Genetic Analysis and Candidate Genes Prediction in Pakistani Families with Retinal Dystrophies

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by

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Submitted in partial fulfilment of the requirements for the MS in Bioinformatics at the faculty of basic and applied sciences department of Bioinformatics International Islamic University Islamabad.

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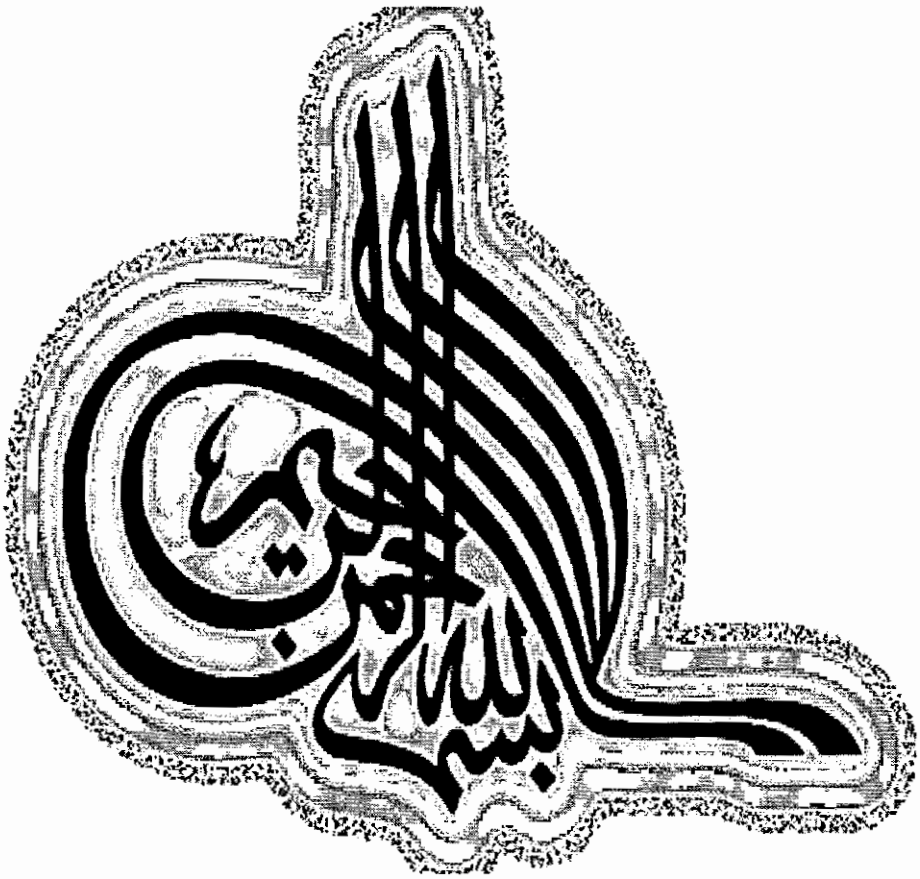
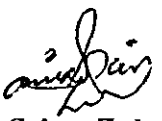


Glory be to Him! When He decreeth a thing, He saith unto it only: Be! and it is.

(Mary-19: 35)

DECLARATION

I hereby declare that the work presented in the following thesis is my own effort, except where otherwise acknowledged, and that the thesis is my own composition. No part of the thesis has been previously presented for any other degree.



Saima Zubair

Dedicated to those chosen servants of Almighty Allah who are serving in the way of their Lord to their best possible capabilities and exposing the true picture of Islam to the whole world.

"Invite (all) to the Way of thy Lord with wisdom and beautiful preaching; and argue with them in ways that are best and most gracious: for thy Lord knoweth best, who have strayed from His path, and who receive guidance."

(Al-Quran 16: 125)

Certificate

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TABLE OF CONTENTS

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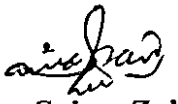
I pray to Almighty Lord that may He bestow me with true success in all fields in all the worlds and shower the knowledge upon me which is beneficial for me, for all Muslims and whole mankind.

Amin



Saima Zubair

LIST OF ABBREVIATIONS

ABCR	ATP-binding cassette transporter-retinal
ACD	Acid Citrate Dextrose
adRP	Autosomal dominant retinitis pigmentosa
arRP	Autosomal recessive retinitis pigmentosa
arSTGD	Autosomal recessive stargardt disease
CA4	Carbonic anhydrase IV
CEP290	Centrosomal protein Cep290
CERKL	Ceramide kinase
CNS	Central nervous system
CRB1	Crumbs protein homolog 1
CRD	Cone-rod dystrophy
CRX	Cone-rod homeobox protein
DNA	Deoxyribonucleic acid
dNTP	Deoxynucleoside triphosphate
EDTA	Ethylene di-ammine tetra acetate
ERG	Electroretinogram
IMPDH1	Inosine 5'- monophosphate dehydrogenase 1
kDa	Kilo Dalton
LCA	Lebers congenital amaurosis
MAP9	Microtubule-associated protein 9
NCBI	National Center for Biotechnology Information
Ng	Nanogram

NRL	Neural retina-specific leucine zipper protein
OD	Optical density
PAGE	Polyaccrylamide gel electrophoresis
PCR	Polymerase chain reaction
PRL	Photoreceptor layer
PRPF8	Pre-mRNA processing splicing factor 8
RBM46	RNA binding motif protein 46
RDH12	Retinol dehydrogenase 12
RHO	Rhodopsin
ROM1	Rod outer segment membrane protein 1
RP	Retinitis pigmentosa
RPE	Retinal pigmented epithelium
Rpm	Revolution per minute
SDS	Sodium Dodecile Sulphate
SNP	Single-nucleotide polymorphism
STE	Saline Tris EDTA
TBE	Tris Boric Acid EDTA
TBE	Tris-borate EDTA
TEMED	N'N'N'N-tetra methyl ethylene diamine
Tris	Trizma Base
USH2A	Usher type II
VAX2	Ventral anterior homeobox 2
xlRP	X-linked retinitis pigmentosa

ZNF638	Zinc finger protein 638
µl	Microliter
µM	Micromolar

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Inherited retinal degenerations are heterogeneous and result in loss of vision due to the premature death of rod and cone photoreceptors, representing the most recurrent forms of inherited visual mutilation in humans, with an approximate prevalence of 1 in 3000 to 1 in 5000. Recessive RP is the main RP type as near about 70% of all the cases of RP are accounted by it. LCA represents the most widespread genetically occurring cause of innate vision destruction in children and infants. LCA accounts for about 5% of all inherited retinopathies and responsible for *10-18%* of congenital blindness. Among the associated symptoms, mental retardation seems to be an important feature of LCA. It is found in around 20% of the LCA population.

