Screening of Genes involved in Non Syndromic Oculocutaneous Hereditary Albinism

By

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FINAL APPROVAL

It is certified that we have read and evaluated the thesis Screening of Genes involved in Non Syndromic Oculocutaneous Hereditary Albinism in families from Pakistan submitted by Ms. Sehrish Sarwar and it is our judgment that this project is of sufficient standard to warrant its acceptance by the INTERNATIONAL ISLAMIC UNIVERSITY, ISLAMABAD, in partial fulfillment of the requirements for the M.S Degree in Biotechnology.

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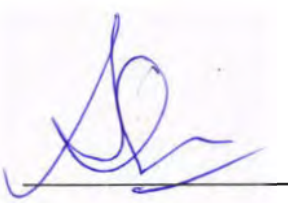

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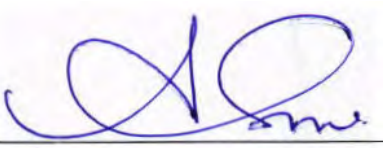

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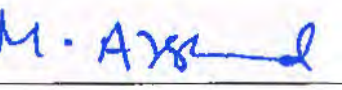

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DEDICATION

I dedicated this dissertation, by the core of my heart, to my beloved family and supervisor. Without their support, bunch of sincere prayers and sacrifices it would not have been possible for me to accomplish my work.

DECLARATION

I hereby declared that the work presented in this thesis is my own effort and hard work and it is written and composed by me. No part of this thesis has been previously published or presented for any other degree or certificate.

Date _____

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LIST OFABREVIATIONS

OCA	Oculocutaneous albinism
TYR	Tyrosinase
TYRP1	Tyrosine related protein
SLC45A2	Solute Carrier Family 45
SLC24A5	Solute carrier family 24
C10orf11	Chromosome 10 Open Reading Frame 11
GPR143	G Protein-coupled receptor
DOPA	Dihydroxy-phenylalanine
EDTA	Ethylene diamine tetra acetate
BOCA	Brown Oculocutaneousalbinisim
ROCA	Red Oculocutaneousalbinisim
MATP	Membrane associated transport protein
P protein	Pink eyed dilution
DCT	Dopachrome tautomerase
TGN	Trans golgi network

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Abstract

One of the most unambiguous visual characteristics found in living organisms is pigmentation. Albinism is an autosomal recessive disorders characterized by reduce pigmentation of the eyes, hair and skin linked with reduced clarity of vision (Montoliu *et al.*, 2014). Four well known explained types of OCA are ranging from OCA1A being the most acute type to the milder forms OCA1B, OCA2, OCA3 and OCA4, that may display some pigment assemble with time. Recently additional OCA types that have been recognized are OCA5, OCA6, and OCA7. The four presently considered families (A, B, C, D) were verified by genotyping polymorphic microsatellite markers for linkage related to different OCA loci. Family A members were affected with the disease known as Oculocutaneous albinism. Reported genes *(OCA2, TYRP1, SLC45A2, and C10ORF11)* which are involved in causing oculocutaneous albinism were tested for homozygosity mapping by using microsatellites markers. Affected individuals of family A exposed that they exhibit different combinations of parent alleles, thus excluding the linkage in this family to the known OCA candidate regions. None of these markers showed linkage to inherited OCA. This concluded that in family A, the involvement of a new gene might be accountable for causing Oculocutaneous albinism. Family B affected individuals showed the phenotypes of OCA. Blood were collected from two affected and two normal member of family B their DNA were subjected for further analysis. Genotyping was done by using highly polymorphic microsatellite markers to figure out affected gene or loci causing hereditary OCA. None of these genes showed linkage to autosomal recessive OCA of family B. This indicates that a new gene is involved in causing OCA in this family. In Family C, DNA of one affected individual (III-1) and two normal (II-1, II-2) were checked for genotyping. The markers linked to Oculocutaneous albinism are *OCA2* gene on chromosome 15q11.2-q12, *SLC45A2* gene on chromosome 5p13.3, *TYRP1* gene on chromosome 9p23, *C10ORF11* gene on chromosome 10q22.2-q22.3. None of these genes show linkage and were excluded. In Family D, DNA of two affected individuals (IV-3, IV-4) and four normal (III-2, 111-3) were tested for linkage by polymorphic microsatellite marker for respective candidate genes. The markers linked to Oculocutaneous albinism are *OCA2* gene on chromosome 15q11.2-q12, *SLC45A2* gene on chromosome 5p13.3, *TYRP1* gene on chromosome 9p23, *C10ORF11* gene on chromosome 10q22.2-q22.3 were studied. These genes were excluded and show no linkage.

CHAPTER 1

INTRODUCTION

Skin pigmentation is an extremely diverse human feature with a range from dark brown, white or pink to tan, or black. It displays a clear difference even within a particular ethnic group. The pigment melanin gives color to hair, skin, and eyes. The retina light-sensitive tissue in the eye also contains melanin therefore it plays a primary role in normal visualization (Castori *et al.*, 2012). In human's eyes, hair and skin melanin production is a vital cellular response in order to defend the cells from harmful ultraviolet light and from the risk of melanoma development (Cichorek *et al.*, 2013).

The studying of physiology and biology of pigment cells, where the melanin is produce is because of the genes, mutation in these genes cause albinism. Amid these genes are *TYR* and *TYRP1* that are considered to be encoding key melanogenic enzymes (Garcia-Borron and Solano, 2002; Sarangarajan and Boissy, 2001; Jimbow *et al.*, 2000; Oetting, 2000).In melanosomes solute carrier, specific receptor, integral proteins are found in the compartments where melanin is synthesized (Lopez *et al.*, 2008; Palmisano *et al.*, 2008; Schiaffino and Tacchetti, 2005; Toyofuku *et al.*, 2002; Newton *et al.*, 2001; Rosemblat *et al.*, 1994).

Melanin the skin color determinant is split up into two groups pheomelanin and eumelanin and its functions is to defend the DNA from damage from ultraviolet light (Ito and Wakamatsu, 2011; Ito and Wakamatsu, 2003). Brown or black human skin and hair color is due to eumelanin (black or brown color). Reddish color of skin is because of pheomelanin (Ito and Wakamatsu, 2011; Meredith and Sarna, 2006). The absence or decrease of pigments in the eye, skin, and hair is called Oculocutaneous albinism (Kamaraj and Purohit, 2014).

In skin, hair and eyes poor melanin production is linked with impaired vision and is easily attacked by sunlight results in Oculocutaneous albinism (Kamaraj and Purohit, 2014) or only diminishes pigments in the eyes is called ocular albinism. Albinism is an autosomal recessive disorders characterized by reduce pigmentation of the eyes, hair and skin linked with reduced clarity of vision (Montoliu *et al.*, 2014).

Partial or complete lack of pigmentation in oculocutaneous albinism is because of uneven enzyme or protein function that peculiarly metabolizes, produce and allocate melanin in melanocytes (Castori *et al.*, 2012).

In persons with albinism the hampering trait is the visual alterations in the absence of melanin in skin and reduced protection against the sun can be potentially overcome with ample protection. Visual problems include photophobia, foveal hypoplasia, nystagmus and lack of pigmentation in the iris (Martinez and Montoliu, 2013).

1.1 Oculocutaneous albinism

Albinism is a heterogeneous genetic disorder with an autosomal recessive, X-linked inheritance pattern and autosomal dominant (Gittler and Marion, 2016). These OCA types are present at different prevalence round the world (Wright *et al.*, 2015). In OCA various genetic heterogeneity has been known and seven known loci have been linked with this disease. Amid these four genes tyrosinase (*TYR*), Pink eyed dilution for P-protein (P), Tyrosinase-related protein (*TYRP1*),Solute carrier 45 subunit A2 (*SLC45A2*) are known to cause diverse types of OCA respectively. *SLC24A5* (Wei *et al.*, 2013) and *C10orf11* (Gronskov *et al.*, 2013) are recently known two novel genes in which mutations results into OCA6 and OCA7 correspondingly. A new locus OCA5 has been exposed but its gene is yet to be identified (Kausar *et al.*, 2013). The *GPR143* (MIM 300808) gene is positioned on chromosome Xp22.32 encodes a 404 amino acid protein predicted to be a membrane protein and known to cause ocular albinism. *SILV, RAB7, TYRP2, SLC24A5,* and *RAB38 are* numerous genes encoding melanosomal proteins including have been well thought-out as fine applicant for OCA. Although in OCA patients pathological mutations of these genes were not reported (Mondal *et al.*, 2012; Suzuki, 2003).

1.2 Prevalence of Oculocutaneous albinism

Albinism has an incidence rate of 1 in 20,000 individuals in which OCA1 is the most commonly responsible locus worldwide (Gargiulo *et al.*, 2011). In the Western populations, the prevalence rate of albinism are best deliberate in North America and Europe, noticed to be 1:17 000 in newborns and in the range of 1:10 000–20 000 (Gargiulo *et al.*, 2011; Gronskov *et al.*, 2009, 2007; Hutton and Spritz, 2008; Rooryck *et al.*, 2008; Zuhlke et al., 2007; Rundshagen *et al.*, 2004; Oetting and King, 1999). Several types of albinism have been described from Asia (Wei and Li, 2013; Wei *et al.*, 2011, 2010; Suzuki and Tomita, 2008 Lin *et al.*, 2006; Inagaki *et al.*, 2004). In Africa whereas the prevalence rate is high is because of consanguinity marriages especially (Cruz-Inigo *et al.*, 2011; Aquaron *et al.*, 2007; Spritz *et al.*, 1995).

1.3 Inheritance Pattern

1.3.1 Recessive Oculocutaneous Albinism (OCA)

In case of recessive OCA disease, one copy of the defective gene is transferred to a child from each parent. Carriers of the disease have only one copy of the defective gene without any symptoms and can transmit the disease to the next generation. Compound heterozygous persons may have mutations in two different alleles.

1.3.2 X-Linked Recessive Inheritance

Hereditary pattern of Ocular albinism (OA) is X-linked recessive. Males have one X and one Y chromosome while females possess two copies of the X chromosome. As an X chromosome is transferred from the mother of a male and a Y chromosome from the father so, an X-linked trait in a male can develop only due to X chromosome from the mother. Due to the need of only one mutated copy of the gene to develop the condition, OA is occurred more frequently in males as compared to females (Nusinowitz & Sarraf 2008; Wapenaar *et al.* 1993).

1.4 Classification of Oculocutaneous Albinism

The known types of autosomal recessive OCA are ranging from OCA1-7 caused by mutations in seven different genes. OCA is inherited as a hereditary autosomal recessive form.

1.4.1 Oculocutaneous albinism type 1

Reduced melanin production in the skin, hair and eyes is linked with OCA1. Two types of OCA1 are OCA1A and OCA1B.Mutations in tyrosinase that produces an entirely inactive or an incomplete tyrosinase enzyme polypeptide results in OCA1A. Melanin biosynthetic pathway blocks the first step due to complete lack of tyrosinase enzyme and results in no melanin forms in melanocyte (Simeonov *et al.*, 2013). The alteration of tyrosine to (DOPA) L-dihydroxy-phenylalanine and then DOPA to DOPA-quinine or 5, 6-dihydroxyindole to indole- 5, 6quinone is catalyzes by tyrosinase the rate limiting step in melanin biosynthesis (Simeonov *et al.*, 2013).Mutations of tyrosinase that produce a partially active or hypomorphic tyrosinase enzyme causes OCA1B (Simeonov *et al.*, 2013; Gronskov *et al.*, 2007).

1.4.2 Oculocutaneous albinism type 2

In Africa OCA2 is more familiar generally but not acute than OCA1 type (Gronskov *et al.*, 2014). P

protein with 12 forecast transmembrane domains is melanocyte-specific transporter protein and is encode by OCA2 gene. It transports proteins, substrates such as tyrosine or tyrosinase as a transporter protein in the melanosome to control pH and stable melanosomal protein complex during melanin synthesis (Hawkes *et al.*, 2013).

1.4.3 Oculocutaneous albinism type 3

Rufous/red OCA (ROCA) also known as OCA3 is a mild form of albinism due to color of pigmentation (Gronskov *et al.*, 2014). In melanin synthesis the function of *TRP1* remains uncertain. However *TRP1* is mainly restricted within melanosomes where it may regulate the melanogensis and exhibit in melanocyte (Ghanem and Fabrice, 2011). *TYR* in large molecular complexes is stabilize by *TYRP1* and without which *TYR* is degraded rapidly (Kobayashi & Hearing, 2007).

1.4.4 Oculocutaneous albinism type 4

OCA4 is rare among Europeans and found in Japanese population (Gronskov *et al.*, 2014). OCA4 is melanocyte differentiation antigen may convey substances required for melanin biosynthesis. *SLC45A2* function as a membrane transporter associated with intracellular trafficking and tyrosinase processing and contains 12 putative transmembrane domains (Inagaki *et al.*, 2006). OCA4 phenotypes results due to mutations in the gene *SLC45A2*.In Turkish origin OCA4 phenotype was recognized in one male. OCA4 is considered to be familiar type of OCA after OCA1 in Japanese individuals (Inagaki *et al.*, 2004). Phenotypically OCA2 and OCA4 are similar so it is not possible to identify OCA4 precisely only on medical findings.

1.4.5 Oculocutaneous albinism type 5

OCA5 a new gene was known in consanguineous Pakistani family accountable for the OCA phenotype (Kausar *et al.*, 2013). This genotype was designated as OCA5 and is mapped on chromosome 4q24 having 14 genes but none of those 14 genes are recognized to have any function in melanin synthesis but to recognize the underlying gene, studies are being made to find out the role of this locus (Montoliu *et al.*, 2014).

