H H G L F N T H W R C P R C S H R L C V A C human hr

C S R C H H G L F N T H W R C S H C S H R L C V A C mouse hr

C S R C H H G L F N T H W R C S H C S H R L C V A C rat hr

Figure 2.2: The six-cysteine zinc-finger domain. It has high evolutionary conservation that is conserved among mouse, rat and human (Ahmad, Irvine et al. 1998).

In skin, the main function of the HR protein is in hair development and growth in humans and mice. It plays an important role in the regulation of hair follicle regeneration through the hair cycle. HR gene is responsible to translate a transcription factor protein that induces telogen stage to transit to anagen stage by Wnt signaling pathway in hair follicles (figure 2.3). Wnt signaling promotes the re-initiation from rest (telogen) to growth (anagen) stages to start a new hair formation (Abbasi 2011). In 2006, Thrompson and colleagues revealed the function of HR protein in the regulation of Wnt signaling. It repressed the expression of Wise protein which worked as an inhibitor for Wnt signaling. So, any mutation or disruption in HR gene prevents

the repression of Wise protein and Wnt signaling will be inhibited. As a result of this inhibition the transition from telogen to anagen will never occur and hair will never regrow again causing alopecia and in some cases with epidermal abnormalities. Thus, HR protein regulates the timing of Wnt signaling that needed for accurate hair cycling (Thompson, Sisk et al. 2006).

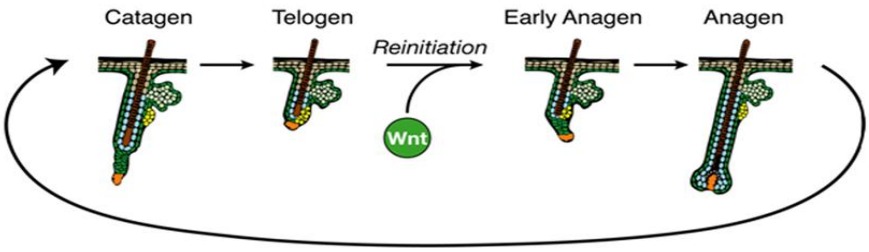


Figure 2.3: diagram illustrates the growth direction of each Hair-Follicle. Wnt signaling plays an essential role in the re-intiation from rest (telogen) stage to growth (anagen) stage to produce new hairs (Thompson 2009).

Moreover, the HR protein plays an essential role in brain. In 1996, Thompson found that the HR gene expression was regulated by thyroid hormone (TH) in the rat brain (Thompson 1996). Then, in 2001, Portter and his colleagues defined HR protein as a new class of nuclear receptor corepressors that interacted with the thyroid hormone receptors and causes repression in the absence of TH (Potter, Beaudoin et al. 2001). The Nuclear receptor proteins have an essential role in the regulation of the expression of different target genes inside cells. So it acts as a transcription factor which works to activate and sometime to repress transcription of genes. Receptors for thyroid hormone, retinoic acid and vitamin D are examples of nuclear receptors that repress transcription in the absence of their ligand. The repression occurs in the present of the co-repressor proteins. These proteins are linked to the nuclear receptors instead of ligand and inhibit the activity of ligand-bound receptors (Thompson 2009). The HR protein connects with unliganded TH receptors (TRs) through two domains: thyroid receptor interacting domains 1 and 2 (TR-ID1 and TR-ID2) (see figure 2.4). TR-ID1 lies from amino acid 786 to amino acid 810 and TR-ID2 from amino acid 1008 to amino acid 1020 (Abbasi 2011).

Also, retinoic acid receptor-related orphan receptor (ROR) is the second type of nuclear receptor that HR proteins interact with. Moraitis et al in 2002 reported that

HR protein worked as a corepressor for ROR receptor in the absence of their ligand. This protein connects with this receptor by two motifs, One is in exon 5 (amino acids 566–570) and the second in exon 10 (amino acids 758–762) (see figure 2.4). These motifs contain LxxLL consensus sequence (Moraitis, Giguere et al. 2002). LXXLL is a short signature motif for this protein where L is leucine and X is any amino acid (Heery, Kalkhoven et al. 1997).

In addition to the previous HR functional domains, Thompson and his colleagues described another functional domain in this protein called repression domain. They showed that hairless protein contained three major repression domains (Thompson 2009). One of these domains is located at the amino acid terminal end (RD1) from amino acid 210 to amino acid 426. The other two domains are located at the carboxyl terminus portion; RD2 from amino acid 730 to amino acid 845 and RD3 from amino acid 845 to amino acid 967 (Azeem, Wasif et al. 2011), (see figure 2.4).

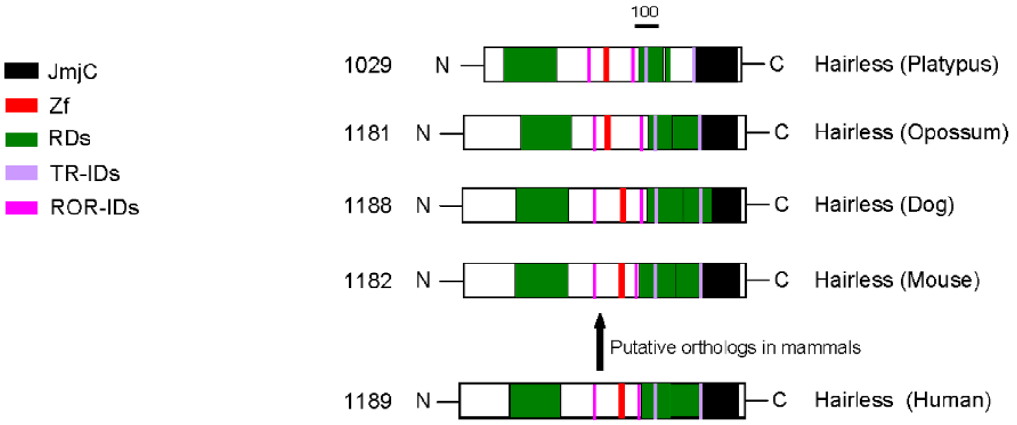


Figure 2.4: Organization of functional domains of HR gene in human and their relative orthologs in mouse, dog, opossum and platypus (Abbasi 2011).

2.7 HR gene mutations in APL.

In humans, a total of forty-six mutations in the hairless gene have been detected so far. Most of these mutations were reported in consanguineous families with high fertility rate especially residents in small geographical regions. However, the number of affected cases that appear in families with unrelated parents has been increased (Wang, Tu et al. 2013). Table 2.1 summarizes the mutations of HR in humans. Until now, families with Congenital Atrichia have been identified from seventeen diversified geographical regions, including Polish, Japanese, German, Israeli, Palestinian, Pakistani, Mexican, Korean, Italian, and Mediterranean populations. These mutations differ from each other, the majority are homozygous missense,

nonsense, deletion, insertion, and splice-site mutations, but also compound heterozygous mutations were reported (O'Regan, Zurada et al. 2007). In 2002, the first compound heterozygous mutation was detected in a non-consanguineous individual (Henn, Zlotogorski et al. 2002).

Table 2.1: **Summary of the HR gene mutations in APL**

Mutation	Location	Protein	Origin	C Or Non-C	Reference
202ins11bp	Exon 2		Pakistani	C	(Wali, Ansar et al. 2006)
431delC	Exon 2		Pakistani	C	(John, Aslam et al. 2005)
2021delG	Exon 8		Pakistani	C	
177del11bp	Exon 2		Jewish Israeli	C	(Zlotogorski, Hochberg et al. 2003)
2147delC	Exon 9		Arab Israeli Arab Palestinian	C C	
C97T	Exon 2	R33X	Mediterranean	Non-C	(Zlotogorski, Panteleyev et al. 2002)
1162ins16bp	Exon 3		Arab	C	(Yip, Horev et al. 2008)
C967T	Exon 3	R323X	Pakistani	Non-C	(Kim, Wajid et al. 2007)
C1504T	Exon 4	Q502X	Pakistani	C	
C2828T	Exon 13	R940X	Pakistani		
1257delC,1263del21bp	Exon 3		Arab Palestinian	C	(Ahmad, Zlotogorski et al. 1999)
C1432T	Exon 4	Q478X	Pakistani	C	(Sprecher, Lestringant et al. 1999)
G1571A	Exon 5	G525E	Korean	Non-C	(Deborah Lee, Ji-Yeon Kim et al. 2011)
A1748T	Exon 5	E583V	Italian	Non-C	(Paradisi, Chuang et al. 2003)
1839delATG	Exon 6		Iranian	C	(Balighi, Lajevardi et al. 2009)
1782delAG	Exon 6		Pakistani	C	(Kraemer, Wajid et al. 2008)
A2909G	Exon 14	N970S	Pakistani	C	
3097delG	Exon 15		Pakistani	C	
A3064G	Exon 15b	T1022A	Pakistani	C	(Ahmad, Faiyaz ul Haque et al. 1998)
G1859A	Exon 6	R620Q	Irish	C	(Ahmad, Irvine et al. 1998)
2001del4bp	Exon 7		Mexican	C	(Kruse, Cichon et al. 1999)
A2909G	Exon 14	N970S	South Tyrolian	Non-C	
C2070A	Exon 8	C690X	Pakistani	C	(Azeem, Wasif et al. 2011)
C2455T	Exon 11	R819X	Pakistani	C	
C3470G	Exon 18	P1157R	Pakistani	C	
2147delC	Exon 9		Arab Palestinian	C	(Zlotogorski, Ahmad et al. 1998)
2776 + 2insT	Intron 12		Irish	Non-C	(O'Regan, Zurada et al. 2007)
G3034A	Exon 15[a]	D1012N	Arab Israeli	C	(Klein, Bergman et al. 2002)
G3166A	Exon 16	V1056M	Arab Palestinian	C	(Zlotogorski, Panteleyev et al. 2002)
1075insGGCC	Exon 17		Albanian	Non-C	(Nucara, Colao et al. 2011)
3434delC	Exon 18		Arab Palestinian	C	(Masse, Martinez-Mir et al. 2005)

Compound Heterozygous Mutations					
189del11bp/C1432T	Exon2/ exon 4		Moroccan	Non-C	(Indelman, Bergman et al. 2003)
1025ins4bp/C2155T	Exon3/ exon 9		Australian	Non-C	(Yip, Horev et al. 2008)
C778T/G2097A	Exon3/ exon 8	Q260X/W699X	Caucasian	Non-C	(Michailidis, Theos et al. 2007)
C1432T/G1557-1T	Exon4/ intron 4	Q478X/splice	Iraq	Non-C	(Paller, Varigos et al. 2003)
C1432T /2776 + 2insT	Exon4/ intron 12		English	Non-C	
T1864G/C2847-3G	Exon6/ intron 13		Russian	Non-C	
2847-2delAG/C3526T	Intron13/ exon 19		German	Non-C	(Henn, Zlotogorski et al. 2002)
c.T2265A/c.3482delCT	Exon10/ exon 18		Chinese	Non-C	(Wang, Tu et al. 2013)

C means consanguineous family, whereas, Non-C means non-consanguineous family. [a] the mutation c.G3034A results appears in both APL and Alopecia Universalis.