In the present study two Pakistani families A and B, with autosomal recessive retinal dystrophy, were ascertained from different regions of Pakistan. In family A six individuals were affected with LCA while in family B five individuals were affected with arRP. Both families were tested for linkage to known loci. Genotyping results showed that the family A was excluded from linkage to known LCA loci. The affected individuals of family B were homozygous at markers D1S1631 and D1S3723, thus establishing linkage to *ABCA4* on chromosome 1p21-13 on RP19.

Bioinformatics approach was used to establish the record of those genes encoded by certain regions which are linked to arRP and LCA, representing our list of strong candidate genes. These genes present an initial point to analyse the mutation as well as linkage for loci refinement within well-characterized families. List of all genes is narrowed down, following the criterion of their retinal expression. Those genes showing retinal expression are further narrowed down by considering a criterion of their physiology in retina. The product and cellular localization of the genes is also considered in this regard. Orthologs along with percent similarity of homologous species to the human candidate genes are also identified. It is a labour-intensive task to perform the mutation analysis of all genes covering a huge area in the genome. For the reduction of the candidate genes to a limited number of mutation analyses, a bioinformatics approach is presented here.

INTRODUCTION

The human eye, a complicated organ, consists of various tissues which are derived from three embryological layers so it is considered among the most frequent sites for genetically inherited disorders. In the developed world, both monogenic and genetically complex eye diseases comprise the most familiar causes of childhood as well as adult blindness (Gregory-Evans and Bhattachararya, *1998*).

The eye is formed by three different layers; the sclera and cornea form the external layer, the intermediate layer is composed of the iris and ciliary body called anterior part and the choroid called posterior part and third layer, the internal layer making retina, a sensory organ of the eye. The posterior part is present behind the retina and absorbs unused radiation. A ring-like muscle called ciliary muscle is connected to the iris and controls the shape of lens by contraction and relaxation of the ciliary muscle. The cornea, present at the front portion of the eye, is responsible for image formation by refracting the light entering the eye. The fovea, a small depression of about 1.5 mm diameter, is the retinal part in which high-resolution vision of fine detail is possible. A colored part of the eye, called iris, regulates the amount of light by adjusting pupil size. The lens is made up of layers of tissue enclosed in a tough capsule. The optic nerve is responsible for transmitting information from the retinal rod and cone cells. The pupil is the aperture through which light and image enters the eye. Its size varies according to the variation in the iris size. The sclera is also known as "white of the eye" as it is a tough white covering outside of the eye-ball. The zonule fibers attach the lens to ciliary muscles. The extraocular muscles keep each eyeball in position.

Development, Anatomy and Physiology of the Human Retina

The human retina is a specialized part of central nervous system (CNS) which has crucial role in vision. It is also easily accessible during embryonic development. Each optic vesicle (formed by the evagination of the embryonic forebrain) forms inner (neural retina) and outer (retinal pigmented epithelium) retinal layers (Graw J, 1996; Saha *et al.*, 1992). The neural retina is comprised of two types of photoreceptors; rods and cones which convert light to nerve impulses. Both classes of photoreceptors contain inner segments (IS), which is the place for metabolic processes and outer segments (OS),

which consist of the opsins, which are visual pigment molecules (Hargrave and McDowell, 1992). The rod photoreceptors work in low light conditions whereas cones function in day light. The fovea which is rich in cone photoreceptors is situated in the macular region of the retina. It is the region showing the highest visual sharpness in humans (Young RW, 1985).

Visual Cascade and Phototransduction

Vision process starts when visual pigments receive and then capture light. The rhodopsin, a photopigment, is embedded in the photoreceptor outer segments membranes' and absorbs the photons in rod photoreceptors (Hargrave and McDowell, 1992). It photoexcites to initiate a GTP-GDP exchange reaction with transducin (T) that is a GTP-binding protein (Figure 1.1). The activation of cGMP phosphodiesterase (PDE) is mediated by activated transducin (*T). This activated PDE (PDE*) then hydrolyzes cGMP as a result of which levels of cytoplasmic cGMP are reduced. The cGMP gated channels are closed due to which hyperpolarization of the plasma membrane occurs resulting in the initiation of the visual transduction channel signal. The recoverin and guanylate cyclase are stimulated causing the elevation in the levels of cGMP, due to which the cGMP channels are re-opened (Shastry BS, 1997; Hargrave and McDowell, 1992). All the same mechanism is involved in the phototransduction in cone photoreceptors but with different proteins (Peng *et al.*, 1992).

Mutations in genes encoding the components of phototransduction cascade i.e; rhodopsin, the tranducin K subunit, the catalytic PDE subunit, the CNG channel subunits, and the GCI/GCAPl modulatory unit have been shown to cause autosomal dominant or recessive retinal degenerations (Andrew *et al.*, 2001).

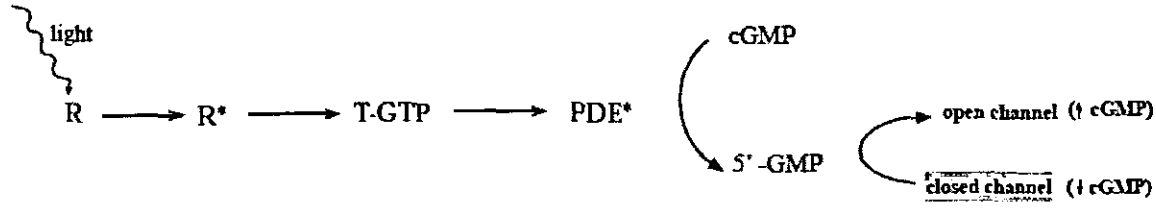


Figure 1.1: Representation of the information flow in visual cascade (Shastry BS, 1997).

Retinal Diseases

The human eye is among the sites showing the genetic diseases frequently, certainly due to its complex structure. Inherited retinal disorders are classified as the developmental disorders, e.g aniridia that is responsible for either hypoplasia or absence of iris because of the PAX6 mutations, and as the gradually degenerative anomalies, e.g retinitis pigmentosa (RP). These hereditary retinal diseases are differentiated into locus and allelic heterogeneity. In locus heterogeneity many genes, which are unrelated, cause the retinitis pigmentosa as a result of mutations in these genes, whereas allelic heterogeneity is associated with various mutations in a particular gene causing the same disorder e.g X-chromosomal retinoschisis (Humphries *et al.*, 1992). Most of the retinal anomalies cause loss of photoreceptors leading to progressive retinal degeneration. Similarly glaucoma along with various forms of optic degenerations is caused due to loss of ganglions. These photoreceptors affecting genetic diseases are also classified on the basis of the cell type which is affected at first (Farber and Danciger, 1997). In cone-rod dystrophies (CRDs) the loss of cone function brings central vision defect in early life while in rod-cone dystrophies, the degeneration occurs due to loss of rod function e.g RP. The night blindness and closure of the visual field is happened when rods are affected initially. Hereditary macular degenerations are the third class of photoreceptors affecting diseases that usually affects central vision in initial age; however the peripheral vision is usually occurred in later life (Weber BH, 1998). As a last the retinal dystrophies can be considered as the example of a complex disease phenotype along with the loss of photoreceptors.