1.4.6 Oculocutaneous albinism type 6

Wei Li led a Chinese team of researchers; expose the new molecular basis of albinism type in an

OCA affected family.In *SLC24A5,* they inaugurate mutations and found a new solute carrier protein and a renowned gene was also linked with a new type of OCA called OCA-6 (Wei *et al.*, 2013). In the maturation of melanosomes part of *SLC24A5* is found by recent results (Wei *et al.*, 2013). Melanosomes assembly of *SLC24A5* show a vital function for the melanosomal design and to make certain that melanin is synthesizing appropriately. Therefore mutation in *SLC24A5* may damage or interrupt melanosomal maturation and consequently, OCA6 phenotype results (Wei *et al.*, 2013).

1.4.7 Oculocutaneous albinism type 7

By founding gene sequencing and homozygosity mapping in a consanguineous family with OCA symptoms on the Faroe Islands of Denmark, Karen Gronskov and Thomas Rosenberg documented OCA7 type in 2013 (Gronskov *et al.*, 2013).OCA7 phenotypes results from mutation in gene *C10orf11* (Montoliu *et al.*, 2014). In melanin biosynthesis the *C10orf11* protein is known to be function in melanocytes distinction (Gronskov *et al.*, 2013).

1.4.8 Ocular albinism

The birth prevalence of X-linked ocular albinism (OA1; MIM 300500) is approximately 1 in 50,000. Ocular abnormalities, including impaired visual acuity, nystagmus, photophobia, foveal hypoplasia, hypopigmentation of iris and fundus are most common in males in OA1 (Preising *et al.*, 2001). *GPR143* is exceedingly found in retinal pigment epithelium and melanocyte encodes a protein that binds to heterotrimeric G proteins (Bassi *et al.*, 1995).

1.1 Proteins involved in OCA along with functions

Albinism types & Genes	Chromosome Location	Proteins	Function of proteins	References
OCA1A/ *TYR* OCA1B/ *TYR*	11q14.3	Tyrosinase	Alteration of tyrosine to DOPA and DOPA to 5,6-dihydroxyindole to indole- 5, 6quinone	(Simeonov *et al.*, 2013)
OCA2/ *OCA2*	15q12-13	Pink eye dilution	It may convey proteins, tyrosine or tyrosinase in melanosome to control melanosomal pH	(Hawkes *et al.*, 2013)
OCA3/ *TYRP1*	9p23	Tyrosinase related protein 1	Restricted within melanosomes where it may regulate the melanogensis	(Ghanem and Fabrice, 2011)
OCA4/ *SLC45A2*	5p13.2	MATP	Membrane transporter associated with intracellular trafficking and tyrosinase processing	(Inagaki *et al.*, 2006)
OCA5/gene Unknown	4q24	unknown	14 genes in chromosomal locus but none of these are directly involve in melanin biosynthesis	(Montoliu *et al.*, 2014)
OCA6/ *SLC24A5*	15q21.1	Solute carrier family 24 protein member 5	Maturation of melanosomes and to make certain that melanin is properly synthesized	(Wei *et al.*, 2013)
OCA7/ *C10orf11*	10q22.2	C10orf11	Play a role in melanocytes distinction	(Gronskov *et al.*, 2013)
OA1/ *GPR143*	Xp22.2	G-protein coupled receptor 143	Receptor for tyrosine, L-DOPA and dopamine, involved in melanosome biogenesis, organization and transport	(Innamorati *et al.* 2006)

1.5 Clinical Description

OCA1 has further two types. No melanin synthesis in any tissue is linked with OCA1A while least amount of melanin in body is linked with OCA1B (Lewis R. A., 2013). White hair brows, lashes and white skin at birth have been found in OCA1A affected individuals. In all ethnic groups the skin stays white throughout life and always burns but does not tan. The blue irides are fully translucent at birth and stay so the whole life. The retinal pigment epithelium does not build up melanin pigment and nystagmus continues. 20/100 and 20/400 is considered to be the best visual acuity. White or very light off white hair at birth and visible light yellow hair color by age one to three years are found in OCA1B affected individuals. In prolonged solar exposure the skin color remains white and burn but may develop some generalized tan.

Hypopigmentation of the skin, hair and the ocular changes nystagmus, reduced iris pigment characterized OCA2 type. Initially in Africans and African Americans brown OCA is known with light brown hair and skin. OCA2 analysis is based on clinical findings. In OCA2 type the amount of cutaneous pigmentation ranges from minimal to near-normal. OCA2 approximately always have lightly pigmented hair, brows, and lashes, with color ranging from light yellow to blond to brown in newborns. Hair color does not vary considerably but may darken with age from teenage years to later life (Lewis R.A, 2013).

Phenotype of affected individuals of OCA3 is a moderate and accrue reddish pigment in their hair and skin mainly clear in families of African origin. Inheritance of OCA3 is also in autosomal recessive manner.

Hypopigmentation of the skin, hair and the ocular characteristics like all other types of albinism characterized OCA4 type. After early childhood vision is probably constant. In OCA4 the amount of cutaneous pigmentation ranges from minimum to near standard. In OCA4 newborns generally have silvery white to light yellow pigment in their hair color. Hair color does not vary considerably but may darken with time from childhood to adulthood.

A new gene positioned on chromosome 4q24 is known as OCA5. Clinical symptoms of OCA5 of affected individuals presented is white skin, golden colored hair, foveal hypoplasia, nystagmus, photophobia and impaired visual perception despite of their age and sex (Montoliu *et al.*, 2014).

The rarest form of non-syndromic autosomal recessive OCA known as OCA6 have light hair color, that darkened with age, reduced visual insight, fovea hypoplasia, iris trans illumination, photophobia and nystagmus. SLC24A5 is found to be role in the maturation of melanosomes (Wei *et al.*, 2013).

OCA7 type of albinism is linked with the gene *C10ORF11*. Karen Gronskov and Thomas Rosenberg in 2013 found a new gene associated with albinism amid OCA individuals from the Faroe Islands (Denmark). Lighter pigmentation in affected individuals is found as compared to relatives but with nystagmus, iris transillumination, reduced visual acuity is predominant eye symptoms. This OCA type has been called as OCA7.

1.2 Phenotypic presentation of different types of OCA

Types of OCA and Genes	Skin color	Hair Color	Eye color	Visual Acuity	Nystagmus	Photophobia
OCA1A *TYR*	White, Pinkish	White	Light blue to pink Gray	Present	Present	Present
OCA1B *TYR*	White, tan with Tim	White to Light yellow blond and then brown	Blue iris, green or brown with time	Present	Present	Present
OCA2 *OCA2*	Light brown	White, light golden, Yellow, light brown	Blue-gray or hazel Irides gray to Tan	Present	Present	Present
OCA3 *TYRRP1*	Reddish brown brick red	Ginger reddish bright copper red	Hazel / brown	Present	Present	Present
OCA4 *SLC45A2*	Yellow skin	White, yellow to brown hair	gray/ blue	Present	Present	Present
OCA5	White skin	golden hair	impair vision	Present	Present	Present
OCA6 *SLC24A5*	White skin	Hair light in color may darken later	Transparent / brown irides	Present	Present	Present
OCA 7 *C10ORF11*	Skin light color	light blond/ dark brown	Iris transillumination	Present	Present	Present

1.6 Risk Factor

A person inherits the faulty genes that cause him incompetent to make the usual amounts of melanin. Based on its particular type various genes are involved. Individuals results from the information that genes carry. Two copies of these autosomal chromosomes genes are present in individuals one inherited from father and the second from mother. A person without being affected by albinism can carry its trait because it is a recessive genetic disorder. To have a child with albinism both mother and father must carry a faulty gene. If both carry a faulty gene but none of the parent has albinism there is a one in four chance that baby will born with albinism (Tandon, 2016).

1.7 Causes

The major cause of Oculocutaneous albinism is hypopigmentation and melanin is considered to be major pigment in skin, hair and eyes. All types of albinism in general have hypopigmentation but its quantity varies in different types. Completely absence of pigment in the eyes, skin and hair causes OCA but some persons have pigments at later stage of life. The eyes of the albino people are mostly of very light color because of a lack of melanin pigment. For normal function of eye melanin is required so due to absence of adequate melanin pigment, vision is diminished. Melanin gives hair color on the scalp and body. Hair appears white, reddish, golden, very light blonde, or of light yellow color due to decreased amount of melanin. Milky white color and fair skin is present in albino peoples. There are more chances of skin cancer by ultraviolet rays and skin damage from sunburn in OCA affected individuals due to the absence or reduced amount of melanin (Dorcy *et al.* 2003).

1.8 Genetic variability of Oculocutaneous albinism

With genetic heterogeneity non syndromic OCA is an autosomal recessive state. One more locus and six genes have been recognized as accountable for seven non syndromic OCA subtypes. Hereditary disorders the melanin biosynthesis that results in varied levels of hypopigmentation of hair, skin and eyes are termed as Oculocutaneous albinism (OCA) (Grønskov *et al.*, 2007). In melanin biosynthesis pathway six genes known for OCA subtypes. These known genes are *tyrosinase* , *OCA2, tyrosinase-like protein 1, solute carrier family 45 member 2, solute carrier family 24 member 5*, and *chromosome 10 open reading frame 11* (Montoliu *et al.*, 2014). By linkage analysis to a genomic locus at 4q24, OCA5 was recognized (Montoliu *et al.*, 2014). The *GPR143* (MIM 300808) gene responsible for x- linked recessive albinism. Phenotypes of OCA subtypes are heterogeneous but all

of these are implicit to be hereditary recessive disorders (Grsønskov *et al.*, 2007; King *et al.*, 2003; Oetting and King, 1999). OCA1 has subtypes, OCA1A and OCA1B. Totally lack of pigment and entire failure of tyrosinase activity is called 'tyrosine negative' or OCA1A. If tyrosinase function partially and permitting pigment to accumulate and produce a phenotype of minimum to close to usual skin pigmentation is termed as OCA1B (Gronskov *et al.*, 2007; King *et al.*, 2003). Pigmentary phenotype of individuals depends on polymorphisms in the OCA genes (Jagirdar *et al.*, 2014; Chiang *et al.*, 2008). An individual's albinism phenotype is greatly influence by ethnic background (Mondal *et al.*, 2016; Chiang *et al.*, 2009).

1.8.1Tyrosinase (*TYR*)

The human *tyrosinase* (MIM 606933) gene positioned on chromosome 11q14–q21 (Khan *et al.*, 2015) MIM 606933 and has 5 exons which translate to produce the 529 amino acids long tyrosinase protein encodes a glycoprotein of 60kDa tyrosinase type I and spans about 65 kb of the genomic DNA (Ray *et al.*, 2007). Mutation studies have found 351 pathogenic mutations (HGMD Professional 2016.1 total) in TYR gene, in which more than 15 are reported in Pakistani families (Khan *et al.*, 2015; Forshew *et al.*, 2005).

1.8.2 Oculocutaneous albinism 2 (*OCA2*)

The *OCA2* gene (MIM 611409) positioned on chromosome region 15q11.2-q12 and encodes 838 amino acids of P protein of 92,850 Da spans 345kb genomic DNA and contain 29 exons. 12 known transmembrane domains of P protein is melanocyte specific transporter protein. It transports tyrosine (Potterf *et al.*, 1998; Gahl *et al.*, 1995) protons (Ancans *et al.*, 2001a; Ancans *et al.*, 2001b; Brilliant and Gardner, 2001; Puri *et al.*, 2000) or tyrosinase in the melanosome as a transporter protein to regulate melanosomal pH or stabilize the melanosomal protein complex during melanin synthesis (Hawkes *et al.*, 2013; Gronskov *et al.*, 2007; Toyofuku *et al.*, 2002).

1.8.3 Tyrosinase related protein 1 (*TRP1*)

The *TYRP1* gene (MIM 115501) positioned on chromosome 9p23 consists of 8 exons and 60,724 Da, of *TRP1* (tyrosinase-related protein 1) of about 16Kb genomic DNA and encodes a 537 amino acids and is mainly confined within melanosomes and expressed in melanocytes where it may regulate the melanogensis (Ghanem and Fabrice, 2011).

1.8.4 Solute carrier family 45 member 2 (*SLC45A2*)

The *SLC45A2* gene (MIM 606202) is positioned on chromosome 5p13.2. SLC45A2 have 7 exons spanning a region of approximately 40 kb and contain 530 amino acids. It may transport substances required for melanin biosynthesis and is melanocyte differentiation antigen (MATP) (Inagaki *et al.*, 2004). Misrouting of tyrosinase results due to mutation in *SLC45A2* (Cullinane *et al.*, 2011; Costin *et al.*, 2003).

1.8.5 Oculocutaneous albinism 5 (OCA5)

OCA5 a new type was known accountable for the OCA phenotype and its locus is positioned on chromosome 4q24 but gene is not found yet. In consanguineous Pakistani family OCA5 phenotype was exposed in the affected members of OCA (Kausar *et al.*, 2013).

1.8.6 Solute carrier family 24 member 5 (*SLC24A5*)

The *SLC24A5* (MIM 609802) positioned on chromosome 15q21.1, genomic DNA spanning a region of 21.4 kb, consists of 9 exons, and solute carrier protein of about 54,888 Da and it encodes a 500 amino acids. Its function is trafficking of proteins to melanosomes regulated by intracellular calcium signaling and calcium homeostasis in melanosomes during melanin biosynthesis (Ginger *et al.*, 2008; Lamason *et al.*, 2005). Melanosomal maturation is disrupt due to mutations in *SLC24A5* and consequently hypopigmentation results in patients with OCA6 (Mondal *et al.*, 2012).

1.8.7 Chromosome 10 open reading frame 11 (*C100RF11*)

The *C10orf11* (MIM 614537) *gene* is positioned on chromosome 10q22.3, spans 1125.9 kb genomic DNA and consists of 11 exons. This gene encodes 198 amino acids, protein of about 22,568 Da that is leucine-rich repeat and in fetal tissue it is highly expressed. In melanin biosynthesis this *C10orf11* encoded protein plays a role in demarcation the melanocytes (Gronskov *et al.*, 2013). OCA7 phenotypes results due to mutations in the *C10orf11* gene.