CHAPTER THREE

Materials and Methods

3.1. Materials

3.1.1. Buffers, Gels and Solutions

- **Red blood cell lysis buffer**

155 mM NH4Cl

10m NH4HCO3

0.1 mM EDTA with (PH=7.4)

- **1X lysis buffer**

50 mM Tris HCL with (PH=7.5)

100 mM NaCl

1mM EDTA with (PH=8)

- **Agarose gel**

0.8%, 1.5%, 3% agarose

1X TBE buffer

Final concentration of 0.01% ethidium bromide

- **Ethidium Bromine**

Ethidium bromide was dissolved in the double distilled sterile water to a final concentration of 1mg/ml. (or bought premade to this concentration)

- **5X loading buffer**

0.25% bromophenol blue

0.25% Xylene cyanol FF

30% Glyserol in water

- **50X TAE Buffer**

2M Tris pH 8.0

1M Acetic acid

0.05M EDTA

Ajust to PH=8.0

- **Proteinase K**

Proteinase K was dissolved in double distilled sterile water to a 5mg/ml final concentration.

3.1.2. Reagents, Instruments, and Kits.

Reagents

Reagents	Supplier	Product specifications
dNTPs 2.5mM	TAMAR	CAT# R0181,4X0.25mM
Oligonucleotide primers	Hylabs	
Super Therm polymerase	Eisenberg Bros	CAT# JMR-801
Q solution	Qiagen	
10x polymerase Buffer	Eisenberg Bros	CAT# JMR-420
100bp DNA ladder H3 RTU	Medipharm	CAT#: DM003-R500
Agarose	aMReSCO®	CAS# 9012-36-6
Exonuclease I	BioLabs	CAT# M0293L,20,000 units/ml
Antaractic Phosphatase	BioLabs	CAT# M0289L, 5000units/ml
20% SDS	aMReSCO®	CAT # 083754-500ml
Proteinas K	aMReSCO®	LOT# 1311C384
Super- DI$_{tm}$ Formamide	MCLAB	CAT#: SDI-100
BDX64, 2*1.25ml	Agentek	CAT#: BDX-100
GENESCAN®-400HD ROX SIZE Standard	Applied Biosystems	CAT#: 402985-394

Kits

Kits	Supplier	Product specification
BigDyeTM Terminators V1.1 Cycle Sequencing Reaction Kit	Applied Biosystems	CAT# 4337451-100

Instruments

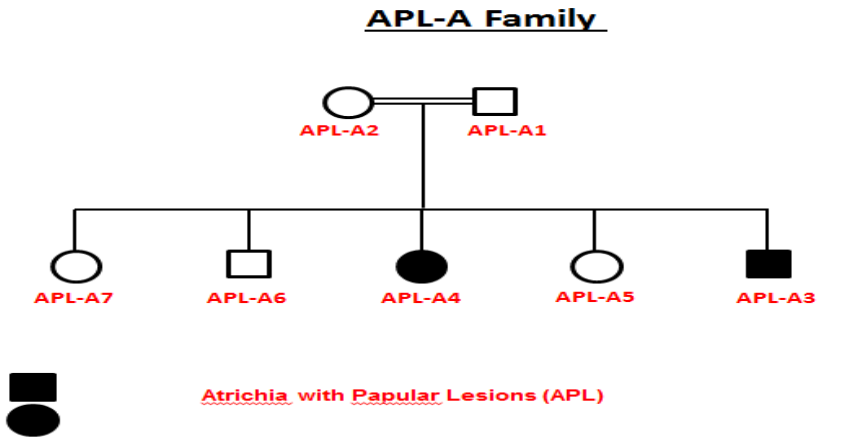
Instruments	Supplier	Instrument Specification
Gel DOCumentation system	BioRad	Molecular Imager, Gel DOC™ XR+ Imaging System
Agarose gel electrophoresis Apparatus	BioRad	SUB-CELL® GT
Agarose gel electrophoresis power supplier	BioRad	Power PAC 300
NanoDrop®		
PCR machine	Applied Biosystems	GeneAmp® PCR System 9700
Sanger Sequencing Machine	Applied Biosystems	ABI 3130XL Genetic Analyzer

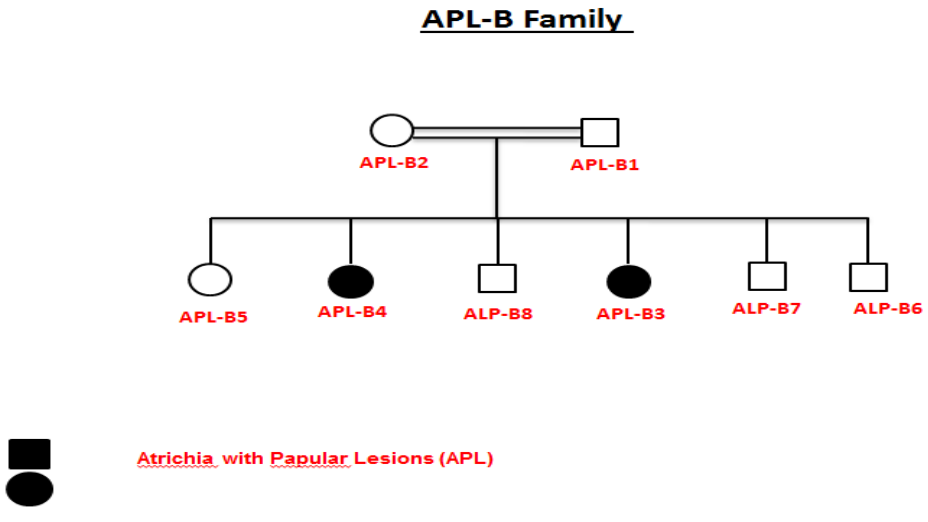
3.2. Methods.

3.2.1. Samples Collection.

Five Palestinian families with Congenital Atrichia from Beit-Fajjar near Bethlehem were collected in this study (see Figure 3.1). We met each family independently at their home to explain the goals of our research and to get agreement for blood sample collection. Before the blood samples were collected a consent form was signed by each family member who agreed to participate in this study. For those individuals who are under 18 years old; the consent form was signed by one of their parents.

Blood samples were collected from 8 affected and 22 unaffected individuals in these families to extract DNA. Five ml blood was put in EDTA tube (Ethylinediaminetetra acetic acid) (0.1 ml 0.5M EDTA for 20 ml blood) to prevent clotting.





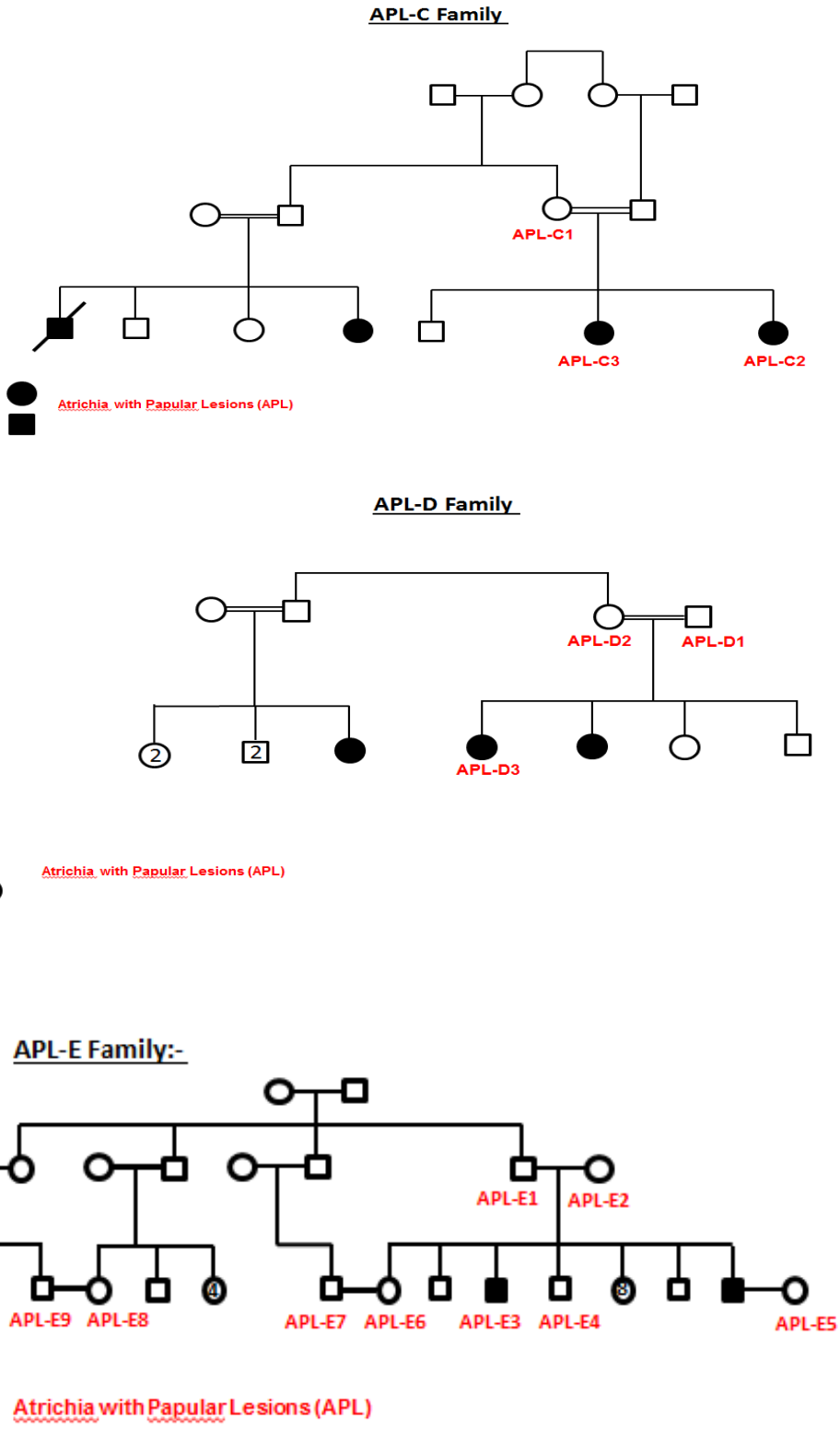


Figure 3.1: pedigrees represented five Arab Palestinian families from Beit-Fajjar near Bethlehem with Papular Atrichia disease.