A disease is an irregular state of an organism which deviates from normal condition and interrupts the usual regular anatomy or physiology of any particular part, organ or system of the body. This abnormal condition of an organism damages various functions of the body which are appeared as particular clinical signs and symptoms. It occurrs because of various factors e.g diet, infection, environmental conditions or it can be inherited. The diagnosis, pathology, etiology and prevalence of a disease may be identified or unknown. Genetic disease appears because of the pathogenic mutation in gene encoding DNA. Usually refers to diseases that are inherited in a Mendalian fashion, although non-inherited forms of cancer also result from DNA mutation.

Inherited Retinal Dystrophies

Hereditary retinal dystrophies and degenerations are heterogeneous clinically as well as genetically. The premature death of the rod and cone photoreceptors causes the loss of vision in various diseases in this class (Rivolta *et al.*, 2002). Showing an estimated occurrence of about 1 in 3000 to 1 in 5000, these represent the most common forms of human visual mutilation collectively (Bunker *et al.*, 1984). The retinal dystrophies are classified into peripheral and central retinal dystrophies. In peripheral retinal dystrophies only the peripheral retina is affected, where as the macular part of the retina is damaged in central retinal dystrophies. The central retinal dystrophies are further sub-classified into those that are purely confined to the macular region are called macular dystrophies and those that eventually spread into the peripheral retina e.g. cone-rod dystrophy. Over 40 loci for human hereditary retinal degenerations involving the photoreceptors and retinal pigment epithelium are known (Morimura *et al.*, 1998).

Peripheral Retinal Dystrophies

Among the peripheral dystrophies, RP is the most prevalent form of inherited retinal degeneration. Other, less common conditions, which may present with night blindness or peripheral visual field loss, include choroideremia, gyrate atrophy, Goldmann-Favre disease and congenital stationary night blindness. In some cases of retinal degenerations, the cone cells die first followed by the degeneration of rods. This type of retinal degeneration is referred to as cone rod dystrophy (CRD). The difference between the two types is the rapid loss of either rods and/or cones respectively. Especially at the later stages of disease, when the patient is completely blind, it is hard to distinguish between the two phenotypes (Rivolta *et al.*, 2002). LCA is the sternest type of retinal degeneration. In this case both rods and cones are dead at birth or early in infancy (Francois J, 1968).

Retinitis Pigmentosa

Retinitis pigmentosa (RP: MIM# 26800), showing an expected occurrence of about 1 in 4000 people, is a such kind of progressive retinal dystrophies which shows heterogeneity (den Hollander *et al.*, 2007). RP is referred to the pigmentation of the bone spicule with in midperipheral fundus which simulates inflammation. It was first time introduced in

1857 by Donders, a German physician (Riazuddin *et al.*, 2005). At first Retinits Pigmentosa affects the physiology of the rod photoreceptors while the cone receptor cells' function is compromised at the later stage of the disease on its progression. In the electroretinograms (ERGs) the individuals showing disease usually have severely abnormal rod responses, even in the initial stage of the disease (Bird AC, 1995). The photoreceptor cells of the retina are damaged due to unidentified mechanisms in the case of RP. Various identified mutations which cause diseases primarily attack the photoreceptor cells. The retinal pigment epithelium (RPE) is primarily attacked in many retinal degenerations, due to which photoreceptors are injured. Apoptosis is usually considered as the final mechanism that leads to the photoreceptors' death (Johnson *et al.*, 2005).

The retinitis pigmentosa affected individuals show an extensive variety in age of onset, harshness, development, hereditary mode and clinical expression (Kondo *et al.*, 2004). RP is caused due to near about 40 identified loci with mutations in more than 25 genes (Khaliq *et al.*, 2005) such as the genes that encode the components of phototransduction cascade, the proteins involved in cell–cell interaction proteins, the retinoid metabolism showing proteins, photoreceptor structural proteins, transcription factors, splicing factors and the intracellular transport proteins. The autosomal dominant RP (adRP) represents 15% to 20% of the cases; autosomal recessive RP (arRP) consists of 20% to 25%; X-linked recessive RP 10% to 15% of all cases, and 40% to 55% of cases, are called simplex RP in which family history is absent (Riazuddin *et al.*, 2005).

Symptoms

RP is described by a progressive constriction of the visual fields that is nyctalopia in which the pigment deposits in the midperipheral retina resultantly the vision perception gradually reduces (den Hollander *et al.*, 2007). On the progression of the disease central vision could also be lost. Various changes occur as a result e.g arteriols and optic disc pallor are tapered along with intra retinal pigmentation (Yong *et al.*, 2005). Due to the progressive degeneration of the retinal photoreceptors, the affected individuals usually observe that the peripheral visual field is lost slowly and night blindness occurs; due to which the central vision is lost consequently (Kondo *et al.*, 2004).

Prevalence

The occurence of retinitis pigmentosa is 19 to 27 per 100,000. Its incidence in the Europe and US is about 1/3,500 to 1/4,000. The risk chance of RP in Denmark is 1/2500. The other remaining populations, however not documented yet, are expected to show the similar frequencies (Haim M, 2002). It is not specific in any ethnic group but it is more common in particular identified and consanguineous populations due to mutations in the specific genes. It is estimated that nearly about 1.5 million individuals are affected with retinitis pigmentosa worldwide (Berson EL, 1993).

Classification

According to a research focused on nonsyndromic forms of retinitis pigmentosa, it is classified as syndromic RP in which other body systems e.g hearing may be affected; nonsyndromic or simple RP with having no affect on other body tissues or organs; and systemic RP in which many tissues are affected at the same time. It is the nonsyndromic RP which can show inheritance in autosomal dominant, autosomal recessive or X-linked modes and some times in digenic forms rarely. This digenic form occurs when individuals show heterozygosity for mutation in RDS as well as ROM1.

http://www.geneclinics.org/profiles/rp-overview/details.html

Genetics of RP

The molecular genetic causes of RP are often intricate according to the research in the field of gene discovery and gene mapping (Rivolta *et al.*, 2002). Those proteins encoded by the RP associated genes are involved in, the conversion of the energy in the light photon to a neuronal signal in the photoreceptors that is the phototransduction; the process in which the rhodopsin chromophore is produced and recycled, that is the visual cycle and also the transcription factors and structure of the photoreceptor cell. But the physiology of many RP associated genes is unidentified yet (Phelan and Bok, 2000).