1.8.8 G protein-coupled receptor 143 (*GPR143*)

The *GPR143* (MIM 300808) gene is positioned on chromosome Xp22.32 encodes a 404 amino acid protein predicted to be a membrane protein essential for development and maturation of melanosomes (Schiaffino *et al.*, 1996).

1.9 Complications

1.9.1 Adverse Cutaneous and ocular Effects of Sun Exposure in OCA

Blistering and erythema is predictable by sunburn and is the commonest acute result of extreme sun contact. Fair skin individuals are at maximum risk of sunburn even though pigmented skin can also practice it (Battie *et al.,* 2013). In OCA persistent ultraviolet rays increase the risk of skin cancer and considered to be the most serious cutaneous effect. Lack of melanin in the iris results in strabismus, reduced stereoscopic vision and iris transillumination in OCA patients (Summers, 2009).

1.9.2 Challenges and Opportunities

A person with OCA faces both psycho-social and physical challenges. Violation of human rights is found in OCA affected individuals in some countries. Loss of bodily dignity, social stigmatization, discrimination, they also practice ostracism as well as more aggressive outcomes body parts removed for witchcraft and including death at birth (Cruz-Inigo *et al.,* 2011). The damaging health effects by contact to solar UVR can be overcome by personal sun protection (Norval *et al.,* 2011). Wearing protective clothing, sunglasses, hats, are sun-protective options and making use of shade may be helpful (Wright *et al.,* 2015).

1.10 Pigmentation

Pigments of the human body are produced by specialized cell melanocytes in the hair and skin and choroidal melanocytes and RPE (retinal pigment epithelium) cells in the eye. The synthesis of the melanin pigment itself takes place in the melanosome, a specialized organelle of the pigment cell which is derived from the lysosome (Schiaffino, 2010; Wasmeier *et al.,* 2008; Dell'Angelica, 2003).

1.11 Melanin

Melanin, hemosiderin (brown), carotene (yellow), hemoglobin (red) and bilin (yellow) are the pigments that determine human color. In determining color of human skin and hair melanin play a key role (Kondo and Hearing, 2011; Simon *et al.,* 2009). Hypopigmentation, hyperpigmentation and mixed hyper/hypopigmentation disorders results due to disruption of pigmented related factors.

1.11.1 Types of Melanin

Two major types of melanin pigments based on their biosynthetic pathways are pheomelanin and eumelanin (Kondo and Hearing, 2011; Simon *et al.,* 2009). Black or brown color is a eumelanin and

is intended for black or brown human hair and skin. Reddish color is a pheomelanin and is accountable for red hair and skin in humans (Ito and Wakamatsu, 2011; Meredith and Sarna, 2006).

1.11.2 Melanosome biology

Melanosomes mature in four steps from immature vesicles to fully mature and pigmented organelles. They begin as stage I pre-melanosomes which bud from the endoplasmic reticulum (ER) (Park *et al.*, 2009) and show a round form with no observable inner structure (Harper *et al.*, 2008).The trans-golgi network (TGN) trafficks structural (PMEL17/GP100) and melanosomal (TYR) proteins to the empty vesicles to form stage II melanosomes. At this stage the melanosomes get elongated and build an organized fibrillar network based on PMEL17/GP100 proteins (Theos *et al.*, 2005). At this point the path between eumelanosomes and pheomelanosomes is parting as pheomelanosomes do not develop further than stage II. TYR is a key enzyme for the pigmentation process, as both melanin types need it for the first step of the synthesis. Only eumelanosomes receive TYRP1 and dopachrome tautomerase (DCT) (needed for the later stages of eumelanin synthesis) through the trans golgi network (TGN) and develop to stage III melanosomes. At this stage pigment is being produced and deposited along the matrix protein fibrils. At stage IV the pigment fills the complete melanosome (Schiaffino, 2010; Hearing, 2005).

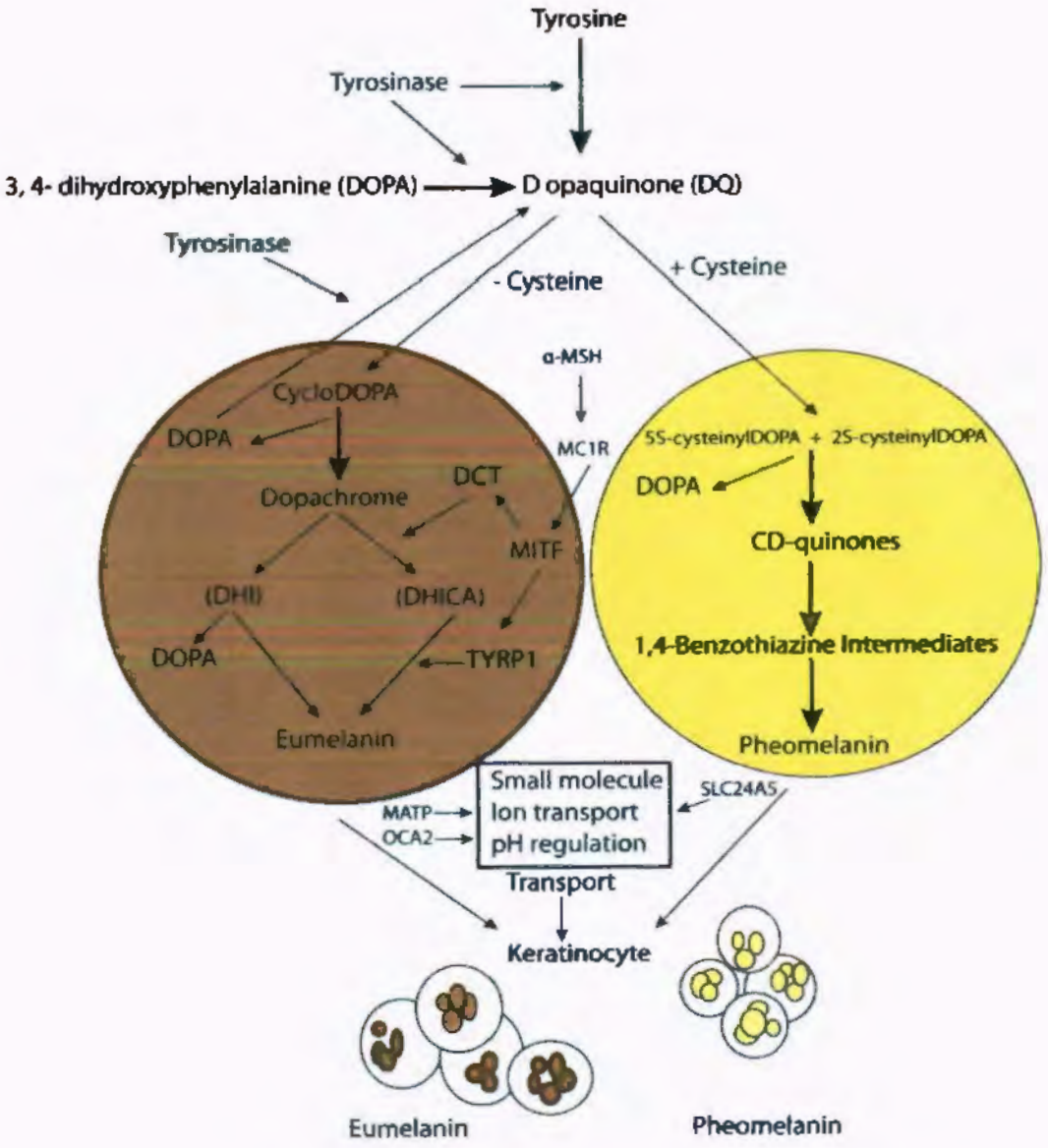


Figure 1.10: Melanin synthesis pathway (Ito & Wakamatsu 2008; Kondo Hearing 2011).

1.12 Objective

The aim of this study was to find the genetic causes of non-syndromic Oculocutaneous albinism in Pakistan

CHAPTER 2

MATERIALS AND METHODS

2.1 Identification, Enrollment and Clinical Assessment of Families

Identification of Oculocutaneous Albinism (OCA) patients was done through school, college, university survey and contacting medical doctors from different areas of Pakistan. Four families having three or more affected members were identified and enrolled for this study. The study was approved by Institutional Review Board at Institute of Biotechnology, International University Islamabad Pakistan.

2.2 Pedigree design

Pedigree was sketched by discussion with elders of families according to standard method given by Bennet *et al.*, 1995.A pedigree of each family was drawn and the relationships within the family were confirmed by interviewing multiple members of the family. Affected members with OCA were assessed based on hypopigmentation of hair, skin and eye color in comparison to the unaffected individuals.

2.3 Blood sample collection

Peripheral blood samples, about 5-6 ml were drawn by 10 ml sterilized syringes (0.7 X 40 mm, 22 G X 1 1/2) and transferred into vacutainers containing potassium EDTA(BD Vacutainers K3 EDTA, Franklin Lakes NJ. USA).These vacutainers (containing blood sample) were stored at 4C. Blood samples or buccal swabs were collected from affected individuals, normal siblings and parents available at the time of enrollment.

2.4 Extraction of Human Genomic DNA

Genomic DNA from peripheral blood samples were extracted by following two methods;

1) Phenol-chloroform DNA extraction or manual extraction of DNA (Sambrook and Russell, 2001).

2) Extraction of DNA using commercially available kits (Sigma-Aldrich MO, USA).

2.4.1 Genomic DNA Extraction (phenol-chloroform Method)

Standard Phenol-Chloroform method (Sambrook and Russell, 2001) was used for the extraction of genomic DNA from blood samples.

- Vacutainers® (BD-Plymouth, UK) having blood samples were incubated at room temperature for 40 minutes.
- Eppendorf tubes (Axygen, Union, USA) were labelled.
- About 0.75 ml of blood was poured in these Eppendorf tubes and was mixed with an equal volume (0.75 ml) of lysis buffer (Solution A).
- To properly mix the blood sample with solution A, tubes were inverted 6-7 times and were incubated for half an hour at 25°C.
- The tubes were centrifuged at 13,000 revolutions per minute (rpm) for 1 minute in a micro centrifuge (Eppendorf, Hamburg, Germany).
- The nuclear pellet was re-suspended in 0.5 ml of solution A after discarding the supernatant.
- The centrifugation step was repeated.
- Supernatant was again discarded.
- The nuclear pellet was suspended in solution B (400 ml), chilled proteinase K (8-10µl) diluted from 8 mg/µl stock bought from Sigma-Aldrich MO, USA, 14 µl of 20% sodium dodecyl sulphate (SDS) bought from BDH, England.
- An overnight incubation of the samples was taken out at 37°C.
- The next day, equal volumes (0.25ml) of freshly prepared of solution C (saturated Phenol)

and solution D (Chloroform: Iso-amyl alcohol in 24: 1 by volumes) were added to the tubes containing samples.

- The tubes were then subjected to centrifugation at 13,000 rpm for 10 minutes after being mixed thoroughly.
- The upper aqueous layer from each tube was picked gently and was transferred to a new tube and was labeled accordingly. Wide bore pipette was used to avoid sharing of DNA.

- These layers were centrifuged at 13,000 rpm for 10 minutes after adding 0.5 ml Solution D to each Eppendorf tube.
- Upper aqueous phases were again transferred into new tubes and the tubes were again labeled respectively.

- 50-55 µl of sodium acetate (3M, pH 6) and (0.5 ml) of chilled Iso-propanol was added in these tubes and DNA was precipitated out. Eppendorf tubes were inverted 3-5 times followed by another centrifugation 13,000 rpm for 10 minutes.

- Supernatant was discarded carefully.

- The pellet of the DNA obtained was the washed with chilled 70% ethanol (200 µl) and centrifuged for 7 minutes at 13,000 rpm.
- Again, the supernatant was discarded and the washed DNA pellet was dried in a vacuum concentrator, 5301 (Eppendorf, Hamburg, Germany) at 45°C for 4-5 minutes. 120-140 µl of dissolving buffer (Tris-EDTA, SigmaAldrich MO, USA) was added to the dried DNA and stored at 4 °C.

Table 2.1 Composition of solution used in genomic DNA extraction

Sr.no	Solution use for DNA Extraction	Chemical composition
1	Solution A	Mgcl2 5mM Tris (pH7.5) 10mM 1% (v/v) Triton X=100 Sucrose 0.32 M
2	Solution B	NaCL 400mM Tris (pH 7.5) 10mM EDTA (pH 8.8) 2mM
3	Solution C	Saturated phenol
4	Solution D	24 volumes of chloroform : 1 Volume of isoamylalchol i-e 24:1
5	DNA Dissolving Buffer	10mM Tris (pH 8.0) 0.1 mM EDTA
6	10% SDS	10g SDS 100ml water
7	Bromophenol blue	0.25g bromophenol blue 40g sucrose

2.4.2 Extraction of Genomic DNA by Kit Method

- 0.2 ml blood was taken from the vacutainer tube in an Eppendorf tube of 1.5 ml volume.
- 200 µl solution C (lysis solution) and 20 µl Proteinase K (SigmaAldrich MO, USA) were added in it.
- A homogenous mixture was obtained after a 20 second short spin.
- This mixture was then incubated at 55 °C for 10 minutes.
- Following the incubation, 100% ethanol (200 µl) was taken in Eppendorf tubes was vortexed for 10 seconds along with lysate.
- Columns were prepared by subjecting 500 µl column preparation solution (Sigma-Aldrich MO, USA) to centrifugation at 12,000 rpm for 60 seconds after adding it to GenEluteTM mini prep binding columns.
- From the Eppendorf tubes, the lysate was then transferred to each prepared column.
- The columns were centrifuged for at 8,000 rpm for 1 minute.
- 500 µl of pre-wash solution (Sigma-Aldrich MO, USA) was added to each column and centrifuged at 8,000 rpm for 1 minute.
- The eluted flow-through was discarded and columns were shifted to new tubes of 2 ml.
- Each column was subjected to centrifugation at 12,000 rpm for 1 minute after adding 500 µl of wash solution (Sigma-Aldrich MO, USA).
- After the eluted flow-through having removed out was subjected to a 3 minutes' empty spin at 12,000 rpm for making the columns free of the wash solution.
- This was followed by transferring each column to new collection

  tubes of 2ml.