3.2.2. DNA Isolation by Salting-Out technique.

About 5-10 ml of the blood was collected in a sterile EDTA tube. The blood sample was transferred to 50 ml conical tube then the tube was filled with RBCs lysis buffer. The tube was kept on ice for 20-30 minutes (min), being shaken by hand from time to time, until blood becomes transparent. Centrifugation was done at 2000 round per minute (rpm) for 10 min at 4C. The supernatant was discarded while the pellet was maintained at the bottom. Then, this pellet was washed by adding 15 ml of RBCs lysis buffer and centrifugation repeated again at 2000 (rpm) for 10 min at 4C. Then supernatant discarded again and the pellet was suspended in a mix of 3 ml of 1x lysis buffer, 100µl of 20% SDS and 100µl of 5 mg/ml proteinase K (section 3.1.1) to break it. After that, the tube was incubated 37°C overnight in shaking incubator. After digestion was complete, one ml of 6M NaCl was added to the homogeneous solution without a clear pellet with vigorously shaken until the solution appeared foamy. Centrifugation was done at 3000 rpm for 20 min at room temperature; the precipitated protein pellet was left at the bottom of the tube and the supernatant containing the DNA was transferred to other clean 15 ml tube. 100% cold ethanol was added to the supernatant (2X volume at least) little by little with gentle inversion. DNA was fished from the solution by using a sterile glass Pasteur pipette, followed by washing in 70% ethanol (in Eppendorf tube) and then air drying for a few minutes on Pasteur pipette. DNA was then transferred into a self-standing sterile screw cap tube that contained 200-600µl 0.02% sodium azide depending on the amount of DNA that was fished and then left at room temperature overnight or for two days to allow DNA to dissolve.

3.2.3. Linkage exclusion analysis.

After we obtained DNA, linkage exclusion analysis was done. Two microsatellite markers [Marker1 (M1) and Marker2 (M2)] were chosen; one before the HR gene and the other after this gene using UCSC genome browser (Figure 3.2).

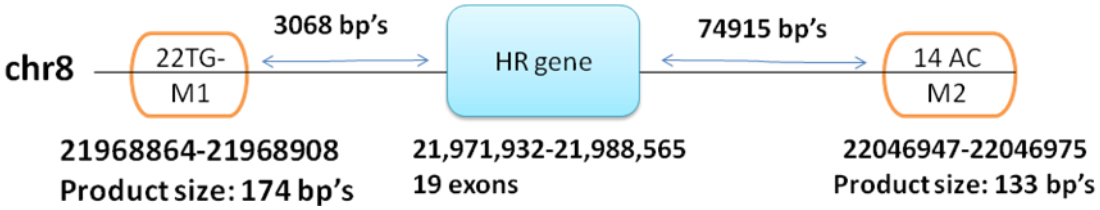


Figure 3.2: M1, M2 and HR gene position on chromosome 8 and the distance between this gene and these two markers.

Also, by using this browser and Primer 3 browser, four primers were designed to amplify this region around the markers for the HR gene for genotyping; two primers for M1; Forward (F) and Reverse (R) primers, and the other two primers for M2 (F and R) (table 3.1 for T.D and primer sequences).

Table 3.1:- Microsatellite markers used to study linkage to Papular Atrichia in APL families:

Microsatellite	FAM-F-primer	R-primer	T.D
M1= 22TG	GTGCAGAGCATCATGCTCCAAATAACTGCAGGCACA	GGTGGGAAAACTGAGGGATT	53°C
M2= 14AC	GTGCAGAGCATCATGCTTATTCAGTTGCCCCCTGAG	CACTGGCTCACTGAATGGAA	58°C

F-primers for M1 and M2 fluorescently labeled with FAM (blue color).

3.2.3.1. Genotyping PCR program.

The sequences of the DNA from 21 members of four families were amplified for genotyping by the polymerase chain reaction (PCR) with M1 and M2 primers. F-primers for M1 and M2 were labeled with FAM florescent (blue color).

Table 3.2: Genotyping PCR reaction mix per 25µl of total volume:

Reagents	**Volume in µl**
10X Polymerase Buffer	2.50
Polymerase enzyme (super therm polymerase)	0.25
Q-Solution	2.00
dNTPs (2.5Mm)	2.00
MgCl2	1.00
Forward primer with FAM tail	0.10
Reverse primer	0.50
Uni FAM	0.50
Nuclease free H2O	15.15
100ng/µl DNA Template	1.00

T.D Program (M1 T.D; 53 Programs):

94 ℃ 5 min
94 ℃ 30sec
61 ℃ 30sec } X2
72 ℃ 30sec
94 ℃ 30sec
59 ℃ 30sec } X2
72 ℃ 30sec
94 ℃ 30sec
57 ℃ 30sec } X2
72 ℃ 30sec
94 ℃ 30sec
55 ℃ 30sec } X2
72 ℃ 30sec
94 ℃ 30sec
53 ℃ 30sec } X35
72 ℃ 30se
72 ℃ 7 min
4 ℃ ∞

Annealing temperature depends on the Melting temperature (Tm) of the primer that we used. Each marker has specific T.D (**M1=53C whereas M2= 58C)**.

3.2.3.2. Genotyping

For genotyping 0.25 µl of 400 Rox, 10.75 µl Hi Di and 1 µl of PCR product were mixed together. This step was done for each PCR product then the mix was incubated for 5 minutes on 95 °C until DNA strands were denatured, followed by incubation for five minutes on ice. Samples were run on 3130XL Genetic Analyzer machine from Applied Biosystem. After loading them on the special optical Reaction Plate from Applied Biosystem, the results were analyzed using the genemapper 4.0. These results were represented in the form of peaks. The number of peaks and their sizes are proportional to the length of repeats and the number of repeats in the PCR product.

Table 3.3: Reaction mix for genotyping per 12 µl of total volume

Reagents	Volume in µl
GS-400HD ROX	0.25 µl
Hi Di	10.75 µl
PCR product	1 µl

3.2.4. Mutation detection

3.2.4.1. Detection of mutation by direct sequencing

3.2.4.1.1. Polymerase Chain Reaction (PCR)

To detect a mutation in the hairless gene, a 244 bps fragment found in Exon 9 was amplified by using the following primer pair:

Primer	Primer sequence
Forward primer	**5'**-TGTGGGTTCTGTTGAATTGTG-**3'**
Reverse primer	**5'**-GAGACTTCCGCGACTGTCC-**3'**

Table 3.4: Standard PCR reaction mix per 25µl of total volume

Reagents	Volume in µl
10X Polymerase Buffer	2.50
Polymerase enzyme (super Therm polymerase)	0.25
Q-Solution	5.00
dNTPs (2.5Mm)	2.00
Forward primer	0.50
Reverse primer	0.50
Nuclease free H2O	13.25
100ng/µl DNA Template	1.00

PCR program (T.D 55 programs)

94 ℃ 5 min

94 ℃ 30sec
63 ℃ 30sec* } X2
72 ℃ 30sec**

94 ℃ 30sec
61 ℃ 30sec } X2
72 ℃ 30sec

94 ℃ 30sec
59 ℃ 30sec } X2
72 ℃ 30sec

94 ℃ 30sec
57 ℃ 30sec } X2
72 ℃ 30sec

94 ℃ 30sec
55 ℃ 30sec } X35
72 ℃ 30se

72 ℃ 7 min

4 ℃ ∞

*Annealing temperature depends on the Tm of the primers that used for PCR Amplification.

**Extension time depends on the size of amplified product (1Kb = 1min).

The Amplification process was done by using our PCR machine GeneAmp-PCR system 9700 from Applied Biosystem.

3.2.4.1.2. PCR product Electrophoresis by using Agarose gels.

Gel electrophoresis was done after PCR step to determine the presence and size of PCR products, also to quantify the amount and length of DNA fragments. An agarose gel was prepared by adding 1.5g agarose powder with 100 ml of 1X TAE buffer in a flask. Then, the flask was put in Microwave to melt the agarose in TAE buffer for 2 min until the solution became clear. The percentage of agarose that dissolved in the TAE buffer was estimated by the size of the PCR product. After that, ethidium bromide was added to the mildly cooled solution. Before the samples were loaded onto the

milky white agarose gel, 4 µl of loading buffer and 4 µl from each PCR product were mixed together very well then the he mixture (PCR products\loading buffer) was loaded onto the gel alongside 4 µl of 100bp DNA ladder in first well. Then the gel was run in 1X TAE running buffer at 120V for 20-30 minutes, depending on the fragment size. DNA fragments were determined by using ultraviolet light and photographed using the (Molecular Imager®, Gel DOC ™ Imaging System, BioRAD).

3.2.4.1.3. Cleaning of PCR products

After gel electrophoresis step was finished and PCR product bands were seen on the gel. PCR product "clean-up" protocol was done which is an important step to each PCR products to chew up excess primers and remove excess unincorporated dNTPs, enzymes, salts and small fragments from these products. Also, this procedure was used to purify the amplified fragments from agarose gel after electrophoresis or directly from the PCR reaction. Antaractic Phosphatase and Exonuclease I enzymes were used to purify the products from the PCR reaction. Antaractic Phosphatase enzyme is responsible to remove the leftover nucleotides, whilethe role of Exonuclease I enzyme is to degrade the remaining primers.

Table 3.5: Master Mix of enzymes with 5 µl of PCR products per 7 µl of total volume

Reagents	**Volume in µl**
Antaractic Phosphatase	0.25
Exonuclease I	0.25
Nuclease free H2O	1.5
PCR Products	5

PCR clean program

37 ^{o}C 30min

80 ^{o}C 20min

4 ^{o}C ∞

3.2.4.1.4. Direct sequencing of purified PCR Products

Sanger method of DNA sequencing was done using Big Dye terminator. The BigDyeTM Terminators V1.1 Cycle Sequencing Reaction Kit (Applied Biosystems) includes ddNTPs (dideoxynucleotides) that inhibit chain-elongating by DNA polymerase. Samples were run on ABI 3130XL Genetic Analyzer (Applied Biosystem) at Bethlehem University. The results analyzed with FINICH TV program.

Table 3.6: Standard Sanger sequencing reaction mix per 16µl total volume

Reagents	**Volume in µl**
Big Dye	0.3
64X Buffer	0.75
5X Buffer	1.5
Forward Primer	0.5
PCR Product	1.5
Nuclease Free H2O	11.45

Sequencing PCR Reaction Program:

96 °C /1min
96 °C /10sec
50 °C / 5sec } X25
60 °C /4min
4 °C /10min

3.2.4.1.5. Sequencing clean of the cycle-sequenced products using EDTA/Ethanol Precipitation method

EDTA/Ethanol Precipitation method was used to purify the cycle-sequenced products from primers, unincorporated dNTPs and excess dyes before performing sequencing by the 3130XL Genetic Analyzer sequence machine.