Mode of Inheritance and Genetic Heterogeneity

Retinitis pigmentosa is among those genetic diseases which are extremely heterogenous (Hims *et al.*, 2003). The inheritance modes are autosomal dominant RP, autosomal recessive RP, X-linked RP, and the last two rare modes as digenic and mitochondrial one, with around 40 known or predicted genes implicated in disease pathology. Of those genes with known functions, some encode proteins involved in the visual transduction cascade

(rhodopsin), others in maintenance of photoreceptor structure (peripherin), and others involved in regeneration of the rhodopsin chromophore (11-*cis*-retinal) in the visual cycle (retinal pigment epithelial protein RPE65) (Farrar *et al.*, 2002).

adRP is reported for about 20% of the whole cases; arRP also accounts the same percentage; xlRP shows 10% of all RP cases; 50% of RP modes are considered among the group of the simplex RP but mostly these are the arRP forms which are not identified (Haim M, 1993). The genetic nature of arRP is ambiguous with indefinable diagnosis due to various technical barriers, in spite of it being the most common form (Kondo *et al.*, 2004).

Autosomal Dominant RP (adRP)

Dominantly inherited RP is heterogeneous, both clinically and genetically. At least 13 loci are reported for adRP (Table 1.1). Rhodopsin (RHO), oxygen-regulated protein 1 (RP1), and peripherin (RDS) are three important genes; nearly about 25-30% of adRP is represented by RHO, while RP1 and RDS both represent equally as about 5-10% (Berson *et al.*, 2001). Among 10% of the American patients with adRP are reported to show more than hundred mutations of RHO, Pro23His. Clinical phenotypes which range from RP to simple and complex macular dystrophies are associated by mutations of RDS. Arg677stop and 2280del5, contribute to the half of adRP cases occurred due to this gene, among the known RP1 mutations. The prevalence is still unknown specifically, however the other cloned adRP genes are the cause of a significant fraction of the RP cases, e.g pre-mRNA splicing factor C8 (PRPF31); the human homologue of yeast (Bader *et al.*, 2003).

RP1

RP1 is the gene for oxygen regulated photoreceptor protein in humans that encodes a protein of 2156 amino acids with the molecular weight of 241 kDa. It is situated at the cones' and rods' photoreceptors connecting cilia (Liu *et al.*, 2002). The morphogenesis of the photoreceptors outer segments and the correct orientation along with appropriate stacking of outer segment disks is carried out by this RP1 protein (Khaliq *et al.*, 2005; Liu *et al.*, 2003; Gao *et al.*, 2002). The actual mechanism, through which *RP1* mutations lead to the death of the photoreceptors, is yet unknown; just as many other genes involved in retinal dystrophies (Liu *et al.*, 2003). It is part of the photoreceptor axoneme

(Liu *et al.*, 2004). This gene gives rise to nearly about 7.1 kb sized transcript consisting of four exons. Its mutations are mostly involved in adRP, are referred as the nonsense codons which exist between 658 and 1053 codons in the last exon of this gene. Nonsense-mediated decay does the degradation of unstable mRNA as a result of the mammalian genes' nonsense mutation (Guillonneau *et al.*, 1999; Hentze and Kulozik, 1999; Sullivan *et al.*, 1999). But if this mutation occurs in the last exon, this decay does not happen. A protein, having an injurious affect leading to dominant inheritance pattern, is formed as a consequent of this gene's disturbance within BIF motif or at once after it. The recessive mode of inheritance is produced as a result of loss of RP1 physiology, when RP1 truncates before the BIF domain or within the protein's terminal part (Riazuddin *et al.*, 2005). In a study of diseased locus mapping in three RP affected consanguineous Pakistani families, the 8q11 locus was mapped. According to this study, the homozygous mutations in the *RP1* gene causes arRP mode to occur for first time (Khaliq *et al.*, 2005).

Rhodopsin (*RHO*)

Many mutations of rhodopsin gene have been reported to be the cause of adRP. The first case of a *RHO* gene mutation, reported in the Singaporean population, was Q344X mutation. A single US family reported the similar mutation, with one case reported in Germany (Kremmer *et al.*, 1997; Sung *et al.*, 1991). Several mutation sites are common in a common mutation region i.e the 3' end of this gene, which also highlights the separate geographical populations reported for the common mutation sites (Yong *et al.*, 2005).

Up till now all the rhodopsin mutations, which have been reported in Asia, are focused on the C-terminal. Hong Kong Chinese identified P347L and 5211delC (Chan *et al.*, 2001), whereas in Japan, China and India P347L, E341X and V345M were reported, respectively (Dikshit *et al.*, 2001; Zhao *et al.*, 2001; Shiono *et al.*, 1992). According to the reports of the Germany, USA, Spain, UK, Lithuania and South Africa, among these mutations P347L has been reported worldwide (Roberts *et al.*, 2000; Kucinskas *et al.*, 1999; Trujillo *et al.*, 1998). V345M has been reported in the USA (Dryja *et al.*, 1991) whereas, an Italian family identified 5211delC (Horn *et al.*, 1992). The Carboxylic end of the rhodopsin is essential for the physiology of the protein opsin, in various populations because of the gatheing of mutation sites at its Carboxylic end (Yong *et al.*, 2005).

HPRP3* and *PRPF3

At the locus RP18 of chromosome 1q21.1 a gene *HPRP3* is located that is responsible for adRP. A large four generation Danish family reported that in a centi Morgan distance of < 2, this locus was identified between markers D1S442 and D1S2858 for the first time (Xu *et al.*, 1998; Xu *et al.*, 1996). This interval at locus RP18 was then reported as a 1cM interval from D1S442 to D1S498 in an English family as a result of the advance study to analyze the haplotype (Inglehearn *et al.*, 1998). On the same interval at chromosome 1q21.1 this gene for *HPRP3* (a U4/U6-associated splicing factor) has been mapped (Heng *et al.*, 1997). It contains a measured molecular weight of 77kDa, encoding a 682 amino acids long protein (Anthony *et al.*, 1997; Wang *et al.*, 1997). *HPRP3*, associated with adRP, is also considered as the strong candidate gene for the locus RP18 (Chakarova *et al.*, 2002).

PRPC8 / PRPF8

According to the results of the linkage study in a huge South African family that is affected with adRP the locus for adRP: RP13 was placed at chromosome 17p13.3.They have reported seven various missense mutations with in the gene *PRPC8,* by applying the candidate gene approach and a strategy of positional cloning. Among these seven mutations, three mutations co-segregate in 3 families linked with RP13 that also includes the pedigree of South African family while the other families with no relation to adRP reported the remaining four mutations. Its last exon contains a stretch of 14 codons, where all these seven mutations are grouped. Here comes an evidence of a new pathway that leads to retinal dystrophies because of the mutations in this highly conserved region (McKie *et al.*, 2001).

RP11

The RP11 locus for adRP has previously been mapped to chromosome 19q13.4 in a large English family. This linkage has been independently confirmed in a Japanese family, and we now report three additional unrelated linked U.K. families, suggesting that this is a major locus for RP (Al-Maghtheh *et al.*, 1996).