- 200 µl of Tris EDTA elution buffer (Sigma-Aldrich, MO, USA) was loaded at the middle of each column to elute out the DNA.
- The eluted-out DNA was incubated for 5 minutes at room temperature and was then subjected to a centrifugation at 12,000 rpm for 2 minutes.

2.5 Agarose Gel Electrophoresis

For the qualitative analysis of extracted DNA, 1% agarose gel was used to resolve the DNA. Mixing distilled water of about 45 ml, 5 ml of 10X Tris -Borate – EDTA and half gram of agarose the required 1% agarose gel and was heated in microwave oven so that the agarose was totally dissolved. After that 6-7 µl staining dye (ethidium bromide) was prepared and added in the conical flask for staining DNA. The combs were placed to their respective slots in the gel tank before pouring the gel into the tank. The gel was set to solidify at room temperature for 25-30 minutes. After being solidified, the combs were removed carefully. Then equal volumes of DNA and bromophenol blue were mixed ad loaded in the gel. The DNA was electrophorized for 20-30 minutes at 120 volts using 1X TBE buffer. The pictures were obtained by using digital camera DC 290 (Kodak, New York, USA) and UV transilluminator (Biometra, Gottingen, Germany was used to visualize the DNA. The image data was then stored.

Table 2.2 Composition of solution used in agarose gel

Solutions	Composition
Gel Loading Dye	40 g Sucrose 0.25 Bromophenol Blue
10X TBE Buffer	0.89Tris M EDTA 0.5 M pH 8.3 0.025 M Borie Acid

2.5 Genotyping and linkage analysis

Polymorphic microsatellite markers of loci known to cause Oculocutaneous albinism in Pakistani families were used for genotyping and linkage analysis. These markers were obtained from Invitrogen Genelink (USA).

2.7 Polymerase Chain Reaction

Specific sequences of DNA were amplified by Polymerase chain reaction.

Undermentioned steps were used to carry out PCR.

- Properly labeled PCR tubes (200 µl) were used to carry out PCR containing a total of 25 µl reaction mixture in each of them.
- Firstly, 1-2 µl dilution (5 µl Stock DNA and 45 µl PCR water) of genomic DNA (template DNA) was loaded in a PCR tube (200 µl).
- 23-24 µl of master mix was added to this Genomic DNA.
- The master mix was prepared by adding 2.5 µl PCR reaction buffer [10X (NH4)2SO4, (MBI Fermentas, Life Sciences, UK)] and 0.5 µl of 10 mM dNTPs mixture to 2µl of 25 mM MgCl2 and 0.3-0.4 µl of each 20 ng/µl markers (forward and reverse). 0.3 µl of the 1 unit Taq DNA polymerase (PerkinElmerectus, Ferments, Burlington, Canada) was added along with 17.3-18.4µl PCR water.
- PCR was performed under standard conditions. T3 Thermocycler (Biometra, Germany) and GENE Amp PCR system 9600 (Perkin Elmer, Wellesley, MA, USA) were used for this purpose.

  In thermocycler following conditions were used for PCR
  - ❖ Early denaturation of whole DNA at 96 C for 5 minutes.
  - ❖ 40 cycles of amplification each consisting of 3 sub steps.

  1) Denaturation of DNA (amplified product) at 96 C for 1 minute.
  2) Annealing of primers at 50-60 C for 30 seconds.
  3) Primer elongation of complementary DNA strands at 72 C for 4 minutes.

Final elongation of remaining incomplete complementary DNA strands at 72 C for 10 minutes.

Table 2.3 Chemicals Used in Polymerase Chain Reaction

Chemical Used	Stock Concentration	Amount used in PCR
DNA dilution	2µl	2 µl
PCR water	17.3-18.4µl	17.3-18.4µl
PCR Buffer	10 X (200 mM Ammonium Sulphate, 750 mM Tris-HCL (pH 8.8), 0.1 % Tween 20)	2.5 µl
$MgCl_2$	25 mM	2 µl
dNTPs	10 mM each dNTPs	0.5 µl
Microsatellite Markers (R & F)	20 ng/µl each	0.3 µl each
Taq Polymerase	0.5 U/µl	0.3 µl

Table 2.4 The standard protocol conditions for thermocycler PCR

Steps	Subcycle	Conditions
Initial Denaturation		96 °C, 5-7 minutes
40 PCR Cycles	1. Denaturation	96 °C, 1 minute
	2. Primer annealing	50 °C-65 °C, 1 minute
	3. Primer Extension	72 °C, 1 minute
Final Extension		72 °C, 10 minutes

Table 2.5 Chemicals Used in Polyacrylamide Gel Electrophoresis (PAGE)

Chemicals	Composition	Amount/ gel
30% Acrylamide	29:1 ratio of acrylamide (MERCK, Darmstadt, Germany) N, N' Methylene-bisacrylamide (BDH, Poole, England	13.5ml
10X TBE	Tris 0.89 M, Borate 0.89 M and EDTA 0.02 M	5ml
10% APS	Ammonium per sulphate (5 g/45 ml distil water) (Sigma Aldrich St Louis, MO, USA)	400ul
TEMED	N, N, N', N'-Tetra methyl ethylene diamine) (Sigma-Aldrich, USA)	25ul
Distilled Water		Up to 50 ml

CHAPTER 3

Results

This chapter deals with the clinical and molecular analysis of four families which showed the symptoms of autosomal and x-linked recessive hereditary Oculocutaneous albinism. These families were enlisted from unconcerned territory of Pakistan.

3.1 Description of the families

3.1.1 Family A

The family A was enrolled in the Muzafarabad Kashmir, Pakistan and has 3 affected individuals in four generations (Fig 3.1). Individual's relation within the family and all required information were obtained and a family pedigree was drawn by consulting multiple people during the visit. Three affected individuals along with their normal siblings participated in this study. Pedigree analysis showed that this family contains three affected individuals including a seventeen years old male (IV-1) and two females (IV-4, IV-5).Clinical examination of skin, hair and iris phenotype of affected individuals showed that they had golden hair along with white to pinkish skin. All the affected individuals had black to brown iris color along with light sensitivity and nystagmus. Ages of the affected individuals varied between 4 and 17 years at the time of study. The normal individuals were fully normal with no sign of disease. Blood samples were collected from three affected (IV-1, IV-4, IV-5) and four normal (III-1, III-2, IV-2, IV-3) individuals including parents and normal siblings. The DNA was extracted using phenol-chloroform method in normal and affected individuals.

3.1.2 Family B

The family B was enrolled in the Mohrian, district Attock Punjab (Fig 3.3). Pedigree analysis showed that this family contains two affected individuals. Clinical examination of skin, hair and iris phenotype of affected individuals showed that they had yellow hair along with pinkish skin and spots on the face. The affected individuals had gray iris color along with light sensitivity and nystagmus. The normal individuals were fully normal with no sign of disease or disease on set in any of them. Blood samples of two affected (III-2, III-3) and two healthy (II-2, III-1) individuals

were collected for the current study. The DNA was extracted using phenol-chloroform method in normal and affected individuals.

3.1.3 Family C

The family C was enrolled in the Nothein district Attock Punjab, Pakistan (Fig 3.5).The family C contains one affected individual (III-1) two normal (II-1, II-2) individuals. Individual's relation within the family and all required information were obtained and a family pedigree was drawn. Clinical examination of skin, hair and iris phenotype of affected individuals showed that they have reddish blond hair along with pinkish skin and brown spots. The affected individual had green iris color along with light sensitivity and nystagmus. The normal individuals have no sign of disease. Blood samples of one affected (III-1) two normal (II-1, II-2) individuals were collected.

3.1.4 Family D

The family D was enrolled in the Rawalakot district Poonch Pakistan (Fig 3.7). Two affected individuals along with their normal siblings participated in this study. Clinical examination of skin, hair and iris phenotype of affected individuals showed that they had brownish to golden hair color and white to pinkish skin with light brown spot in one affected individual. The affected individuals had brown iris color along with light sensitivity and nystagmus. Blood samples were collected from two affected (IV-3, IV-4) and four normal (III-2, III-3, IV-1, IV-2) individuals including parents and siblings. The DNA was extracted using phenol-chloroform method in normal and affected individuals.

3.2 Genetic Mapping of Candidate Genes Responsible for Hereditary Oculocutaneous albinism

The candidate genes involved in hereditary Oculocutaneous albinism were checked for linkage in four families. Microsatellite markers were used for genotyping through PCR to check linkage or exclusion prior to embarking on genome wide scan. The amplified PCR products were then loaded on 8% non-denaturing polyacrylamide gel electrophoresis. The gel was stained with ethidium bromide and visualized. Markers with homozygous bands in affected individuals were supposed to be linked for that locus or gene.

The relevant families were not considered linked if the affected individuals exhibited heterozygous banding pattern.

In Family A, seven DNA samples including four normal (III-1, III-2, IV-2, IV-3) and three affected (IV-1, IV-4, IV-5) were checked for linkage with the help of microsatellite markers linked to *OCA2, TYRP1, SLC45A2, C10ORF11* genes. The results obtained substantiated on exclusion of family from linkage to any of the known OCA loci. Therefore, a novel genetic factor may be responsible for the disease phenotype in this particular family.

In Family B, DNA of two normal (II-2, III-1) and two affected individuals (III-2, III-3) were tested for linkage by polymorphic microsatellite marker for respective candidate genes. The markers linked to Oculocutaneous albinism are *OCA2* gene on chromosome 15q11.2-q12, *SLC45A2* gene on chromosome 5p13.3, *TYRP1* gene on chromosome 9p23, *C10ORF11* gene on chromosome 10q22.2-q22.3 were studied. These genes were excluded and show no linkage.

In Family C, DNA of two normal (II-1, II-2) and one affected individuals (III-1) were used for genotyping. The markers linked to Oculocutaneous albinism are *OCA2* gene on chromosome 15q11.2-q12, *SLC45A2* gene on chromosome 5p13.3, *TYRP1* gene on chromosome 9p23, *C10ORF11* gene on chromosome 10q22.2-q22.3. None of these genes show linkage and were excluded.

In Family D, DNA of four normal (III-2, 111-3) and two affected individuals (IV-3, IV-4) were tested for linkage by polymorphic microsatellite marker for respective candidate genes. The markers linked to Oculocutaneous albinism are *OCA2* gene on chromosome 15q11.2-q12, *SLC45A2* gene on chromosome 5p13.3, *TYRP1* gene on chromosome 9p23, *C10ORF11* gene on chromosome 10q22.2-q22.3 were studied. These genes were excluded and show no linkage.

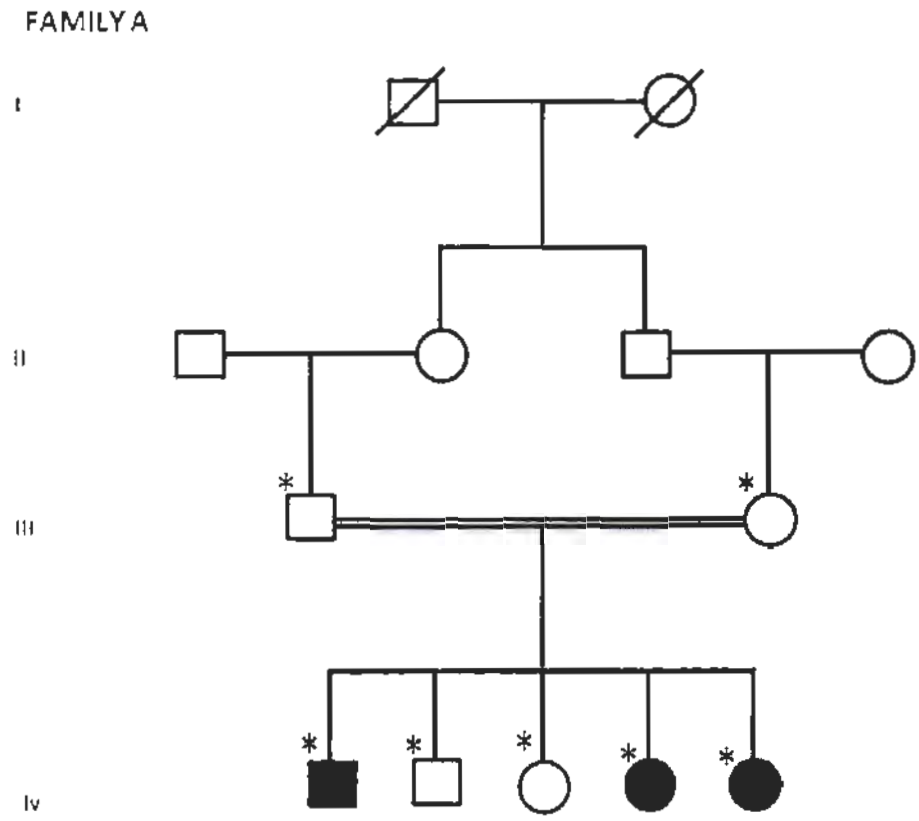


Fig 3.1 Pedigree of Family A showing hereditary Oculocutaneous albinism with an autosomal recessive pattern of inheritance. Squares and circles represent male and female members respectively. Blank shapes are used to indicate the normal individuals while filled shapes represent the affected individuals for the given disorder. Double lines between the two individuals indicate consanguineous marriage. Cross lines represent deceased individuals. Generation numbers are indicated by Roman numerals. The members whose blood was sampled for the study are shown by the Asterisk (*) symbol over the respective shapes.