For each 16µl of the cycle-sequenced products 5µl of 125mM EDTA and 100µl of absolute ethanol were added and mixed very well. The mixture was incubated in -20°C freezer for 30 min or in -80°C for 8 min. Then samples were centrifuged at 3800 RPM for 30 min at 4°C. After centrifugation the supernatant was discarded and 60µl of 70% ethanol were added to each sample and centrifuged again at 3800 RPM for 20

min at 4C°. The supernatant was discarded again and the PCR tubes or plate was inverted upside down on tissue paper and centrifuged at 500 RPM for 1 min. The samples were put on 95C° hotplate for 5min to evaporate the ethanol totally. Finally the samples were resuspended in 10µl Hi Di Formamide (CAT#4311320, Applied Biosystem). Samples were then put on 95C° for 5min for denaturation followed by 5min incubation on ice. Samples were loaded on the 96 –well Optical Reaction Plate from Applied Biosystems and run on sequencing machine (3130XL Genetic Analyzer from Applied Biosystems).

3.2.5. Healthy Controls

100 healthy, unaffected Palestinian individuals controls from Hebron were collected, DNA was extracted from their whole blood samples, and the sequence that contain the mutation was amplified using the same primer set, and then sequenced and analyzed to detect the presence of the mutation.

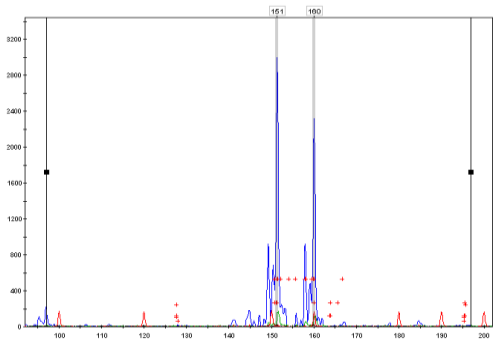
CHAPTER FOUR

Results

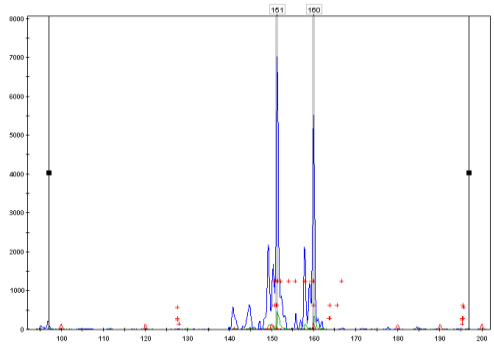
4.1 Linkage Exclusion Analysis Results

Linkage Exclusion Analysis was used at the beginning of the study. We searched one megabase around the HR gene and we found two highly polymorphic microsatellite markers one before the HR gene (M1) and the other after it (M2). M1 was 22TG repeat located 3068 bps just before the HR gene, while M2 was 14AC repeat located 74915bps after the gene. The initial results showed that this gene could not be excluded as the cause of this disease. In A and B families the two markers were informative, but in C and D families, M1 was informative but M2 was semi-informative. The two markers were homozygous in affected members in all families, but unaffected parents and the unaffected members (carrier) were heterozygous and wild type members were homozygous. Fragment analysis of two of HR microsatellite markers of unaffected parents and one affected individual and one unaffected individual of Congenital Atrichia from B-family are shown in figure 4.1. Figure 4.2 is also shown Pedigrees of four families used for linkage analysis with genotyping results.

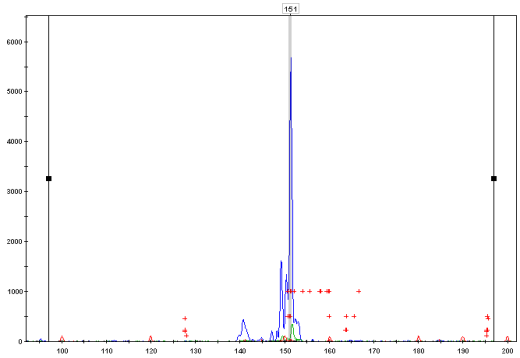
APL-B1-M1: Unaffected father.



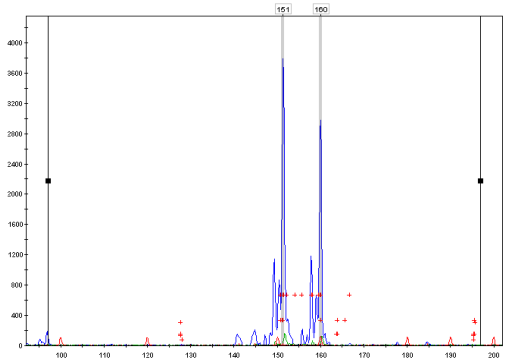
APL-B2-M1: Unaffected mother.



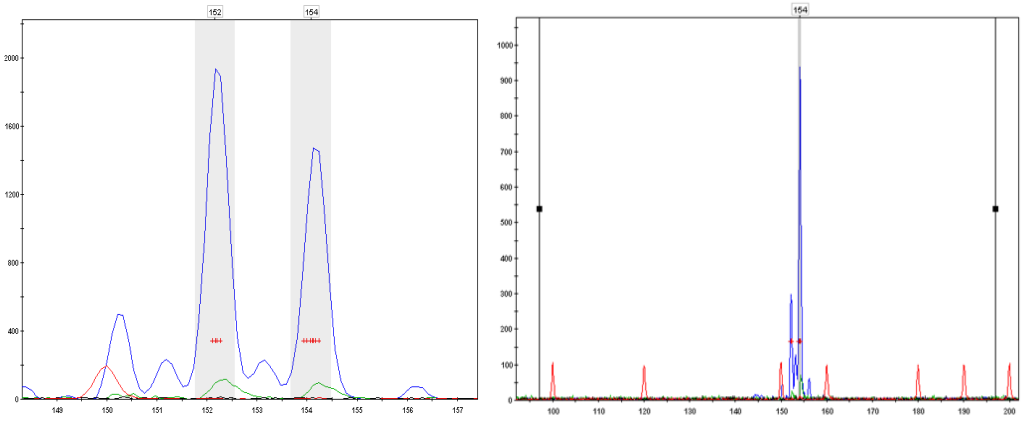
APL-B3-M1: affected child.



APL-B5-M1: Unaffected child.



APL-B1-M2: Unaffected father. **APL-B2-M2: Unaffected mother.**



APL-B3-M2: affected child. **APL-B7-M2: Unaffected child.**

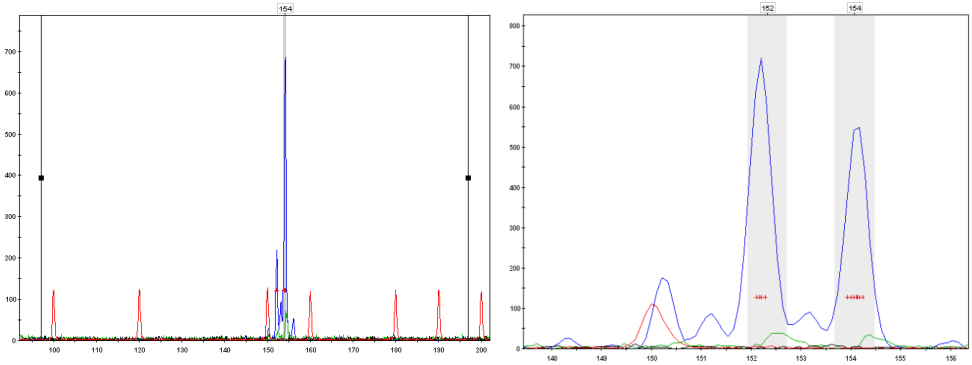
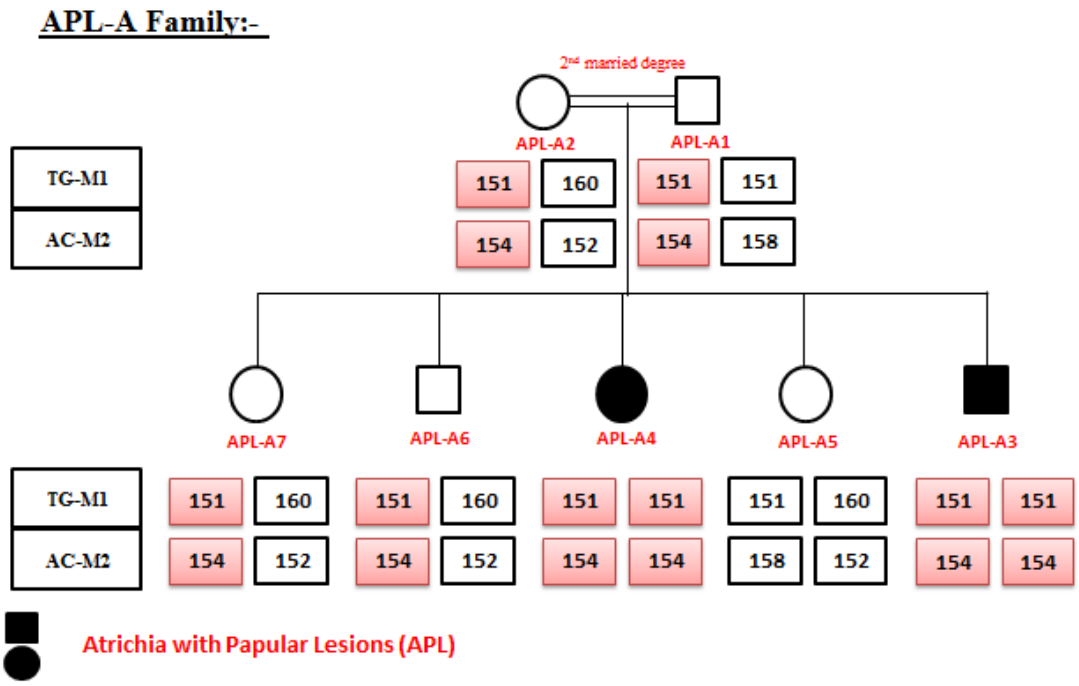
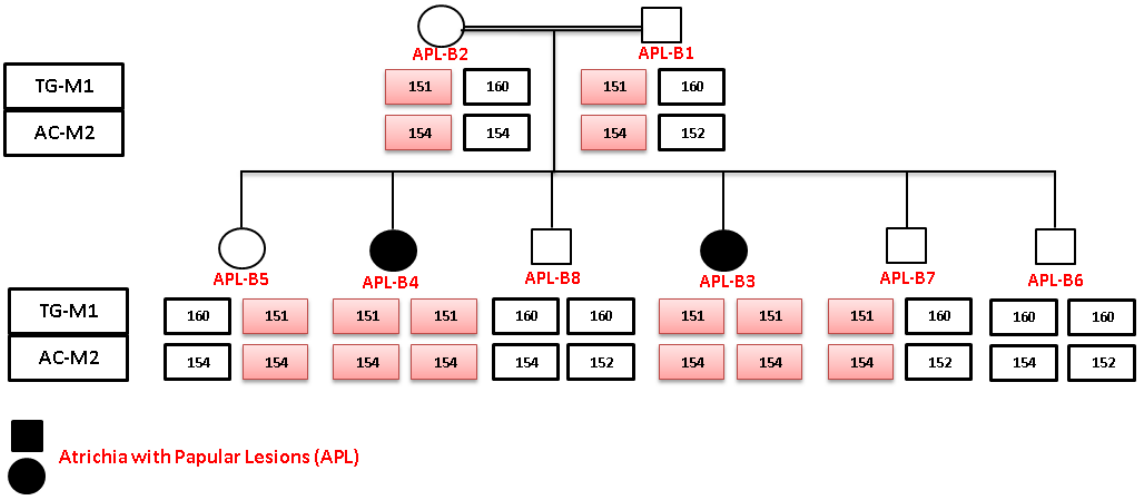


Figure 4.1: Fragment analysis of M1 and M2 from B-family.