Autosomal Recessive RP (arRP)

In the consanguineous families affected with autosomal recessive RP, the affected individuals must show homozygous pattern for the regions linked to disease causing loci

(Kondo *et al.*, 2004). Accounting for about 70% of the total cases of RP,this form of RP is considered as the most important one (Jay M, 1982). There are no distinguished clinical differences between arRP, adRP or xlRP (X-linked RP). The arRP usually shows clinical similarities with other forms of retinal degenerations like early-onset RP and LCA (Lebers congenital amaurosis). Autosomal recessive RP also shows extensive genetic heterogeneity. Over 19 loci/genes are reported to be associated with arRP. Among these loci 5 are reported to contain the unidentified genes affecting the individuals (den Hollander *et al.*, 2007) (Table 1.2). The most genes for arRP cause 1% cases or even less than that, so considered as rare, however retinal pigment epithelium-specific 65kD protein (*RPE65*), rod cGMP phosphodiestrase alpha and beta subunit (*PDE6A and PDE6B*) are considered as the common genes as these cover 2-5% cases of all the cases for arRP. *USH2A* gene is the gene causing both Usher syndrome and arRP. About 5% of arRP cases are accounted by this gene. There are few examples for genes' mutations being the frequent cause of autosomal recessive RP such as, *RP25* is involved in 10-20% of arRP in Spain and rarely associated with the disease anywhere else in the world. Similarly, mutations in nuclear receptor subfamily 2 group E3 (*NR2E3*) are only reported in Sephardic Jews in Portugal (Bader *et al.*, 2003).

CERKL

A novel autosomal recessive RP (arRP) locus, RP26, was defined within an 11-cM interval (17.4 Mb) on 2q31.2-q32.3 in a linkage analysis study in a Spanish consanguineous family (Tuson *et al.*, 2004). RP26 locus was further refined down to 2.5 Mb, by microsatellite and single-nucleotide polymorphism (SNP) homozygosity mapping. A novel gene encoding a ceramide kinase (CERKL) was finally identified, which encompassed 13 exons. Human CERKL is expressed in the retina, among other adult and fetal tissues. A more detailed analysis by in situ hybridization on adult murine retina sections shows expression of CERKL in the cell layer of the ganglion. The sphingolipid metabolite ceramide is converted into ceramide-1-phosphate by ceramide kinases. The neuronal cells containing sphingolipids in their membrane are controlled under ceramide metabolism. Therefore, CERKL deficiency could shift the relative levels of the signaling sphingolipid metabolites and increase sensitivity of photoreceptor and other retinal cells to apoptotic stimuli. This is the first genetic report suggesting a direct

link between retinal neurodegeneration in RP and sphingolipid-mediated apoptosis (Tuson *et al.*, 2004).

USH2A

Most of the cases of Usher type II occur due to mutations in the gene USH2A at the chromosome 1q41 (Eudy *et al.*, 1998). It encodes a protein usherin showing its anatomical similarity with a protein called laminin. This gene containing 21 exons is physiologically unidentified yet, however it has been hypothesized that it is a sort of molecule showing adhesion in the cell. It is also assumed it having a portion of the basement membrane. But its anatomy has been established along with some novel USH2A mutations (Pieke-Dahl *et al.*, 2000).

TULP1

Around 1% to 2% of the the autosomal recessive RP affected individuals show mutations in the gene *TULP1*, a member of the tubby-like protein family (Mandal *et al.*, 2005; Kondo *et al.*, 2004; Paloma *et al.*, 2000). The people affected with arRP due to mutation in this particular gene, are handicap visually the most, even in the age of childhood so better illustrated as LCA (Hanein *et al.*, 2004). *TULP1* locates at the IS i.e the inner segments at first and then connecting cilium of photoreceptors, so its expression is only limited to the retina of eye. It is required for the travelling of the protein and it also transports the rhodopsin gene from the inner segments that is the synthesis site to the outer segments through the connecting cilium (den Hollander *et al.*, 2007; Xi *et al.*, 2005). It has been reported that 22 distinctive *TULP1* mutations, which are disease causing, occur in the individuals affected with LCA or early-onset RP. A study on a consanguineous Israeli Muslim Arab family affected with arRP reported c.1495+2_1495+3insT as a novel splice-site mutation of *TULP1* (Abbasi *et al.*, 2008). Its mutations with in humans as well as mice are the cause of retinal dystrophies (Ikeda *et al.*, 2000).

ABCA4

Many forms of hereditary autosomal recessive retinal degenerations are caused due to the *ABCA4*, a retinal-specific ATPbinding cassette gene (Gerber *et al.*, 1998). Its mutations lead to many of the autosomal recessive cone–rod dystrophies (arCRDs); all the cases of both early- as well as late-onset arSTGD (autosomal recessive Stargardt) disease; and few

cases of RP (Ducroq *et al.*, 2002; Rozet *et al.*, 1999). Apart from it, *ABCA4* shows a very high carrier frequency in general population having the range from 1/50 to 1/10 (Ducroq *et al.*, 2006).

RP28

In a study, a consanguineous Indian family affected with arRP has been reported to show linkage to the RP28 locus of chromosome 2p11.2-p15. This locus was previously mapped to between marker D2S1337 and D2S286, a region of16 cM. This region has been further narrowed down to a 1.06 cM region between D2S2225 and D2S296, as a consequent of the haplotype analysis in the current family (Gu *et al.*, 1999). In a second Indian family arRP showed positive linkage, with a maximum two-point lod score of 3.96 at theta=0 for D2S380, to the same locus mapped on chromosome 2p14-p15. 14 genes of the total 15 reported genes in MCR show expression in either eye or the retina; these genes are *MDH1*, *KIAA0903*, *KIAA0582*, *SPRED2*, *OTX1*, *UGP2*, *ACTR2*, *VPS54*, *PELI1*, *TRIPBr2*, *RAB1A*, *HSPC159*, *FLJ20080* and *SLC1A4* (Kumar *et al.*, 2004).

ABCR

The exon–intron anatomy of the ATP-binding cassette transporter-retinal gene, *ABCR* has been completed. According to it a study on the four RP affected individuals, IVS30+1G → T, a homozygous 5'splice site mutation, was detected (Cremers *et al.*, 2002).

X-linked RP (xlRP)

The clinical symptoms, both in terms of earlier age of onset and rapid progression observed in males affected with any form of xlRP are usually more severe than the ones associated with adRP or arRP. The age of onset of the disease typically occurs in the first decade of life and the disease progresses to partial or complete blindness by the third or fourth decade of life (Bird AC, 1975). Mutations in retinitis pigmentosa GTPase regulator (RPGR) and retinitis pigmentosa 2 (RP2) genes are the most common causes of xlRP, showing the occurrence, for the X-linked RP, of about 70-90% and 10-20%, respectively. The studies on most of the families could not identify the mutations in this RPGR gene, however the rate of the detection of mutations has now been increased alarmingly due to the identification of ORF15, an additional exon of RPGR (Bader *et al.*, 2003) (Table 1.3).