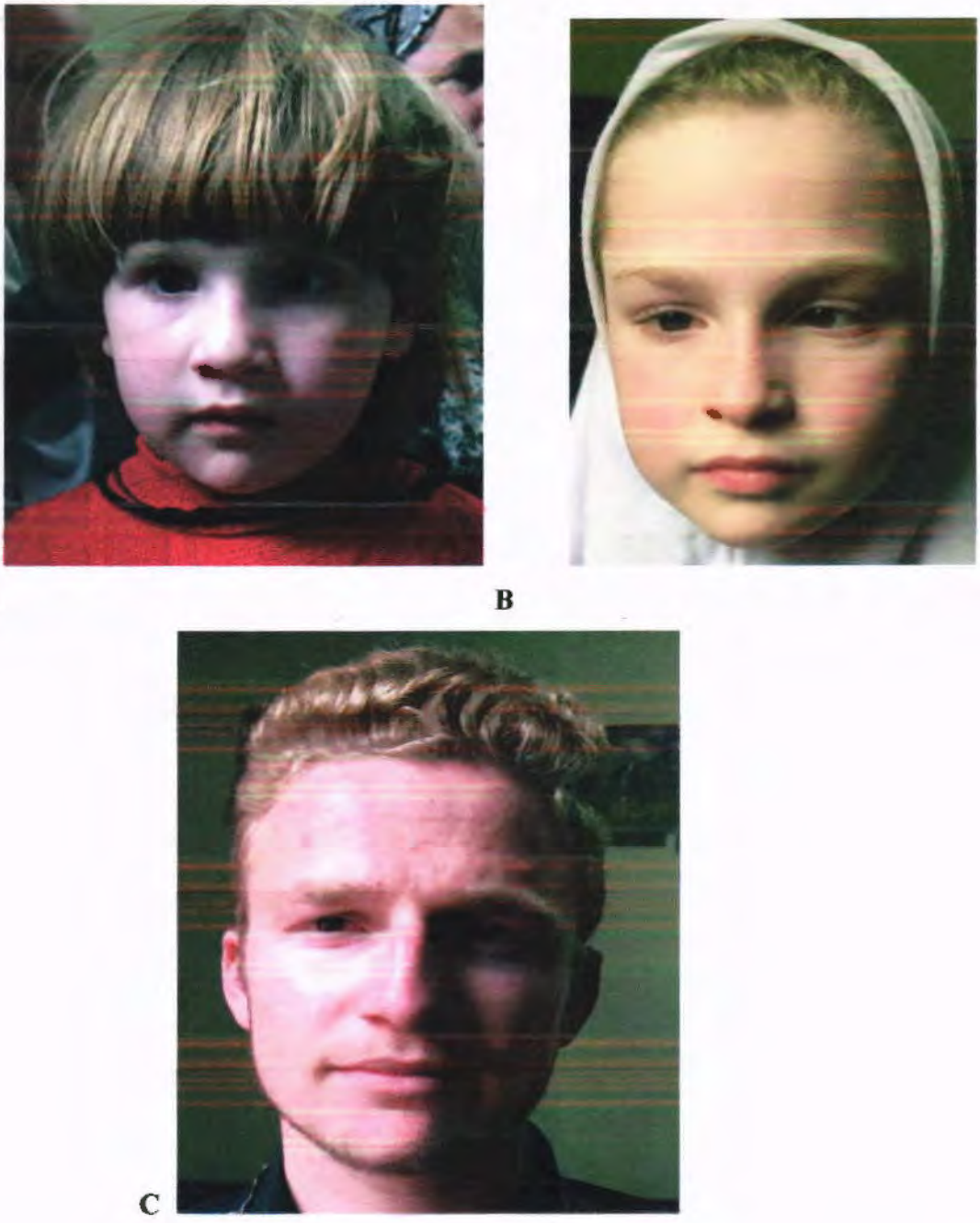


Fig 3.2 Clinically presentation of autosomal recessive Oculocutaneous albinism in three (IV-1, IV-4, IV-5) affected members of family A

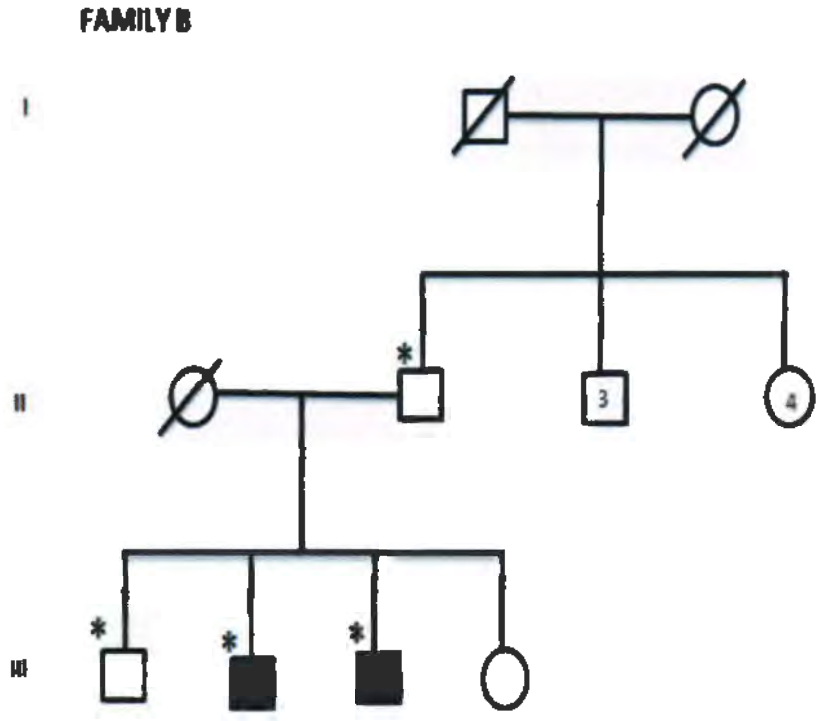
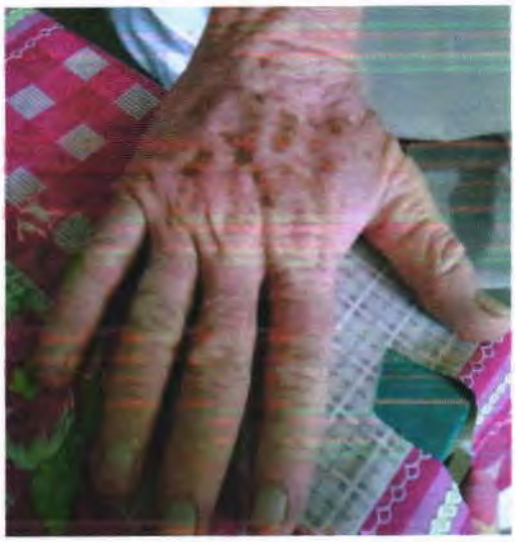


Figure 3.3: Pedigree of Family B exhibits phenotypes of Oculocutaneous albinism. Squares and circles represent male and female members respectively. Blank shapes are used to indicate the normal individuals while filled shapes represent the affected individuals for the given disorder. Cross lines represent deceased individuals. Generation numbers are indicated by Roman numerals. The members whose blood was sampled for the study are shown by the Asterisk (*) symbol over the respective shapes.

A



B

C

Figure 3.4: Affected Individual (III-2, III-3) of family B showing phenotypes of Oculocutaneous albinism.

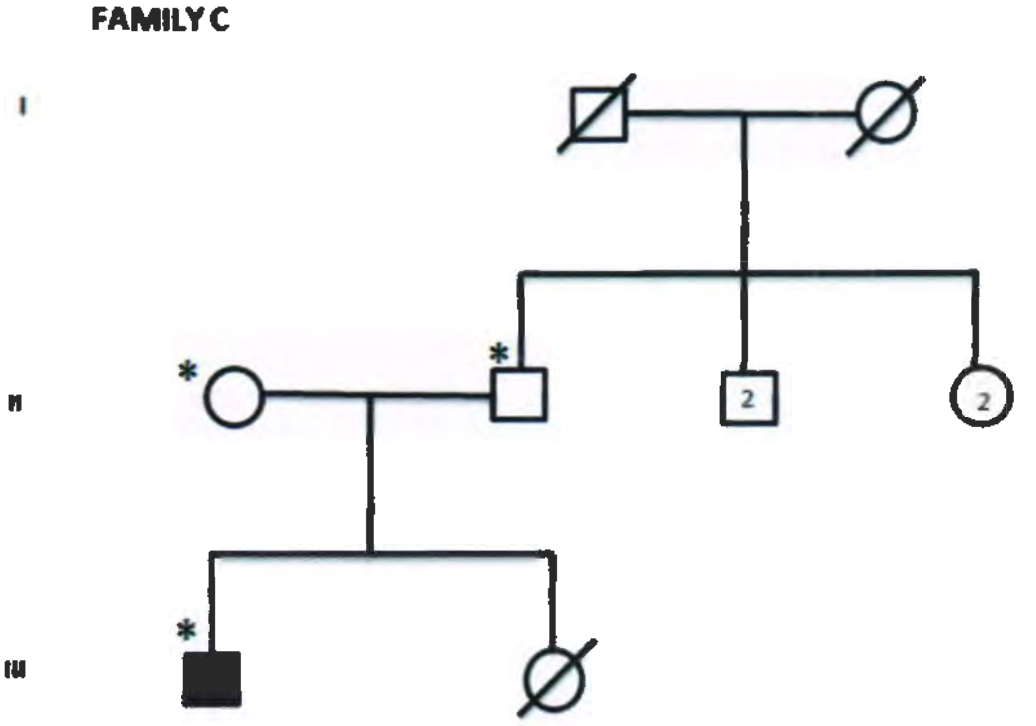


Figure 3.5: Pedigree of Family C showing phenotypes of Oculocutaneous albinism. Squares and circles represent male and female members respectively. Blank shapes are used to indicate the normal individuals while filled shapes represent the affected individuals for the given disorder. Cross lines represent deceased individuals. Generation numbers are indicated by Roman numerals. The members whose blood was sampled for the study are shown by the Asterisk (*) symbol over the respective shapes.

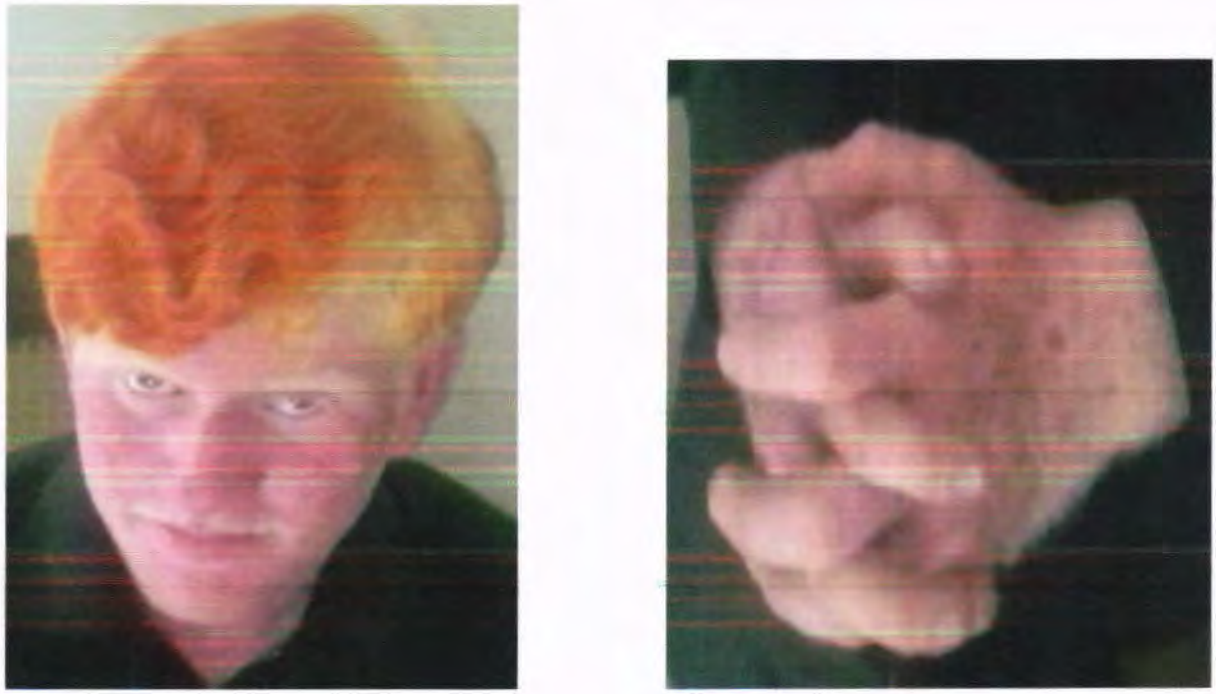


Figure 3.6: Affected Individual (III-1) of family C showing phenotypes of recessive Oculocutaneous albinism.