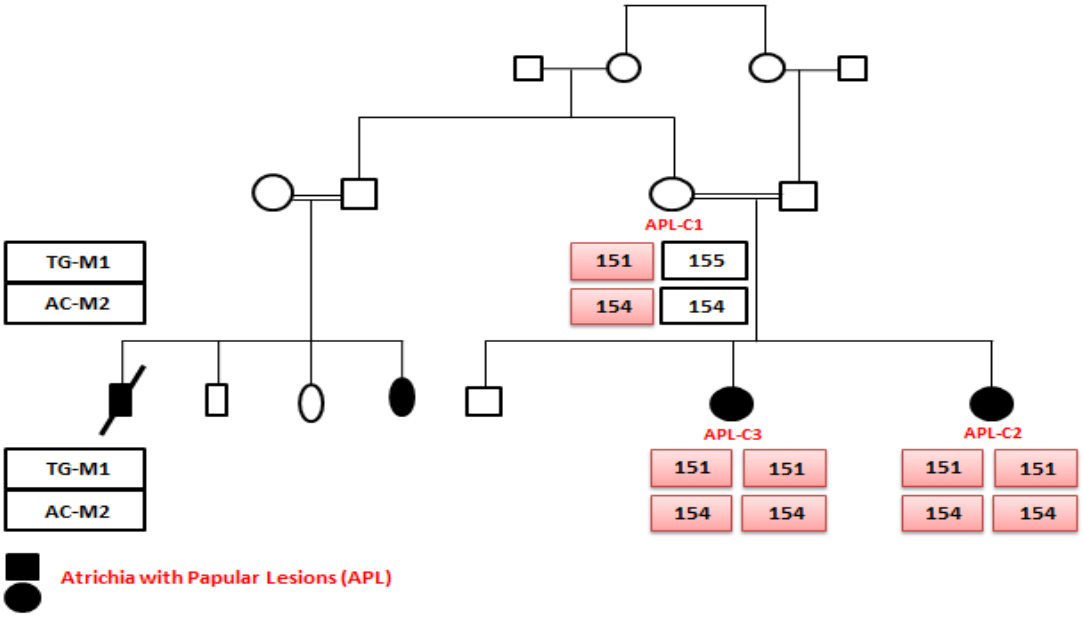


APL-B Family:-



Atrichia with Papular Lesions (APL)

APL-C Family:-



Atrichia with Papular Lesions (APL)

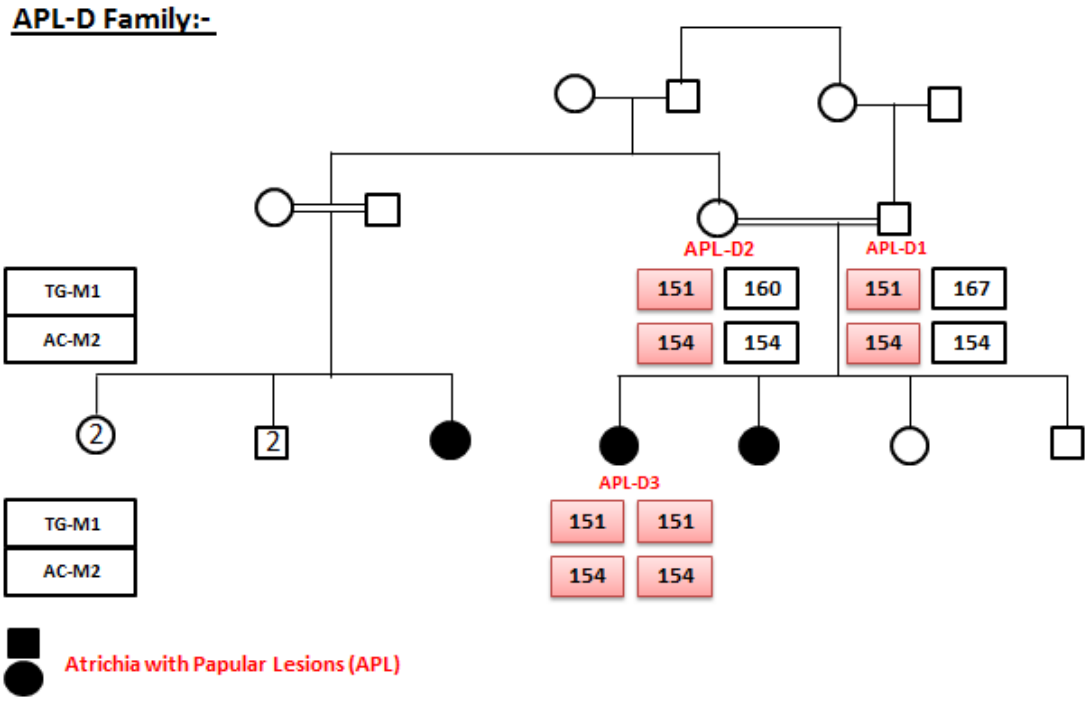


Figure 4.2: Pedigrees of families used for linkage analysis with Haplotypes.
(1) Haplotypes of TG-M1 and AC-M2 around HR gene in APL-A family.
(2) Haplotypes of TG-M1 and AC-M2 around HR gene in APL-B family.
(3) Haplotypes of TG-M1 and AC-M2 around HR gene in APL-C family.
(4) Haplotypes of TG-M1 and AC-M2 around HR gene in APL-D family.

4.2 Sanger sequencing result of the Exon 9 in HR gene.

Sanger sequencing was done to find the specific mutation in this gene. We started with exon 9 since this exon harbored most of the mutations seen in the Arab population. Sequencing results revealed that there is a single base pair deletion mutation at position 2147 (2147delC), which leads to a frameshift and premature termination codon. This stop codon is 544 bp downstream in exon 12. So this mutation is predicted to disrupt the structure of the putative single zinc-finger transcription factor protein. We found this mutation in all affected members in APL-B family (APL-B3 and APL-B4).

4.3 Segregation of the 2147delC mutation with the Congenital Atrichia phenotype in all families, Sanger Sequencing Results.

Sanger sequencing results of all members in the five families, 8 affected and 22 unaffected individuals, showed that this mutation in HR gene segregates perfectly in a

recessive mode of inheritance in all families (see Figure 4.4). It also revealed that the unaffected parents were heterozygous for the deletion and the affected members were homozygous, while the unaffected siblings were wild type or heterozygous for the mutation. In the mutant sequence, the homozygous and heterozygous deletion of a nucleotide C shows four consecutive Cytosines instead of five consecutive C nucleotides that appeared in the wild type as in Figure 4.3; the electrophyrogram of the parents and affected child and unaffected (wild type) of APL-B family.

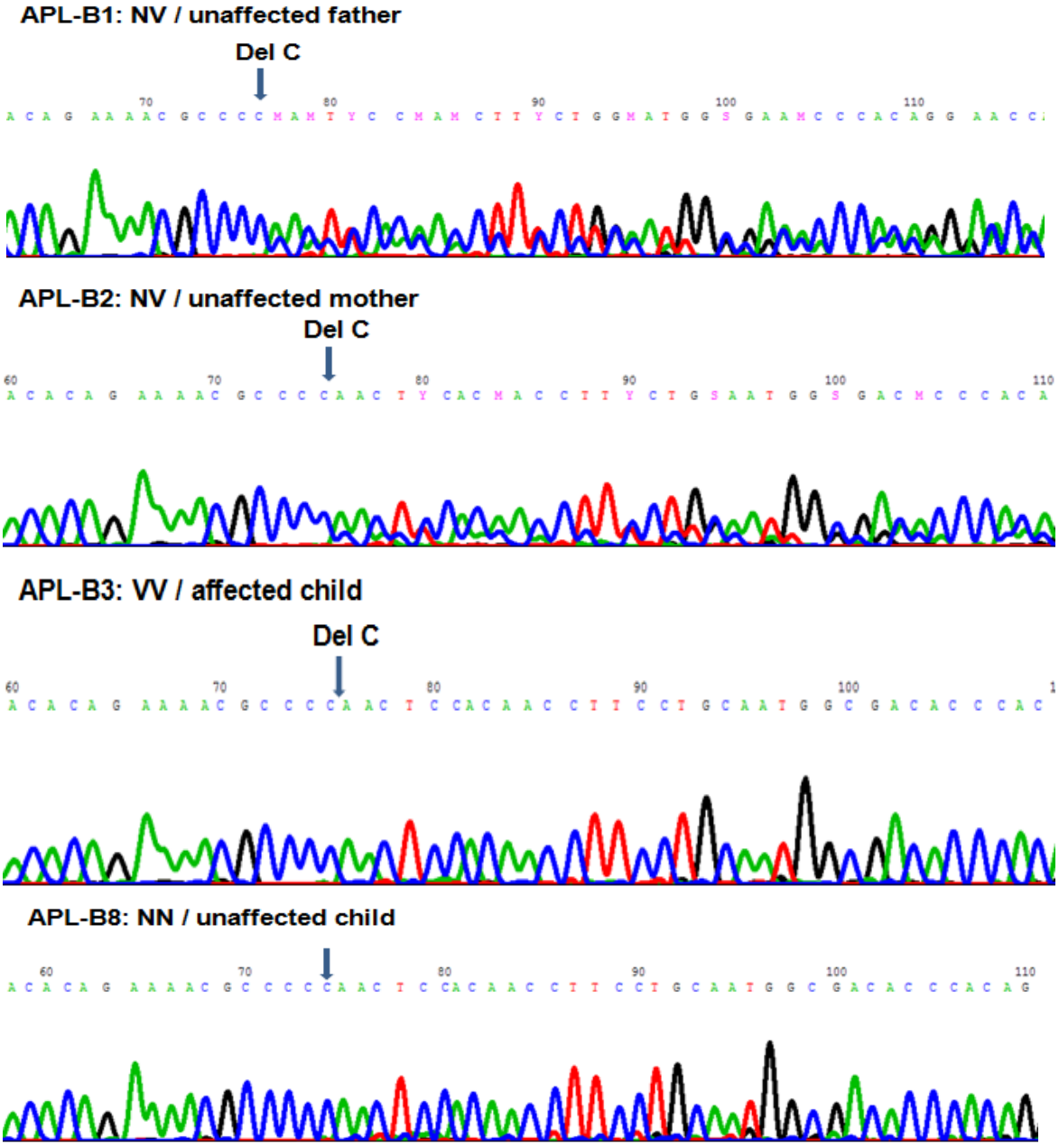
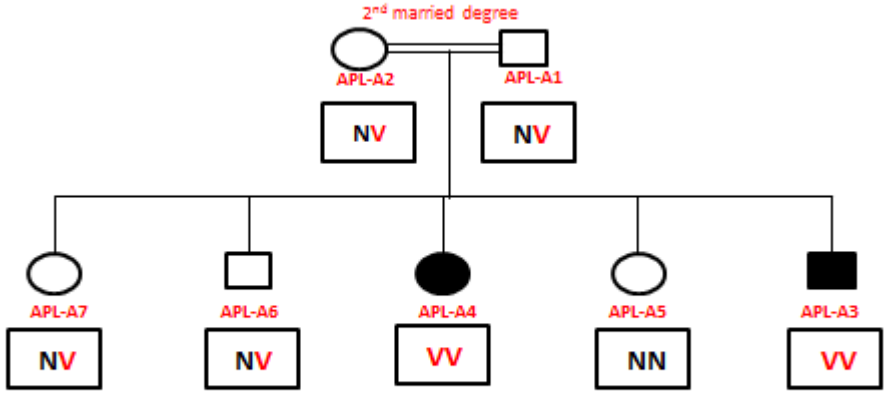