Digenic RP

The concurrent inheritance of the heterozygous mutations in two different genes is the cause of the digenic RP. These two genes are *ROM1* that is retinal outer segment membrane protein 1 and *RDS*-peripherin. *ROM1* and *RDS*-peripherin covalently link to form homodimers, which then interact non-covalently to form a tetrameric complex. The formation tetrameric complex is essential for the morphogenesis and structural stability of disc rims of rod photoreceptor cells (Bascom *et al.*, 1992). The mutated *ROM1* and *RDS*-peripherin may unable to form tetrameric complexes and thus can result into the structural instability of photoreceptor cells which can lead to a disease phenotype.

To date, four families are reported, where RP patients were double heterozygous for mutations at the unlinked *RDS*-peripherin (6p21) and *ROM1* (11q13.1) loci. In each case, a Leu185Pro substitution was found in *RDS*-peripherin and it was associated with one of the three different 1 base pair (bp) insertions situated early in the *ROM1* coding region. All these frame-shifts due to insertion mutations lead to a premature termination of protein product (Kajiwara *et al.*, 1994). The RP family individuals with either the *RDS*-peripherin or the *ROM1* mutation alone were found to be asymptomatic (Bascom *et al.*, 1992).

Disease Progression

In the majority of RP cases, blindness can occur at around the age of 60 years. Although many authors have suggested that the retinal and choroidal circulation decreased in RP (Bergen *et al.*, 1995; Teague *et al.*, 1994), the exact mechanism responsible for these changes remains unclear. The retinal pigment epithelium (RPE) cells migrate to the inner retina close to retinal vessels following death and disappearance of photoreceptors. The extracellular matrix between the translocated RPE cells and retinal vessels can be quite thick, and the retinal vascular endothelial cells become thin and fenestrated. Another possible mechanism that could lead to a decreased retinal blood flow in RP is the photoreceptor loss, which causes a decreased metabolism in the inner retina. This may induce a decrease in the blood flow to the residual retinal tissue (Meindl *et al.*, 1996).

Leber's Congenital Amaurosis

Leber congenital amaurosis (LCA; MIM # 204000) is a group of conditions that result in early onset retinal dystrophy, which over time may be accompanied by pigmentary changes in the retina, hence "amaurosis" (Greek for darken) (Hooser *et al.,* 2002). A large number of visual autosomal recessive retinal degenerations are labeled as LCA collectively. It represents the most frequently occurring genetic causes of congenital visual impairment in children along with infants. The mutations with in the gene that encodes *RPE65* are the cause of about 10% cases of the all LCA cases (Pang *et al.,* 2005). LCA patients are diagnosed usually at the time of birth or during initial months of life, with extremely damaged eye vision or the complete blindness. The pathophysiology of the disease LCA is little known. It is considered as a result of either defect in the photoreceptor cells' development or deterioration of normally developed cells at very initial stage (Perrault *et al.,* 1999). LCA accounts for about 5% of all inherited retinopathies and responsible for *10-18%* of congenital blindness (Graw J, 2003). Some other diseases are also developed frequently during this disease like hypermetropia and keratoconus (Hameed *et al.,* 2000).

Symptoms

Along with the damage in development of rod and cone photoreceptor cells, the affected people with LCA might retain visual physiology, in spite of progressive retinal changes, which supports the aplasia hypothesis (Fulton *et al.,* 1996). This hypothesis has also been supported due to some pathological discoveries showing the range from diffuse retinal atrophy to abnormalities in the ganglion cell, absent rods, or cones, or abnormal rods and cones (Koenekoop *et al.,* 1999).

Clinical Description

LCA, the sternest form of the hereditary retinal degeneration occurring in very early age of life, is responsible for harsh visual impairment, in other words the congenital blindness. In 1869, it was introduced by Theodore Leber for the first time. The clinical signs presented in LCA are sluggish papillary response, infantile nystagmus, roving eye movement, squint, occasional photophobia, cataract, keratoconus and keratoglobus. LCA phenotype is also reported to be associated with many syndromic anomalies of the renal, cardiac, skeletal and central nervous system. Among the associated symptoms, mental

retardation seems to be an important feature. It is found in around 20% of the LCA population (Fazzi *et al.,* 2003). The fundoscopy analysis shows that the fundus and optic discs are not abnormal apparently, at the infant stage. But as the time progresses, the structural and functional disabilities of fundus are made obvious, such as the retinal vasculature and the optic nerve shrinks, the retinal pigment epithelium (RPE) deteriorates, the bone cells show pigmentation. Some times the peripheral and mid-peripheral parts of retina show abnormal yellow pigmentation. As some times it is difficult to observe the LCA signs and disabilities at the stage of infants, so electroretinographic (ERG) evaluation is used here to distinguish it from other retinal degenerations showing early age onset. In addition to LCA, some other clinical disturbances also occur, the most common of which is the psychomotor retardation (Lambert *et al.,* 1989; Moore and Taylor, 1984).

Molecular genetics of Leber's congenital amaurosis (LCA)

LCA is the disorder that usually follows the autosomal recessive mode of inheritance, showing the genetic heterogeneity (Alstrom CH, 1957). AIthough infrequent accounts of dominant inheritance have been reported (Rivolta *et al.,* 2001; Perrault *et al.,* 2000). To date, eight causative genes have been identified in LCA (Table 1.4).

RetGC-1 or GUCY2D

It is the first gene mapped to chromosome 17p13.1 in humans (Camazut *et al.,* 1996 and 1995). *RetGC-1*, a vital element of the phototransduction cascade, is translated to *RetGC-1 or GUCY2D*, a photoreceptor-specific guanylate cyclase. Its mutations destroy the dark state's recuperation after the photoreceptors are photo excited (Perrault *et al.,* 1999 and 1996). It has been identified that individuals affected with the locus *LCA1* show 22 various mutation; among these, half i.e 11 mutations were missense. 9 of these missense mutations are reported as; W21R, L41F, R313C, M1I, N129K, R976, H1019P, M1009L and R995W. The other half that is, 11 of these 22 mutations were thought to destroy the cyclase activity of *RetGC-1* hence called as truncating mutations (Rozet *et al.,* 2001; Perrault *et al.,* 2000).

PEDF

Eight families showing linkage to 17p13.1 were analyzed for mutatios; among these 8 families only 4 showed mutations in the RetGC gene. Another gene, which enhances the

differentiation of photoreceptors and neuronal endurance, also localizes to 17p13.1 that is *PEDF*, a Pigment Epithelium-Derived Factor (Tombran-Tink *et al.*, 1994). This gene, showing expression with in the retinal pigment epithelial cells of the fetus, is serpin that is a serine protease inhibitor (Steele *et al.*, 1993). It is proposed that there might be the possibility of more than single gene can be the cause of LCA, in the same particular region; as *PEDF* is considered as an evident and stimulating candidate gene for the degenerations with in photoreceptor cells, specifically LCA according to data presented by linkage study as well as physiological report (Koenekoop *et al.*, 1999).