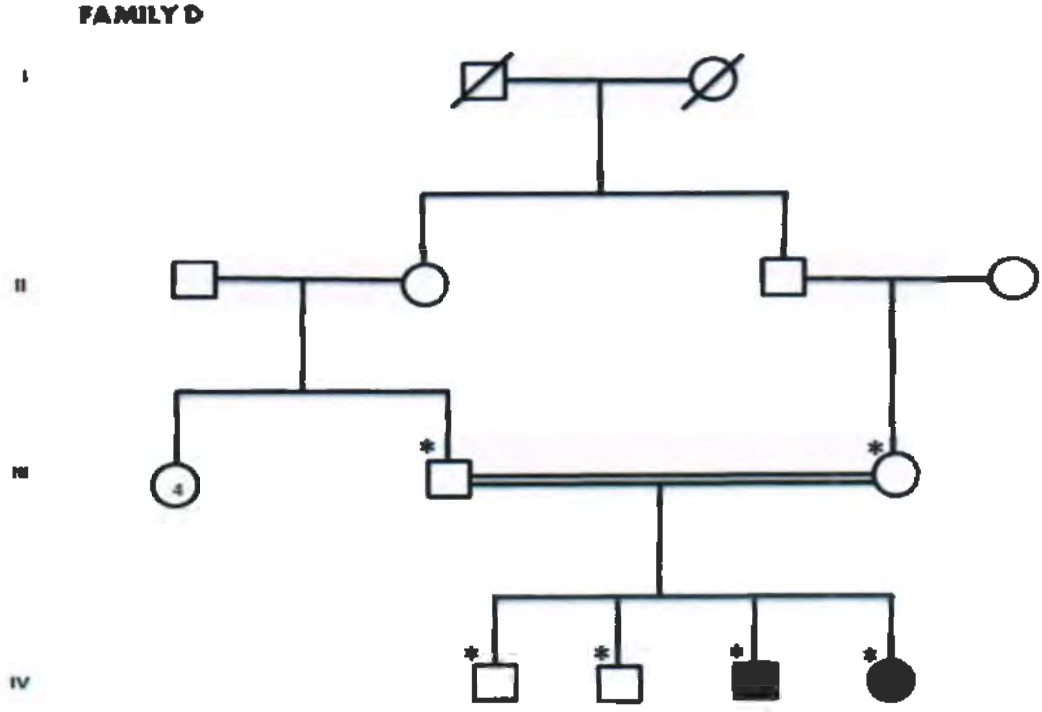


Figure 3.7: Pedigree of Family D showing phenotypes of Oculocutaneous albinism. Squares and circles represent male and female members respectively. Double lines between the two individuals indicate consanguineous marriage. Blank shapes are used to indicate the normal individuals while filled shapes represent the affected individuals for the given disorder. Cross lines represent deceased individuals. Generation numbers are indicated by Roman numerals. The members whose blood was sampled for the study are shown by the Asterisk (*) symbol over the respective shapes.

Figure 3.8: Affected Individual (IV-3, IV-4) of family D showing phenotypes of autosomal recessive Oculocutaneous albinism.

Family A: *OCA2* gene

Marker id	Cytogenetic Position (cM)	N	N	N	N	A	A	A
D15S976	21.01 29339626							
D15S976	21.01 29339626							
D15S1010	23.74 30877609							

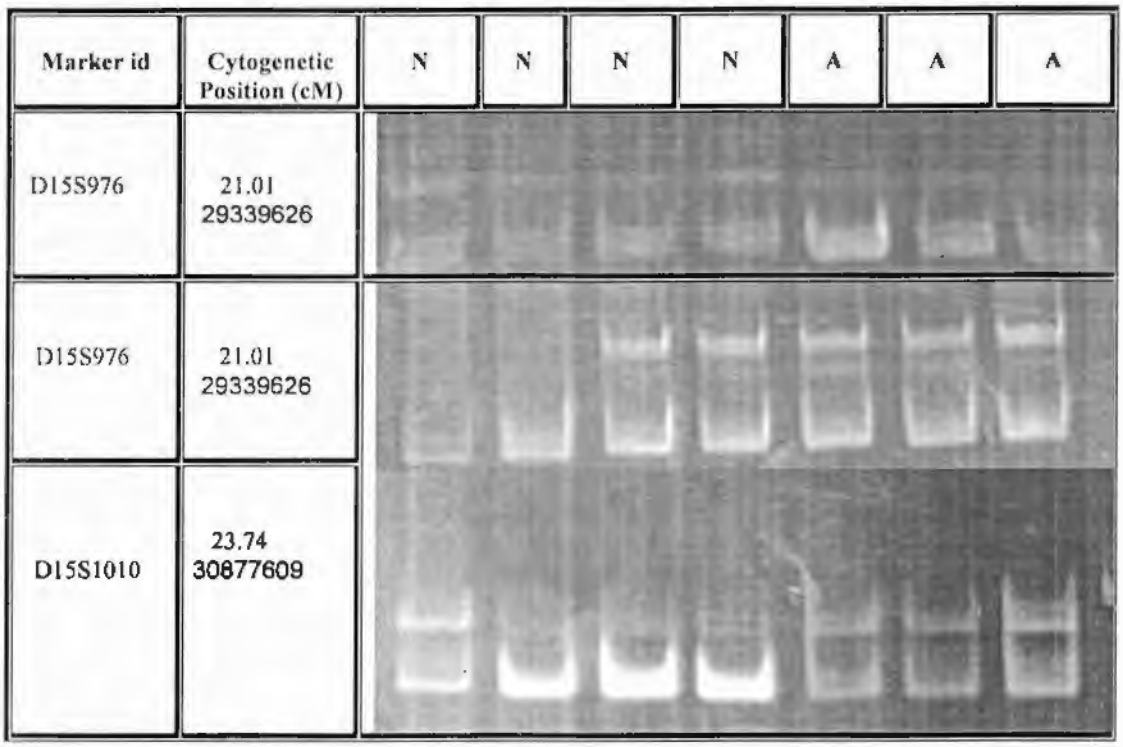


Figure 3.9: Arrangement of the alleles for the markers corresponding to chromosomal location 15q11.2-q12 form the gene *OCA2* obtained through electropherogram of 8% Polyacrylamide gel stained with ethidium bromide.

Family A: *SLC45A2* gene

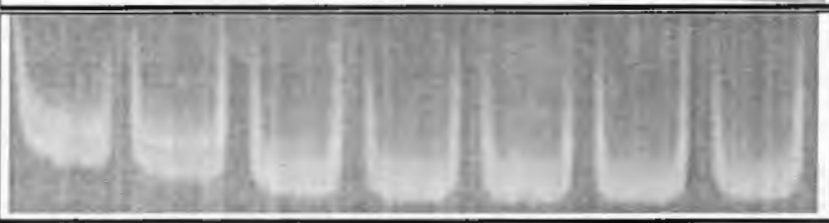
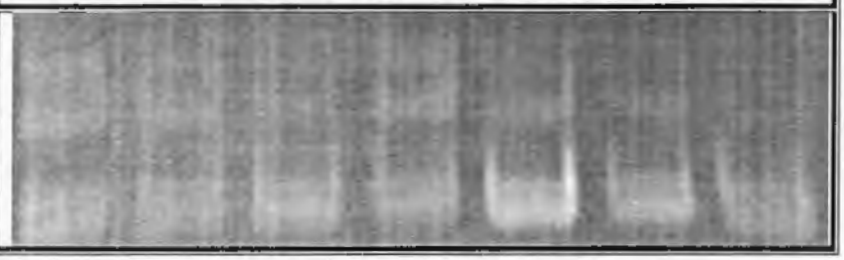
Marker id	Cytogenic position	N	N	N	N	A	A	A
D5S674	55.19 33626790							
D5S1506	56.26 33863399							
D5S2843	56.26							

Figure 3.10 Arrangement of the alleles for the markers corresponding to chromosomal location 5p13.3 form the gene *SLC45A2* obtained through electropherogram of 8% Polyacrylamide gel stained with ethidium bromide.

FamilyA: *C10ORF11* gene

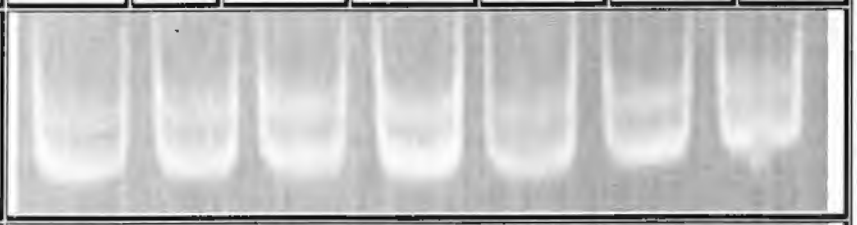
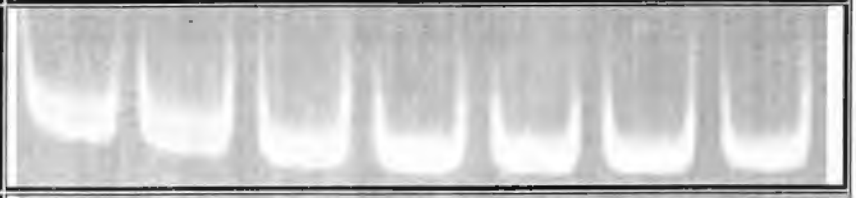
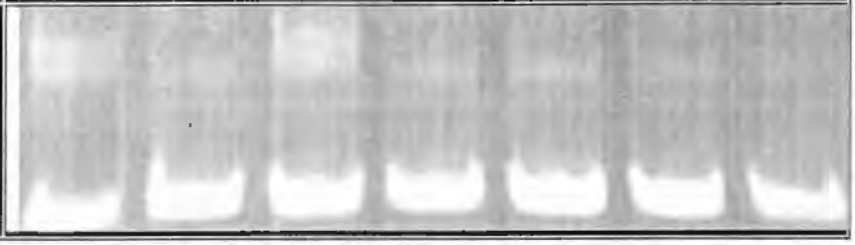
Marker Id	Cytogenic position	N	N	N	N	A	A	A
D10S1694	92.13 73097085							
D10S202	95.19 78232903							
D10S1730	96.62 78601039							

Figure 3.11 Arrangement of the alleles for the markers corresponding to chromosomal location 10q22.2-q22.3 form the gene *C10ORF11* obtained through electropherogram of 8% Polyacrylamide gel stained with ethidium bromide.

Family A: *TYRPI* gene

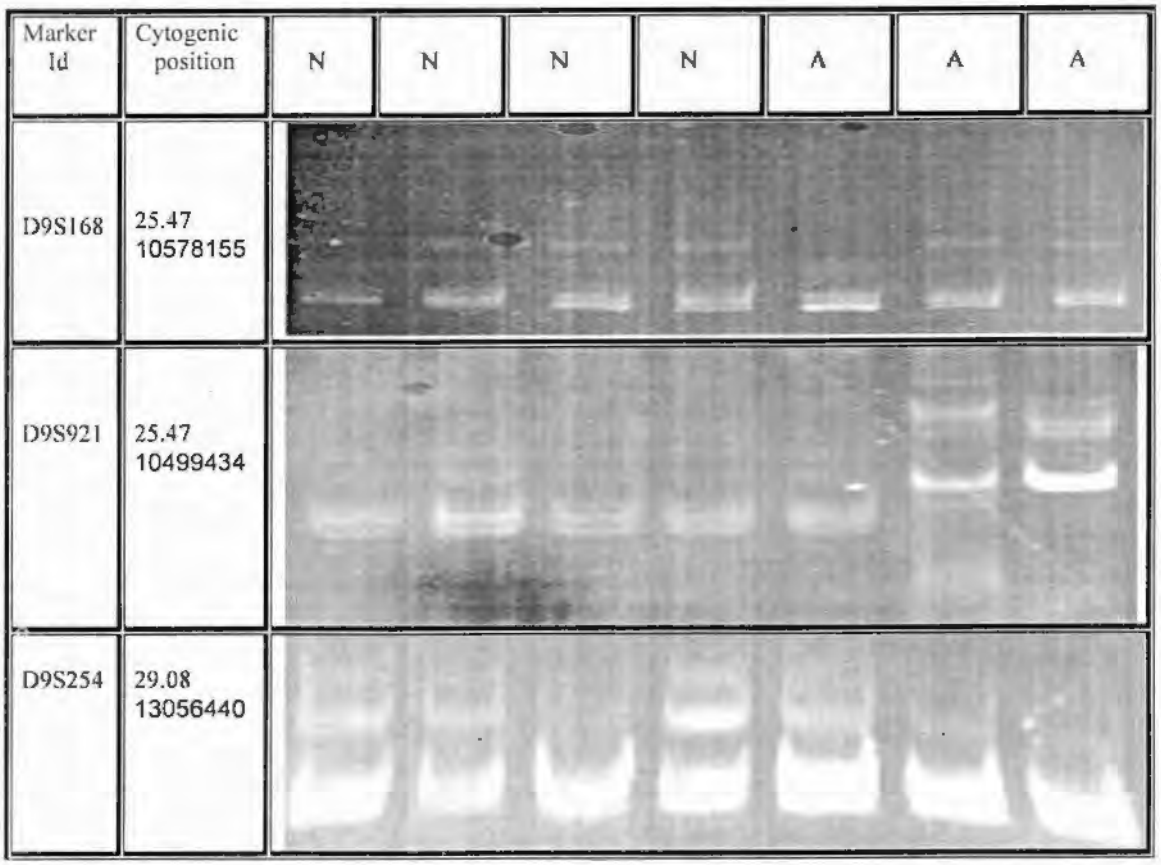


Figure 3.12 Arrangement of the alleles for the markers corresponding to chromosomal location 9p23 form the gene *TYRP1* obtained through electropherogram of 8% Polyacrylamide gel stained with ethidium bromide.

Family B: *OCA2* gene

Marker Id	Cytogenic Position	N	N	A	A
D15S1031	22.86 29875415				
D15S1010	23.74 30877609				
D15S231	24.67 31037782				

Figure 3.13 Arrangement of the alleles for the markers corresponding to chromosomal location 15q11.2-q12 form the gene *OCA2* obtained through electropherogram of 8% Polyacrylamide gel stained with ethidium bromide.