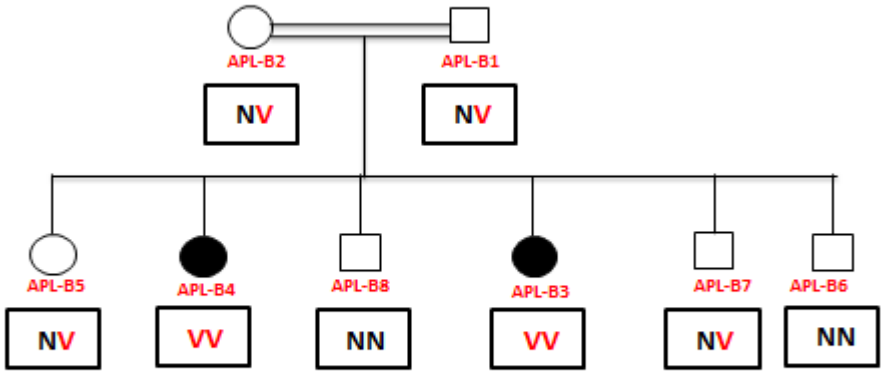
Figure 4.3: Electrophyrogram of the parents and affected child and unaffected (wild type) of APL-B family.

APL-A Family:-



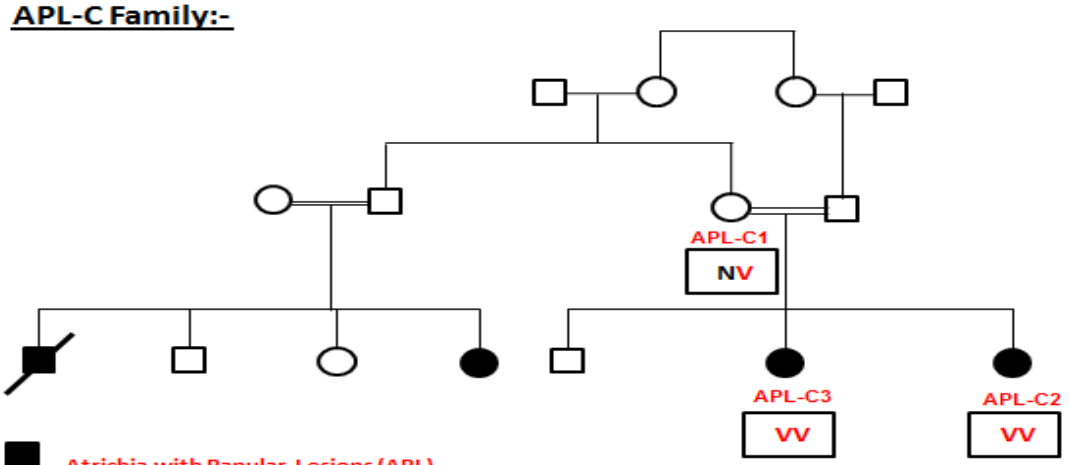
Atrichia with Papular Lesions (APL)

APL-B Family:-



Atrichia with Papular Lesions (APL)

APL-C Family:-



Atrichia with Papular Lesions (APL)

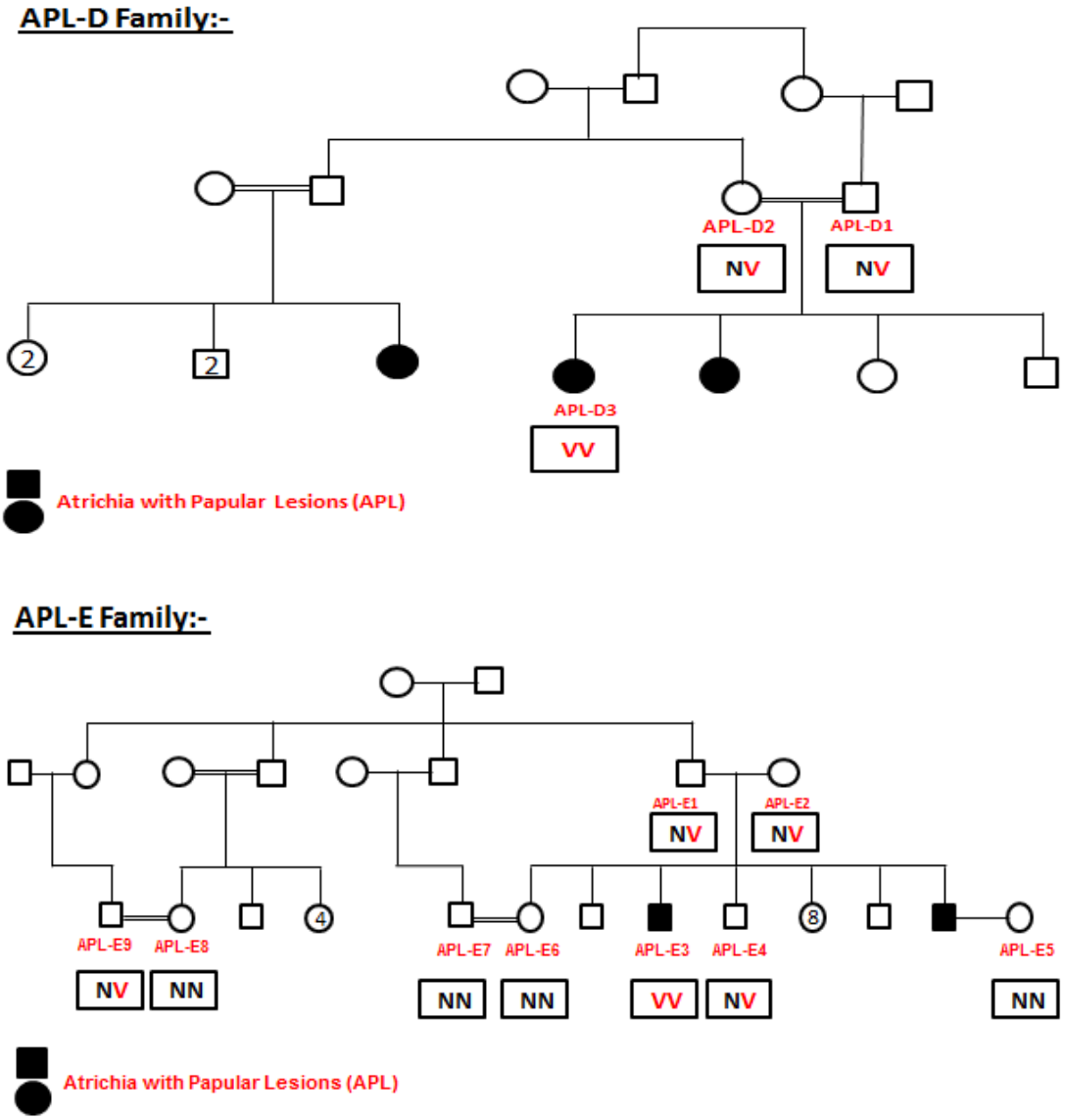


Figure 4.4: Sanger Sequencing Results that showed segregation of the 2147delC mutation with the Congenital Atrichia phenotype in APL-A, APL-B, APL-C, APL-D and APL-E families.
(1) Segregation of 2147delC mutation with the phenotype in APL-A family
(2) Segregation of 2147delC mutation with the phenotype in APL-B family.
(3) Segregation of 2147delC mutation with the phenotype in APL-C family.
(4) Segregation of 2147delC mutation with the phenotype in APL-D family.
(5) Segregation of 2147delC mutation with the phenotype in APL-E family.

4.4 Sanger Sequencing Results of Healthy Controls.

Samples from 100 healthy, unaffected Palestinian controls from Hebron were amplified and sequenced by using the same primers that we used to detect the presence of the 2147delC mutation. Results showed no one of these individuals carrying this mutation either in homozygous or heterozygous forms.

4.5 Bioinformatics Tools Results.

After we did Sanger sequencing, our result revealed that the causative mutation for this disease in all affected members in the five families is a single base pair deletion of C at position 2147 (2147delC) that leads to a frameshift and premature termination codon. By using mutation taster database (http://www.mutationtaster.org/), we found that this mutation is located at chr8:21979182. It is a deletion of C which caused a frameshift at amino acid 716 (P 716 Q fS*186). As a result of this change the JmjC domain is lost (see Figure 4.5).

alteration (phys. location)	chr8:21979182_21979182delG
HGNC symbol	HR
Ensembl transcript ID	ENST00000381418
Genbank transcript ID	NM_005144
UniProt peptide	O43593
alteration type	deletion
alteration region	CDS
DNA changes	c.2147_2147delC cDNA.3628_3628delC g.11716_11716delC
AA changes	P716Qfs*186
position(s) of altered AA if AA alteration in CDS	716 (frameshift or PTC - further changes downstream)
frameshift	yes
protein features	start (aa) end (aa) feature details 946 1157 DOMAIN JmjC. lost
length of protein	NMD
AA sequence altered	yes
position of stopcodon in wt / mu CDS	3570 / 2703
position (AA) of stopcodon in wt / mu AA sequence	1190 / 901
position of stopcodon in wt / mu cDNA	5051 / 4184
original gDNA sequence snippet	ATCAACACAGAAAACGCCCCCAACTCCACAACCTTCCTGCA
altered gDNA sequence snippet	ATCAACACAGAAAACGCCCCAACTCCACAACCTTCCTGCA
original cDNA sequence snippet	ATCAACACAGAAAACGCCCCCAACTCCACAACCTTCCTGCA
altered cDNA sequence snippet	ATCAACACAGAAAACGCCCCAACTCCACAACCTTCCTGCA

Figure 4.5: this figure illustrate our results that we get from the mutation taster database about our mutation in exon 9 of HR gene (http://www.mutationtaster.org/).