RPE65

The second locus for LCA was assigned to chromosome lp31 (Hamel *et al.*, 1994) and the affected gene encodes a microsomal membrane protein (*RPE65*) of the RPE (Marlhens *et al.*, 1997). The cDNA sequence predicts a protein with 533 amino acid residues and a molecular mass of 61 kDa (Hamel *et al.*, 1993). *RPE65* is expressed with in an organelle of RPE that is the smooth endoplasmic reticulum (Pang *et al.*, 2005), and is essential for the retinoid cycle. Retinoid cycle is responsible to convert vitamin A from all-*trans* retinol to the visual pigments chromophore that is 11-*cis* retinal (Thompson *et al.*, 2004). Although the biochemical function of the protein product is currently obscure, the tissue-specific expression of the *RPE65* gene made it an attractive candidate as a cause for some retinal degeneration because the retinal pigment epithelium has an essential role in maintaining the viability of the neighboring photoreceptor cells (Morimura *et al.*, 1998). The sensitive examination revealed that the individuals, affected with LCA which occurred due to the mutation in this particular gene *RPE65*, show the clinical signs of moderate hyperopia, low myopia or no hyperopia and some times low myopia (Perrault *et al.*, 1999).

CRX

A gene mapped to chromosome 19q13.3 is the third gene causing LCA in humans that is a cone-rod homeobox *(CRX)* gene (McInnes *et al.*, 1998). It encodes a protein that helps to differentiate, develop and maintain the photoreceptors by the process of the transactivation of numerous gene promoters which are photoreceptor-specific; such as rhodopsin. This is the CRX protein, a photoreceptor-specific homeodomain transcription factor (Chen *et al.*, 1997; Furukawa *et al.*, 1997).

AIPLI

Another gene that causes LCA is the gene *AIPLI*, aryl hydrocarbon receptor interacting protein-like 1, which is mapped on chromosome 17p13.1 of human with in the interval of 2.5 Mb to the locus for retGC-1 (Sohocki *et al.*, 2000b). This particular gen encodes a protein containing 384 amino acids (Blatch and Lassle, 1999). Mutations in several genes, including *AIPL1*, cause LCA (Koenekoop *et al.*, 2004). *AIPL1* is expressed specifically in adult rod photoreceptors (Dharmaraj *et al.*, 2004), where its function is essential but not understood. It possesses one peptidyl-prolyl isomerase (PPI) domain and three consecutive tetratricopeptide repeats (TPR). AIPL1 might fulfill a molecular chaperone function for retinal protein folding (Chapple *et al.*, 2001). Sequence comparison places *AIPL1* in the FK-506-binding protein (FKBP) family. These proteins are "specialized chaperones" that assist specific client proteins in later stages of maturation, subunit assembly, transport, and degradation (Young *et al.*, 2003; Petrulis and Perdew, 2002). Many such client proteins are components of signal transduction pathways. Therefore, the primary sequence of *AIPL1* suggests a possible role as a specialized accessory chaperone important for photoreceptor protein(s) (Liu *et al.*, 2004). It has been proposed that there are 2 options of AIPL1 showing a vital role for the photoreceptors of retina (Ramamurthy *et al.*, 2003). Firstly, this gene promotes a vital reaction of farnesylation which is a definite kind of prenylation; this is the process of the accumulation of a farnesyl residue to the proteins of particular kinds. Numerous retinal proteins are transducin, cGMP phosphodiesterase and rhodopsin (Rho) kinase (RK) (Anant *et al.*, 1992). The cyto-architecture and anatomy of the photoreceptor cells in retina are maintained by the prenylation process of proteins of retina while the deterioration of these photoreceptors occurs when prenylation is hindered (Pittler *et al.*, 1995). A retinal protein vital for the life of photoreceptor cells that is cGMP PDE remains stable because of prenylation process; postulating that *AIPL1* is important for photoreceptors' maintenance (Qin *et al.*, 1994). Another possibility is *AIPL1* having role in the development that is differentiation and proliferation of the photoreceptor cells. *AIPL1* shows interaction with a protein showing a vital contribution for the regulation of cell cycle progression that is a Nedd8-ultimate buster 1 (*NUB1*) (Akey *et al.*, 2002). So *AIPL1* is assumed vital for the photoreceptors' development at early stage, on the basis of

AIPL1 expression with in retina at initial stage, interaction of *NUB1* and the brutality of those mutations of *AIPL1* which are associated with LCA (Ramamurthy *et al.,* 2004).

CRB1

CRB1 is another LCA causative gene, which maps on 1q31.3 (den Hollander *et al.,* 2001; Lotery *et al.,* 2001). *CRB1* a well conserved gene with homologues across multiple phyla is the human homologue of Drosophila Crumbs (Richard *et al.,* 2006). This gene, of 40kb size containing 11 exons, encodes a protein which is expressed with in retina and the central nervous system (CNS) and is important in cell polarity. The *CRB1* gene shows its expression particularly with in the retina and the brain. Human *CRB1* is a transmembrane protein containing a large extracellular part. It contains three laminin G domains and nineteen epidermal growth-factor-like domain. The conserved intracellular part consists of 37 amino acids C-terminal PDZ-binding motif (Meuleman *et al.,* 2004). Functions of the crumbs protein has extensively been studied in Drosophila, although some mouse models now been reported. In Drosophila it is localized on the apical membrane of the epithelial cells, and also in the stalk membrane apical to the zonula-adherens in the photoreceptor cells (Davis *et al.,* 2007). Thus it has been identified that the crumbs gene of the drosophila is essential for the morphogenesis of the photoreceptor cells (Pellikka *et al.,* 2002).

RPGRIP1

RPGRIP1, mapped on 14q11.2 of LCA6, is considered to be located on the connecting cilium of the photoreceptor cells controlling the movement from IS to OS and from OS to IS. It is recognized as a related partner of RPGR, an X-linked retinitis pigmentosa protein (Dryja *et al.,* 2001). *SEMA4A* and *RPGRIP1* are needed for photoreceptor subcellular protein translocation (Abid *et al.,* 2006; Cremers *et al.,* 2002).

TULP1 and *LRAT* are also known to cause LCA but their roles in the pathology of the disease are poorly understood. Besides these disease causing genes, two other disease loci have been identified on chromosome 14q24 and 6ql I-ql6, for which the genes are still unknown (Dharmaraj *et al.,* 2000).

LCA5

In another study to perform genetic analysis of a single consanguineous family, a linkage was observed on the chromosome 6q11-16 to the locus LCA5 locus. Further analysis may expose more detail upon the gene identification and characterization regarding LCA5 (Mohamed *et al.,* 2003).