Family B: *TYRP1* gene

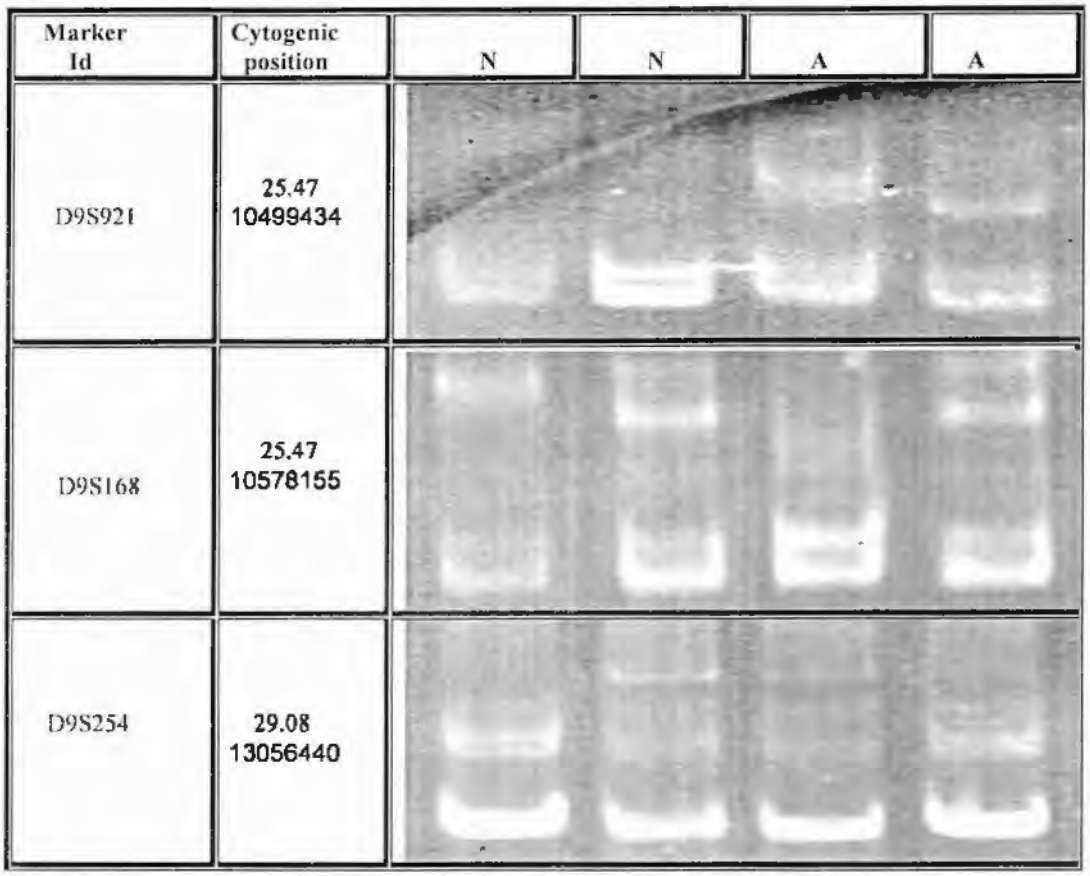


Figure 3.14 Arrangement of the alleles for the markers corresponding to chromosomal location 9p23 form the gene *TYRP1* obtained through electropherogram of 8% Polyacrylamide gel stained with ethidium bromide.

Family B: *C10ORF11* gene

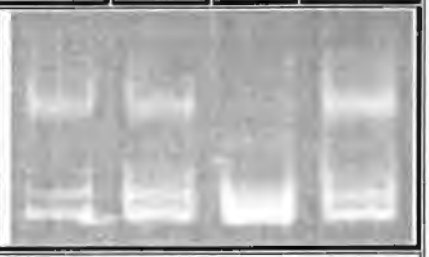
Marker Id	Cytogenic position	N	N	A	A
D10S606	92.13 73039635				
D10S1694	92.13 73097085				
D10S580	94.95 77728884				

Figure 3.15 Arrangement of the alleles for the markers corresponding to chromosomal location 10q22.2-q22.3 form the gene *C10ORF11* obtained through electropherogram of 8% Polyacrylamide gel stained with ethidium bromide.

Family B: *SLC45A2* gene

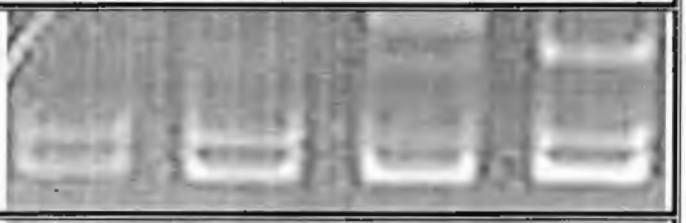
Marker Id	Cytogenic Position	N	N	A	A
D5S1350	53.94 32381428				
D5S674	55.19 33626790				
D5S493	57.76 34987917				

Figure 3.16 Arrangement of the alleles for the markers corresponding to chromosomal location 5p13.3 form the gene *SLC45A2* obtained through electropherogram of 8% Polyacrylamide gel stained with ethidium bromide.

Family C: *OCA2* gene

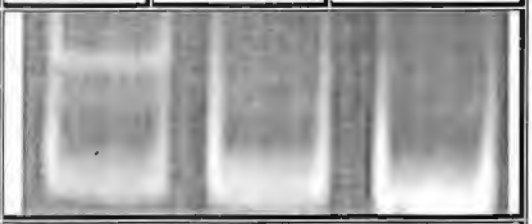
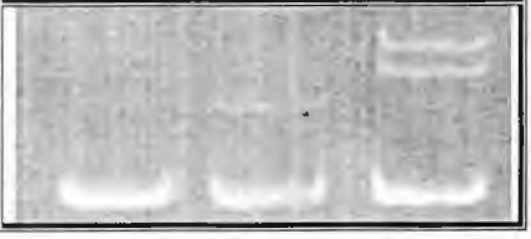
Marker Id	Cytogenic Position	N	N	A
D15S1031	22.86 29875415			
D15S1010	23.74 30877609			
D15S231	24.67 31037782			

Figure 3.17: Arrangement of the alleles for the markers corresponding to chromosomal location 15q11.2-q12 form the gene *OCA2* obtained through electropherogram of 8% Polyacrylamide gel stained with ethidium bromide.

Family C: *C10ORF11* gene

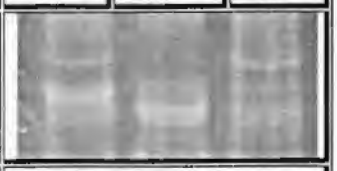
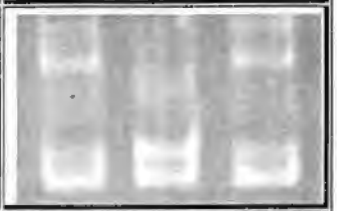
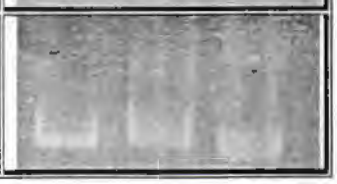
Marker Id	Cytogenic Position	N	N	A
D10S1694	92.13 73097085			
D10S580	94.95 77728884			
D10S202	95.19 78232903			

Figure 3.18 Arrangement of the alleles for the markers corresponding to chromosomal location 10q22.2-q22.3 form the gene *C10ORF11* obtained through electropherogram of 8% Polyacrylamide gel stained with ethidium bromide.

Family C: *TYRP1* gene

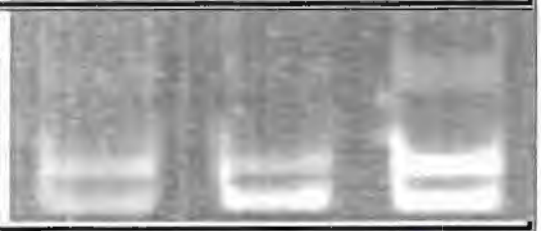
Marker Id	Cytogenic Position	N	N	A
D9S921	25.47			
D9S168	25.47			
D9S254	29.08			

Figure 3.19 Arrangement of the alleles for the markers corresponding to chromosomal location 9p23 form the gene *TYRP1* obtained through electropherogram of 8% Polyacrylamide gel stained with ethidium bromide.

Family C: *SLC45A2* gene

Marker Id	Cytogenic position	N	N	A
D5S1350	53.94 32381428			
D5S1470	54.04 32528047			
D5S674	55.19 33626790			
D5S631	56.95 34674317			

Figure 3.20 Arrangement of the alleles for the markers corresponding to chromosomal location 5p13.3 form the gene *SLC45A2* obtained through electropherogram of 8% Polyacrylamide gel stained with ethidium bromide.

Family D: *OCA2* gene

Marker Id	Cytogenic position	N	N	N	N	A	A
D15S165	20.5 29047841						
D15S1010	23.74 30877609						
D15S231	24.67 31037782						

Figure 3.21 Arrangement of the alleles for the markers corresponding to chromosomal location 15q11.2-q12 form the gene *OCA2* obtained through electropherogram of 8% Polyacrylamide gel stained with ethidium bromide.

Family D: *SLC45A2* gene

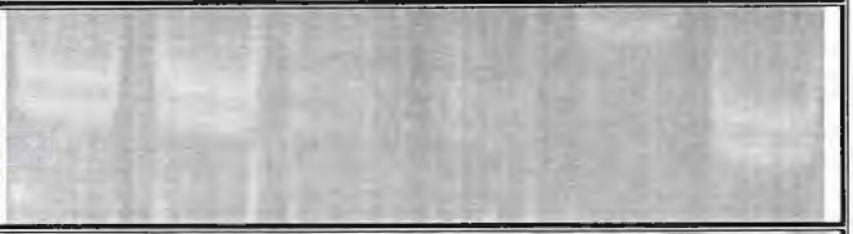
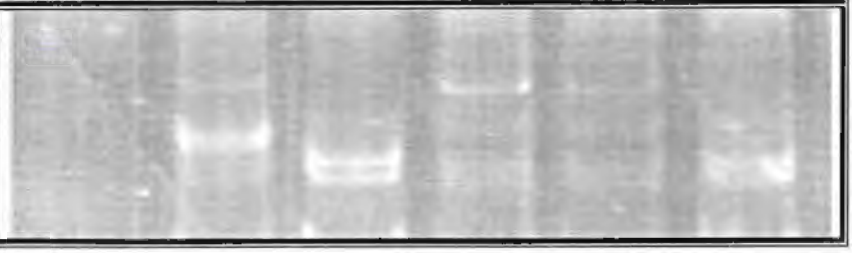
Marker Id	Cytogenic position	N	N	N	N	A	A
D5S1350	53.94 32381428						
D5S631	56.95 34674317						
D5S493	57.76 34987917						

Figure 3.22 Arrangement of the alleles for the markers corresponding to chromosomal location 5p13.3 form the gene *SLC45A2* obtained through electropherogram of 8% Polyacrylamide gel stained with ethidium bromide.

Family D: *TYR P1* gene

Marker Id	Cytogenic position	N	N	N	N	A	A
D9S921	25.47 10499434						
D9S254	29.08 10578155						
D9S1869	30.91 14264014						

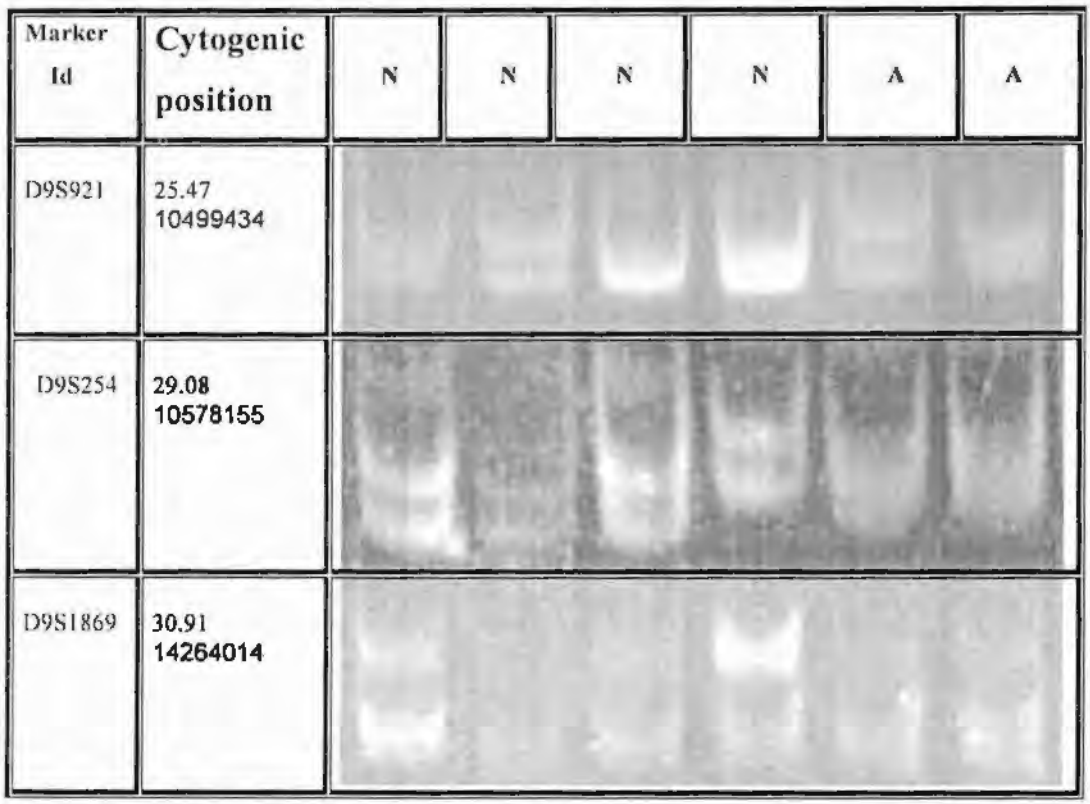


Figure 3.23 Arrangement of the alleles for the markers corresponding to chromosomal location 9p23 form the gene *TYRP1* obtained through electropherogram of 8% Polyacrylamide gel stained with ethidium bromide..