Also, by using the UCSC genome browser, we found the amino acid Proline is highly conserved among the species from human to platypus. So, any changes in this amino acid will be harmful and will lead to nonfunctional hairless protein.

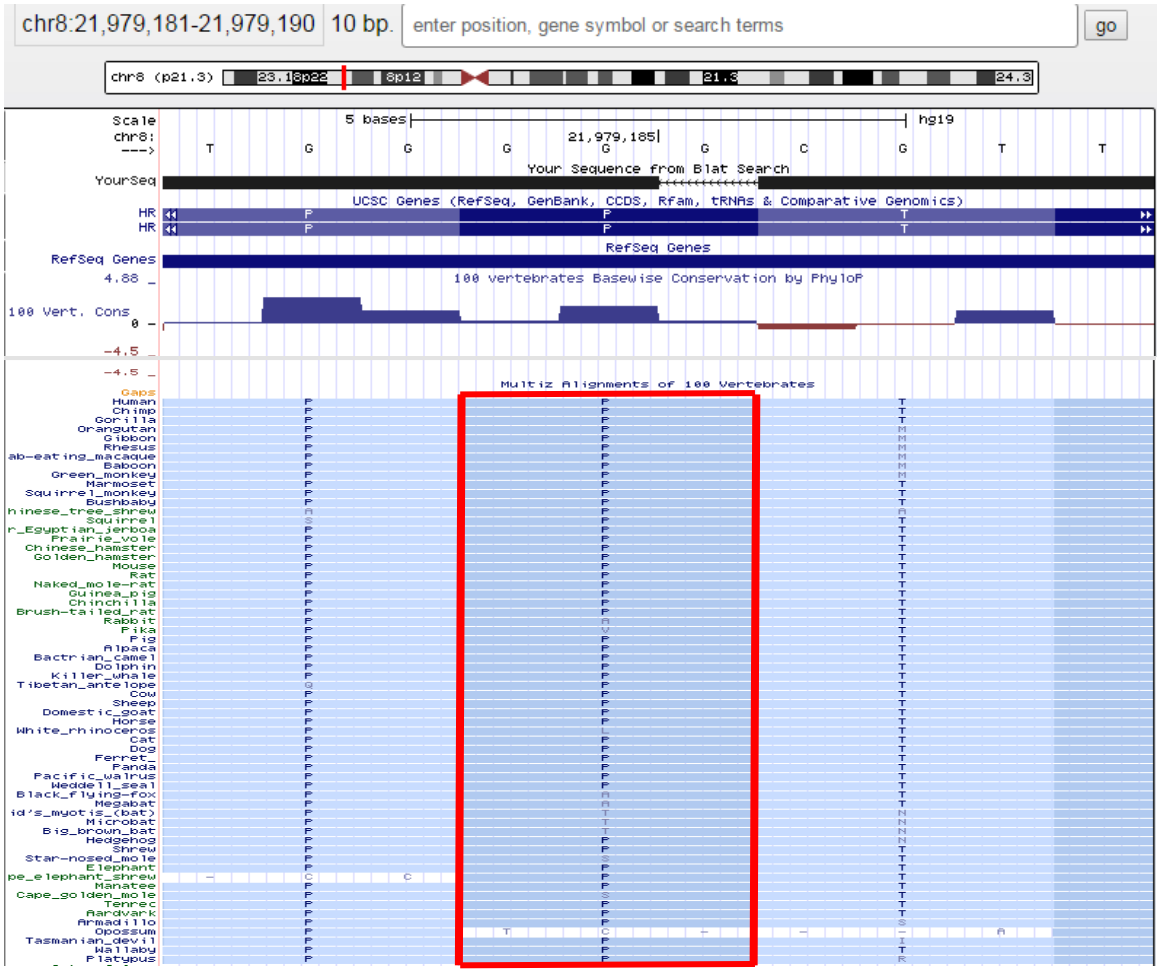


Figure 4.6:- Multiple sequence alignment of the amino acid residue of HR in human with other 50 vertebrate species. The red rectangle shows the amino acid Proline at position 716 is highly conserved across the species from human to platypus.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

In our study we describe a rare hair loss human disorder known as Congenital Atrichia or Atrichia with Papular Lesions (APL). This rare disease differs from other diseases that are related to hair loss. Usually, most patients with APL have the same degree of clinical severity and symptoms. People with Congenital Atrichia can be born with a head full of hair like any normal baby, but in early childhood they lose all their hair, and it never regrows again. The followed phenotype is widespread of papules all over the body which is a distinguished symptom for this inherited disease. Histologically, mature hair follicles are completely absence in APL patients with normal present of sebaceous glands. Also in some cases, the malformed follicles appear instead of mature hair follicle which is in line with the findings of scientists about the occurrence of the problem after the initial hair follicle differentiation (see figure 5.1) (Wang, Tu et al. 2013).

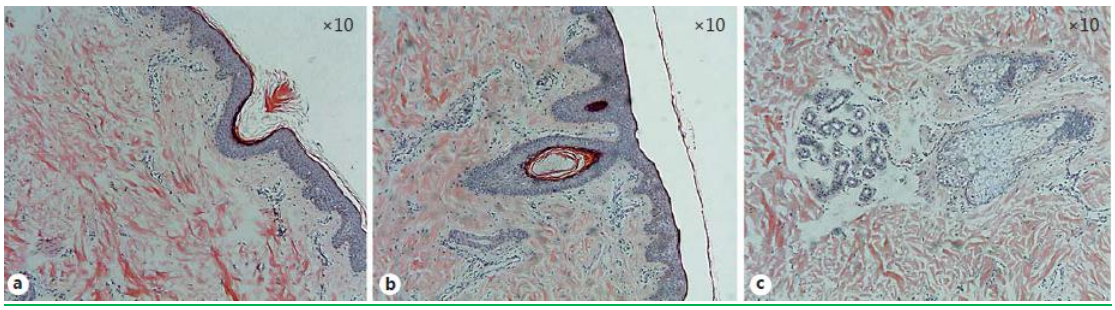


Figure 5.1: (**a)** biopsy from the scalp of APL affected one that showed complete absence of hair follicle structures. (**b)** Malformed follicle filled with amorphous keratinous material. (**c)** Normal presented of sebaceous glands without connection with the malformed hair follicles. (Wang, Tu et al. 2013).

In 1998, studies established that this genetic disease was linked to chromosome 8p12 in a region containing the human HR gene (Ahmad, Irvine et al. 1998, Nothen, Cichon et al. 1998, Sprecher, Bergman et al. 1998). This gene is found in all hairy animals and the mammalian HR gene was not found in any non-mammalian vertebrate animals. This supported the key role of this gene in hair follicles cycling (Abbasi 2011). Therefore, mutations in this gene could be the causative reason for the appearance of this disorder in humans and mice. A large number of mutations have been reported in this gene especially in consanguineous families with high percentage

in Pakistani and Arab Palestinian families. To date, most mutations that were identified in HR gene are 24% missense, 32% frameshift, 13% splice-site and 31% nonsense mutations (Wang, Tu et al. 2013).

In the presents study, we identified five Palestinian families with APL from Beit-Fajjar village south of Bethlehem. We studied 30 members of these families, including 22 unaffected and 8 affected individuals (figure 3.1). All of the affected siblings exhibited typical features of Congenital Atrichia. They showed total hair loss, characterized by complete irreversible hair loss on the scalp, axillary, pubic region and other parts of the body during their first months of life. Also, papules were observed on the skin within the first years of life especially on the knees, scalp and elbows. Moreover, these patients showed normal nails, teeth, hearing and sweating. They also had normal growth and development. Figure 5.2, shows one affected member from the identified families who has no scalp hair with whitish hypopigmented layer.

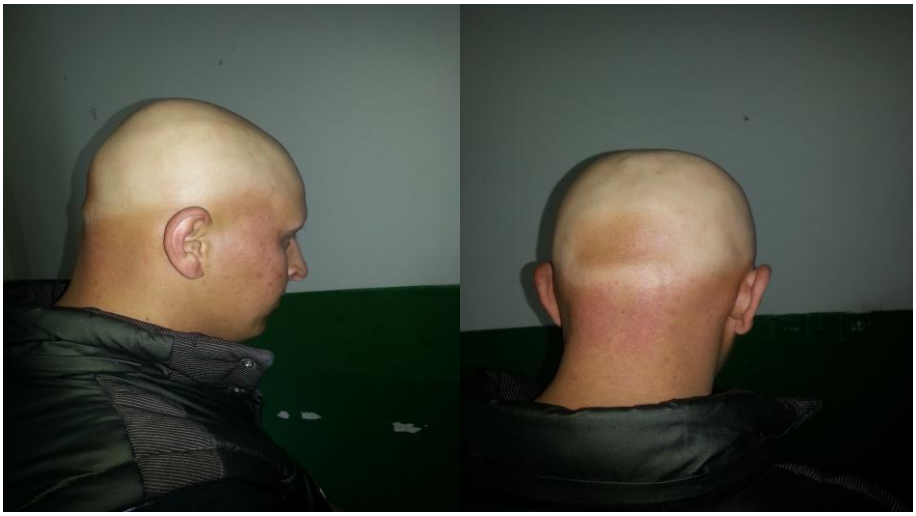


Figure 5.2: picture for one affected member from our families.

To find the causative mutation in these families, Linkage Exclusion Analysis was used at the beginning of the study to indicate that HR gene might be involved in causing this phenotype before we perform detailed molecular analysis. Two highly polymorphic microsatellite markers were used one before the HR gene (M1) and the other after it (M2). The two markers were homozygous in affected members in all families, while unaffected parents and unaffected members who are carriers were heterozygous and non-carrier members were homozygous.

After that, Sanger sequencing was done to find the specific mutation in this gene. The Homo sapiens hair growth associated (HR) gene is composed 19 exons, exon 1 is non-coding but the other 18 exons are coding. To identify the mutation/s in the HR gene these five families, we first PCR amplified and directly sequenced exon 9 in APL-B (APL-B3 and APL-B4) family to look for the most common 2147delC mutation identified in this exon. The results revealed that this mutation is present in all affected members of the five families. A single base pair deletion mutation was found at position 2147 (2147delC) that causes a frameshift (fS) and changed the reading frame. It causes a change from Proline (P) to Glutamine (Q) at position 716 (P 716 Q fS*186). This frameshift causes an early appearance of alternative stopcodon at 901 instead of 1190. It is in position 186 after the changed amino acid glutamine (Q) (figure 5.3). This stopcodon is 544 bps downstream in exon 12 (Wang, Tu et al. 2013).