Family D: *C10ORF11* gene

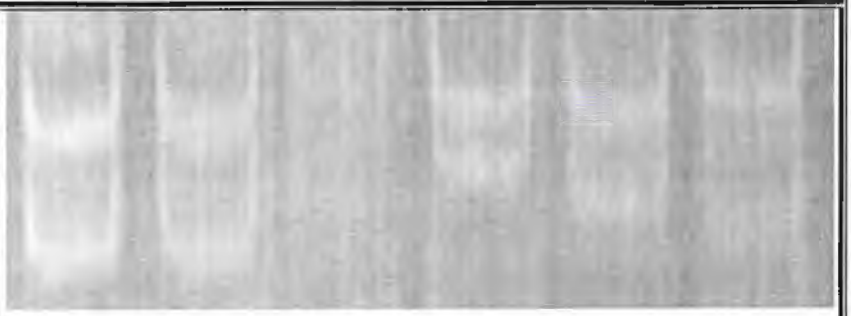
Marker Id	Cytogenic position	N	N	N	N	A	A
D10S606	92.13 73039635						
D10S1694	92.13 73097085						
D10S202	95.19 78232903						

Figure 3.24 Arrangement of the alleles for the markers corresponding to chromosomal location 10q22.2-q22.3 form the gene *C10ORF11* obtained through electropherogram of 8% Polyacrylamide gel stained with ethidium bromide.

CHAPTER 4

DISCUSSION

Complete or partial loss of pigmentation in the iris, skin and hair is autosomal and x-linked recessive non-syndromic oculocutaneous albinism (nsOCA) disorder this is due to a decrease or absence of melanin production in body (Tomita & Suzuki, 2004; Spritz *et al.*, 2003). Melanin occurs in specialized cells called melanocytes and its production is regulated in body. Melanocytes derived from a separate ectodermal line it may be extracutaneous or cutaneous. Albinism nomenclature is complex by the actuality that the terms albinism, oculocutaneous albinism and ocular albinism can be used both as a phenotypic descriptions and explain precise disease. Impaired eye development plus variable hair, skin and ocular hypopigmentation illustrate Oculocutaneous albinism. Totally lack of tyrosinase function has no apparent melanin pigmentation while fractional OCA is distinct by hypopigmentation. An increased UV sensitivity results in the health consequences and visual disruption in OCA patients. UV radiations are absorbed by melanin and thus reducing DNA damage by ultraviolet rays in the skin and eyes. There is high risk for developing skin cancer in individuals with OCA unless enough sun shields are worn (Lund and Taylor, 2008). Also for the normal visual development melanin is critical (Summers, 2009). Disrupt fovea development and changes in the optic nerves and tracts is linked with diminish melanin synthesis in the eye during development. Lack of vision (decreased visual acuity), light sensitivity, nystagmus and decreased profundity are the visual phenotype in OCA patients. Melanocytes are present in other tissues as well but do not have documented harmful health effect in the setting of OCA. Like in cochlea melanocytes are present. Melanocytes in cochlea have play a main role in the conservation of adjoining cochlear cells (Zhang *et al.*, 2012; Uehara *et al.*, 2009).To date pathogenic mutations in six genes, *TYR, OCA2, TYRP1, SLC45A2, SLC24A5,* and *C10orf11*, have been identified in individuals with nsOCA (Montoliu *et al.*, 2014).We previously mapped a new locus for non syndromic OCA on chromosome 4q24 in a large consanguineous Pakistani family (Kausar *et al.*, 2013). The *GPR143* (MIM 300808) gene is positioned on chromosome Xp22.32 encodes a 404 amino acid protein predicted to be a membrane protein and known to cause ocular albinism. For which the gene is currently unknown. In published population studies however the detection rate of alleles causing albinism varies from 60% to 90% (Mauri *et al.*, 2016; Simeonov *et al.*, 2013; Park *et al.*, 2012; Hutton & Spritz, 2008; Rooryck *et al.*, 2008;

Tomita *et al.*, 2000).The method involves the detection of the disease locus by simply comparing homozygous pattern of affected gene with heterozygous pattern of gene present in normal member of family.

Six known genes of non syndromic OCA are *TYR* (tyrosinase), *OCA2*, *TYRP1* (tyrosinase-like protein 1), *SLC45A2* (solute carrier family 45 member 2), *SLC24A5* (solute carrier family 24 member 5), and *C10orf11* (chromosome 10 open reading frame 11) (Montoliu *et al.*, 2014). *TYR* positioned on chromosome 11q14–q21, *OCA2* gene positioned on chromosome region 15q11.2-q12 *TYRP1* gene positioned on chromosome 9p23*SLC45A2* gene positioned on chromosome 5p13. The *SLC24A5* positioned on chromosome 15q21.1, *C10orf11* gene is positioned on chromosome 10q22.3.

Current research focused on the genetic and clinical study of four families (A, B, C, D) recruited from different area of Pakistan. These families were affected with Oculocutaneous albinism. To full fill this purpose the blood samples of normal and affected individual of families were collected genomic DNA was extracted by stander extraction protocol for homozygosity mapping to figure out the homozygous region of candidate gene by the help polymorphic microsatellite markers. If linkage found the linked gene were further subjected for Sanger sequencing to figure out pathogenic variant that causes disease.

Family A members were affected with the disease known as Oculocutaneous albinism. Reported genes *(OCA2, TYRP1, SLC45A2, and C10ORF11)* which are involved in causing oculocutaneous albinism were tested for homozygosity mapping by using microsatellites markers. Affected individuals of family A exposed that they exhibit different combinations of parent alleles, thus excluding the linkage in this family to the known OCA regions. None of these markers showed linkage to inherited OCA. This concluded the involvement of a novel gene might be responsible for causing Oculocutaneous albinism in family A.

In family B affected individuals showed the phenotypes of OCA. Blood were collected from two affected and two normal member of family B their DNA were subjected for further analysis. Genotyping was done by using highly polymorphic microsatellite markers to figure out affected gene or loci causing hereditary OCA. None of these genes showed linkage to recessive OCA of family B. This indicates that a new gene is involved in causing OCA in this family.

In Family C, DNA of two normal (II-1, II-2) and one affected individuals (III-1) were checked for

linkage analysis. The markers linked to Oculocutaneous albinism are *OCA2* gene on chromosome 15q11.2-q12, *SLC45A2* gene on chromosome 5p13.3, *TYRP1* gene on chromosome 9p23, *C10ORF11* gene on chromosome 10q22.2-q22.3. None of these genes show linkage and were excluded.

In Family D, DNA of two normal (III-2, III-3) and one affected individuals (IV-1, IV-2) were tested for linkage by polymorphic microsatellite marker for respective candidate genes. The markers linked to Oculocutaneous albinism are *OCA2* gene on chromosome 15q11.2-q12, *SLC45A2* gene on chromosome 5p13.3, *TYRP1* gene on chromosome 9p23, *C10ORF11* gene on chromosome 10q22.2-q22.3 were studied. These genes were excluded and show no linkage.

CHAPTER 5

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Basic Clinical Proforma

Family "A"

PATIENT NAME: Suhrab, Qurat-ul-ain, Noor

DATE OF BIRTH: 2000, 2007, 2010

DATE OF VISIT: Feb 2017

What are their main medical problems?

They had visual problems, hypopigmentation of skin and hair as well

What age did these problems start?

At the time of birth

Have these problems got worse, better or stayed the same?

Stayed the same

Were they born at the right time, were there any problems when they were born?

Yes, no they have only phenotype that is affected

What did they weigh at birth?

Suhrab	6 pounds
Qurat-ul-ain	5 pounds 8 ounces
Noor	7 pounds

What age did they walk?

one year

When did they start talking?

one year - 8 months

Do they have learning difficulties, and if so please describe?

No, they don't have

Have they had any seizures, and if so when did they start?

No

Do they have any other health problems, for example is there a heart or kidney defect (and if so what is this)?

No, they have n't any health problems

Have they ever had any operations or hospital procedure (if so what)?

No

Have they ever had any stays in hospital (if so why)?

No

Have they had any clinical investigations (eg blood tests, metabolic tests, brain scans)

No, they had not any clinical investigations

Can we have any copies of any hospital investigations, for example MRIs or metabolic tests (if undertaken)?

No.

Do they see any doctors regularly (neurologist, paediatrician etc), and if so why?

No

Do they have any unusual physical characteristics and look different from other members of their family, for example finger/toe abnormalities or birthmarks?

No, only phenotype is affected

Please obtain height, weight and head circumference measurements and state the age (years, months) that these were obtained

Suhrab	5" 4 inch	47 kg
Qurat-ul-ain	3" 6	19 kg
Noor	3" 1	16 kg

Do they take any regular medication, and if so what for?

No

In addition to the above, please obtain photographs or videos of affected individuals

Attached in results

Basic Clinical Proforma

Family "B"

PATIENT NAME: Arshad, Khalid

DATE OF BIRTH: 1982, 1979

DATE OF VISIT: September 2016

What are their main medical problems?

Hypopigmentation of Skin, Hairs & eyes
Brown spots on face & hands

What age did these problems start?

From birth

Have these problems got worse, better or stayed the same?

Stayed same

Were they born at the right time, were there any problems when they were born?

yes, they born at the right time. Hypopigmentation is From birth

What did they weigh at birth?

Arshad 7 pounds

Khalid 6 pounds

What age did they walk?

Arshad 11 month

Khalid 12 month

When did they start talking?

Arshad 2 years

Ichalid 1 year . 8 month

Do they have learning difficulties, and if so please describe?

No, Learning difficulties found

Have they had any seizures, and if so when did they start?

No, they had not any seizures

Do they have any other health problems, for example is there a heart or kidney defect (and if so what is this)?

They had not any other health Problems

Have they ever had any operations or hospital procedure (if so what)?

No

Have they ever had any stays in hospital (if so why)?

No

Have they had any clinical investigations (eg blood tests, metabolic tests, brain scans)

No, they had not

Can we have any copies of any hospital investigations, for example MRIs or metabolic tests (if undertaken)?

No

Do they see any doctors regularly (neurologist, paediatrician etc), and if so why?

No

Do they have any unusual physical characteristics and look different from other members of their family, for example finger/toe abnormalities or birthmarks?

No, they had not any unusual physical characteristics

Please obtain height, weight and head circumference measurements and state the age (years, months) that these were obtained

Arshad 5"7 inch 66kg

Ichalid 5"8 70kg

Do they take any regular medication, and if so what for?

No

In addition to the above, please obtain photographs or videos of affected individuals

Photographs were attached in thesis

Family "C"

Basic Clinical Proforma

PATIENT NAME: Shakeel

DATE OF BIRTH: 1996

DATE OF VISIT: 1 December 2016

What are their main medical problems?

Hypopigmentation of Skin, Hair and Eyes

What age did these problems start?

At the time of birth

Have these problems got worse, better or stayed the same?

Stayed the same

Were they born at the right time, were there any problems when they were born?

Yes, only phenotype is affected

What did they weigh at birth?

6 pounds 3 ounces

What age did they walk?

11 months

When did they start talking?

one year & 10 month

Do they have learning difficulties, and if so please describe?

No

Have they had any seizures, and if so when did they start?

No

Do they have any other health problems, for example is there a heart or kidney defect (and if so what is this)?

No

Have they ever had any operations or hospital procedure (if so what)?

No, they had not any operations

Have they ever had any stays in hospital (if so why)?

No

Have they had any clinical investigations (eg blood tests, metabolic tests, brain scans)

No

Can we have any copies of any hospital investigations, for example MRIs or metabolic tests (if undertaken)?

No

Do they see any doctors regularly (neurologist, paediatrician etc), and if so why?

No

Do they have any unusual physical characteristics and look different from other members of their family, for example finger/toe abnormalities or birthmarks?

No, he has not unusual physical characteristics only phenotype is affected.

Please obtain height, weight and head circumference measurements and state the age (years, months) that these were obtained

Height 5" 1 inch

Weight 40 kg

Do they take any regular medication, and if so what for?

No

In addition to the above, please obtain photographs or videos of affected Individuals

Attached in Thesis

Family D"

Basic Clinical Proforma

PATIENT NAME: Hassan, Noor

DATE OF BIRTH: 2004, 2006

DATE OF VISIT: January 2017

What are their main medical problems?

Hypopigmentation of skin, hair and eyes. Brown spots on the Face of Hassan

What age did these problems start?

From birth

Have these problems got worse, better or stayed the same?

Stayed same

Were they born at the right time, were there any problems when they were born?

Yes, phenotype is affected

What did they weigh at birth?

Hassan 25kg

Noor 19kg

What age did they walk?

one year, 2 month

When did they start talking?

one year & 11 months

Do they have learning difficulties, and if so please describe?

No

Have they had any seizures, and if so when did they start?

No

Do they have any other health problems, for example is there a heart or kidney defect (and if so what is this)?

No they have not

Have they ever had any operations or hospital procedure (if so what)?

No

Have they ever had any stays in hospital (if so why)?

No

Have they had any clinical investigations (eg blood tests, metabolic tests, brain scans)

No

Can we have any copies of any hospital investigations, for example MRIs or metabolic tests (if undertaken)?

No

Do they see any doctors regularly (neurologist, paediatrician etc), and if so why?

No

Do they have any unusual physical characteristics and look different from other members of their family, for example finger/toe abnormalities or birthmarks?

No, only phenotype is affected

Please obtain height, weight and head circumference measurements and state the age (years, months) that these were obtained

Hassan 25kg 4"2 inch

Noor 19kg 3"6

Do they take any regular medication, and if so what for?

No

In addition to the above, please obtain photographs or videos of affected individuals

Attached in Thesis