Wild type amino acid sequence

MESTPSFLKG TPTWEKTAPE NGIVRQEPGS PPRDGLHHGP LCLGEPAPFW RGVLSTPDSW
LPPGFPQGPK DMLPLVEGEG PQNGERKVNW LGSKEGLRWK EAMLTHPLAF CGPACPPRCG
PLMPEHSGGH LKSDPVAFRP WHCPFLLETK ILERAPFWVP TCLPPYLVSG LPPEHPCDWP
LTPHPWVYSG GQPKVPSAFS LGSKGFYYKD PSIPRLAKEP LAAAEPGLFG LNSGGHLQRA
GEAERPSLHQ RDGEMGAGRQ QNPCPLFLGQ PDTVPWTSWP ACPPGLVHTL GNVWAGPGDG
NLGYQLGPPA TPRCPSPEPP VTQRGCCSSY PPTKGGGLGP CGKCQEGLEG GASGASEPSE
EVNKASGPRA CPPSHHTKLK KTWLTRHSEQ FECPRGCPEV EERPVARLRA LKRAGSPEVQ
GAMGSPAPKR PPDPFPGTAE QGAGGWQEVR DTSIGNKDVD SGQHDEQKGP QDGQASLQDP
GLQDIPCLAL PAKLAQCQSC AQAAGEGGGH ACHSQQVRRS PLGGELQQEE DTATNSSSEE
GPGSGPDSRL STGLAKHLLS GLGDRLCRLL RREREALAWA QREGQGPAVT EDSPGIPRCC
SRCHHGLFNT HWRCPRCSHR LCVACGRVAG TGRAREKAGF QEQSAEECTQ EAGHAACSLM
LTQFVSSQAL AELSTAMHQV WVKFDIRGHC PCQADARVWA PGDAGQQKES TQKTPPTPQP
SCNGDTHRTK SIKEETPDSA ETPAEDRAGR GPLPCPSLCE LLASTAVKLC LGHERIHMAF
APVTPALPSD DRITNILDSI IAQVVERKIQ EKALGPGLRA GPGLRKGLGL PLSPVRPRLP
PPGALLWLQE PQPCPRRGFH LFQEHWRQGQ PVLVSGIQRT LQGNLWGTEA LGALGGQVQA
LSPLGPPQPS SLGSTTFWEG FSWPELRPKS DEGSVLLLHR ALGDEDTSRV ENLAASLPLP
EYCALHGKLN LASYLPPGLA LRPLEPQLWA AYGVSPHRGH LGTKNLCVEV ADLVSILVHA
DTPLPAWHRA QKDFLSGLDG EGLWSPGSQV STVWHVFRAQ DAQRIRRFLQ MVCPAGAGAL
EPGAPGSCYL DAGLRRRLRE EWGVSCWTLL QAPGEAVLVP AGAPHQVQGL VSTVSVTQHF
LSPETSALSA QLCHQGPSLP PDCHLLYAQM DWAVFQAVKV AVGTLQEAK*

mutated amino acid sequence

MESTPSFLKG TPTWEKTAPE NGIVRQEPGS PPRDGLHHGP LCLGEPAPFW RGVLSTPDSW
LPPGFPQGPK DMLPLVEGEG PQNGERKVNW LGSKEGLRWK EAMLTHPLAF CGPACPPRCG
PLMPEHSGGH LKSDPVAFRP WHCPFLLETK ILERAPFWVP TCLPPYLVSG LPPEHPCDWP
LTPHPWVYSG GQPKVPSAFS LGSKGFYYKD PSIPRLAKEP LAAAEPGLFG LNSGGHLQRA
GEAERPSLHQ RDGEMGAGRQ QNPCPLFLGQ PDTVPWTSWP ACPPGLVHTL GNVWAGPGDG
NLGYQLGPPA TPRCPSPEPP VTQRGCCSSY PPTKGGGLGP CGKCQEGLEG GASGASEPSE
EVNKASGPRA CPPSHHTKLK KTWLTRHSEQ FECPRGCPEV EERPVARLRA LKRAGSPEVQ
GAMGSPAPKR PPDPFPGTAE QGAGGWQEVR DTSIGNKDVD SGQHDEQKGP QDGQASLQDP
GLQDIPCLAL PAKLAQCQSC AQAAGEGGGH ACHSQQVRRS PLGGELQQEE DTATNSSSEE
GPGSGPDSRL STGLAKHLLS GLGDRLCRLL RREREALAWA QREGQGPAVT EDSPGIPRCC
SRCHHGLFNT HWRCPRCSHR LCVACGRVAG TGRAREKAGF QEQSAEECTQ EAGHAACSLM
LTQFVSSQAL AELSTAMHQV WVKFDIRGHC PCQADARVWA PGDAGQQKES TQKTPQLHNL
PAMATPTGPR ASKRRPPIPL RPQQRTVLAE GPCLVLLSAN CWLLPRSNSA WAMSEYTWPS
PPSLRPCPVM TASPTSWTAL SHRWWNGRSR RKPWGRGFEL ARVCARAWAC PSLQCGPGCL
PQGLCCGCRS PSLALGVAST SSRSTGGRAS LCWCQGSKGH CRATCGGQKL LGHLEARCRR
*

Figure 5.3: amino acid sequences for the wild type and mutant hairless protein (http://www.mutationtaster.org/).

Sanger sequencing results showed that this deletion segregated perfectly with APL all tested families APL-A, APL-B, APL-C, APL-D, and APL-E. We reported this deletion most probably responsible for the Papular Atrichia in those Palestinian families. All eight affected individuals who were tested in this study were homozygous for the deletion and all twenty two unaffected individuals were either heterozygous or homozygous normal. Our results propose that HR gene is the causative gene of Congenital Atrichia in the five Palestinian families.

In HR gene, the 2147delC caused the loss of JmjC domain. It is located at the carboxyl terminus of the human hairless gene (946–1157 amino acids). It is composed of 212 amino acids (figure 5.4). This domain is highly conserved in different species including human, platypus, dog, rat, mouse, opossum and rabbit genomes (figure 2.4). This high conservation gives an indication that this domain may be important to the hairless protein function. With the absent of this domain, the protein lose demethylase activity. Which plays an important function in histone demethylation mechanism that is conserved from yeast to human (Abbasi 2011). Loss of demethylase activity will lead to consistent methylation that represses transcription of the HR interacting signaling pathways genes (Azeem, Wasif et al. 2011). Any protein that possesses this domain works as a candidate for enzymes that regulates chromatin remodeling (Clissold and Ponting 2001). Moreover, this domain is involved in the production of NAD+ precursors that are responsible for protection against apoptosis. Consequently, without this domain as a result of mutation in HR gene, the matrix cells in the follicle undergo apoptosis and hair will not grow again (Clissold and Ponting 2001).

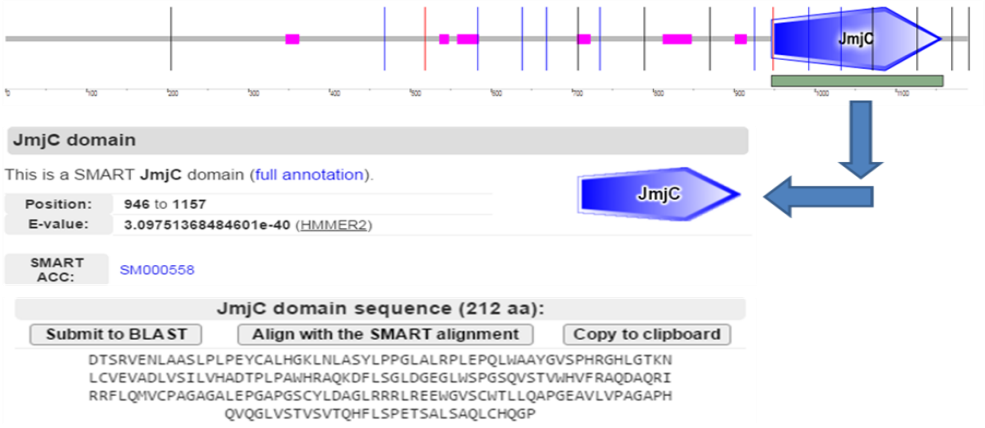


Figure 5.4: the description of JmjC domain by using SMART database (http://smart.embl.de/).

The 2147delC mutation that was reported in our study is a recurrent mutation. It has been previously identified in five Arab Palestinian families with Congenital Atrichia. These families lived near Jerusalem. They had the same phenotypes, irreversible haring loss with irruption of papules on skin (Zlotogorski, Ahmad et al. 1998). This mutation was considered as a 'founder mutation' which means it came from one ancestor (Wang, Tu et al. 2013).

Among the Palestinian population consanguineous marriages are very common and it constitutes about 40% of all marriages in the west bank according to Palestinian central Bureau in 2009. This type of marriage leads to increase the occurrence of many of genetic diseases such as autosomal recessive disorders. All of our families have first and second cousin marriages. That explains the appearance of the APL phenotype in the affected individuals of these families. Also, these affected siblings in the five families shared the same mutation which suggests that these families may have the same ancestor especially they belong to the same geographical area.

Until now, Congenital Atrichia is misdiagnosed with its phenocopy Alopecia Universalis (AU). This occurs as a result of lack of awareness, lack of known diagnostic criteria and the belief that patients with APL are found only in consanguineous families and rarely. Also, APL patients fail to respond to AU manufactured therapies, and sometimes these drugs cause side effects for APL patients (Indelman, Bergman et al. 2003). Eventually, to solve this problem, it is important to condense research in this area to determine the genetic background and causative mutations for this disorder which leads to correct diagnosis for this genetic disease in affected Palestinian families. Moreover right dealing by the community with this illness will highly help patients to coexist with the disease.

5.2 Concluding Remarks

- Five consanguineous Palestinian families suffer from Atrichia of Papular Lesions disorder. In this phenotype the affected members are characterized by irreversible hair lose after birth with widespread of papules on skin during the first years of life.
- Our study reports a single base pair deletion of in exon 9 of HR gene mutation at position 2147 (2147delC) in a homozygous form in all affected siblings and heterozygous or wildtype in all unaffected members of five consanguineous Palestinian families with Congenital Atrichia.
- The identified frame shift mutation in this study (P 716 Q fS*186) leads to premature termination codon at 544 bps downstream in exon 12. It is responsible for Papular Atrichia disease in these Families.
- This mutation in HR gene segregates perfectly in a recessive mode of inheritance in all families.
- The mutation was not detected in 100 healthy Palestinian controls.
- This gene also has been discovered to cause APL in people from different ethnic backgrounds

CHAPTER SIX

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