LCA9

A new locus for LCA is LCA9 that has been found in a single Pakistani family which maps to chromosome 1p36 (7–14Mb). This is the initial step to further analyze LCA families linked to this locus which will consequently make it possible to identify the related genes showing vital role in the retinal development as well as maintenance (Keen *et al.,* 2003).

LCA is not a curable disorder, Research, at molecular level is in progress. Genes responsible for this disorder have been reported from different populations. Pakistani population is an interesting one because of close relative marriage and particularly in first cousin marriages, which are preferred here. Because of inbreeding in human population it is expected that recessive form of disorder shall appear. The aim of the present study is to search for any new locus for this disorder and how consanguinity influences in the appearance of this disorder. These contributions in this regard may be helpful in future research regarding gene therapy of this disorder.

In the present study two Pakistani families have been ascertained, one family with congenital Blindness (LCA) and other with Autosomal Recessive retinitis pigmentosa (arRP). The data presented here includes mapping of LCA and arRP loci in two families and finding candidates genes for retinal dystrophies using Bioinformatics Approach.

Table 1.1: The known Loci and Genes, of Autosomal Dominant RP (adRP), with their Products and Percentages

Locus ID	Chromosomal Locus	Gene/Product	Also causes	Percentage
RP18	1q21.2	PRPF3, U4/U6 small nuclear ribonucleoprotein Prp3	Unknown	Several families
RP4	3q21-q24	RHO, Rhodopsin	Recessive RP; dominant CSNB	25%-30%
RP7	6p21.1-cen	RDS, Peripherin	Dominant MD; digenic RP with ROM1	5%-10%
RP9	7p14.2	RP9, Retinitis pigmentosa 9 protein	Unknown	Unknown
RP10	7q31.3-q32	IMPDH1, Inosine 5'-monophosphate dehydrogenase 1	Unknown	3%-5%
RP1	8q11-q13	RP1, Oxygen-regulated protein 1	Unknown	5%-10%
	11q13	ROM1, Rod outer segment membrane protein 1	Digenic RP with RDS	Rare
RP27	14q11.1-q11.2	NRL, Neural retina-specific leucine zipper protein	Autosomal recessive RP	Rare
RP13	17p13.3	PRPF8, Pre-mRNA processing splicing factor 8	Unknown	Unknown
RP17	17q23	CA4, Carbonic anhydrase IV	Unknown	Unknown
RP30	17q25	FSCN2, Fascin 2	Unknown	3% of Japanese (adRP)
	19q13.3	CRX, Cone-rod homeobox protein	Dominant CORD, dominant and recessive LCA	Rare
RP11	19q13.4	PRPF31, U4/U6 snRNP-associated 61-kD protein	Unknown	15%-20%

(RetNet)

http://www.geneclinics.org/profiles/rp-overview/details.html

Table 1.2: The known Loci and Genes, of Autosomal Recessive RP (arRP), with their Products and Percentages

Locus ID	Chromosomal Locus	Gene/ Product	Also Causes	Percent-age
RP20/ LCA2	1p31	RPE65, Retinal pigment epithelium-specific 65-kD protein	LCA (7%-16%)	2%
RP19	1p21-p13	ABCA4, Retinal-specific ATP-binding cassette transporter	Recessive Stargardt disease, and cone-rod dystrophy	~5%
	1p22.1	ABCR (ATP-binding cassette transporter-retinal)	Unknown	Unknown
RP12	1q31-q32.1	CRB1, Crumbs protein homolog 1	Recessive RP with para-arteriolar preservation of the RPE (PPRPE); LCA (9%-13%)	Rare
	1q41	USH2A, Usher syndrome type IIa protein	Usher syndrome type II	4%-5%
RP28	2p14-p15	Unknown	Unknown	2nd Indian family
	2q14.1	MERTK, Proto-oncogene tyrosine-protein kinase MER tyrosine kinase	Unknown	Rare
RP26	2q31.2-q32.3	CERKL, Ceramide kinase-like protein	Unknown	Rare
	2q37.1	SAG, S-arrestin	Recessive Oguchi disease	Rare
RP4	3q21-q24	RHO, Rhodopsin	Dominant RP; Dominant CSNB	Rare
CSNB 3	4p16.3	PDE6B, Rod cGMP-specific 3', 5'-cyclic phosphodiesterase beta-subunit	Dominant CSNB	3%-4%
	4p12-cen	CNGA1, cGMP-gated cation channel alpha 1	Unknown	Rare
RP29	4q32-q34	Unknown	Unknown	Rare; 4 families

	4q31	LRAT, Lecithin retinol acyltransferase	Unknown	Unknown
	5q31.2-q34	PDE6A, Rod cGMP-specific 3', 5'-cyclic phosphodiesterase alpha-subunit	Unknown	3%-4%
RP14	6p21.3	TULP1, Tubby-related protein 1	Unknown	Rare
RP25	6q14-q21	Unknown	Unknown	10%-20% of arRP in Spain
	10q23	RGR, RPE-retinal G protein-coupled receptor	Dominant choroidal sclerosis	Unknown
RP27	14q11.1-q11.2	NRL, Neural retina-specific leucine zipper protein	Dominant RP	Unknown
	15q23	NR2E3, Photoreceptor-specific nuclear receptor	Recessive enhanced S-cone syndrome	Rare; found in Sephardic Jews in Portugal
	15q26	RLBP1, Cellular retinaldehyde-binding protein	Recessive Bothnia dystrophy; recessive retinitis punctata albescans; recessive Newfoundland rod-cone dystrophy	Unknown
RP22	16p12.3-p12.1	Unknown	Unknown	Rare
	16q13	CNGB1, Cyclic-nucleotide-gated cation channel 4	Unknown	Unknown

(Kumar *et al.*, 2004)

(RetNet)

http://www.geneclinics.org/profiles/rp-overview/details.html

Table 1.3: The known Loci and Genes, X-linked Recessive RP (xlRP), with their Products and Percentages

Locus ID	Chromosomal Locus	Gene/ Product	Also Causes	Percentage
RP23	Xp22	Unknown	Unknown	Unknown
RP6	Xp21.3-p21.2	Unknown	Unknown	Unknown
RP3	Xp21.1	RPGR, X-linked retinitis pigmentosa GTPase regulator	X-linked CSNB; X-linked cone dystrophy 1; X-linked atrophic MD, recessive; RP plus sensorineural hearing loss, recurrent sinopulmonary infection	70%
RP2	Xp11.3	RP2, XRP2 protein	Peripapillary and macular atrophy	8%
RP24	Xq26-q27	Unknown	Unknown	Unknown

(Dandekar *et al.*, 2004; Bader *et al.*, 2003)

(RetNet)

http://www.geneclinics.org/profiles/rp-overview/details.html

Table 1.4: The known Loci, Genes and Frequency of Leber's Congenital Amaurosis (LCA)

Locus ID	Locus	Gene/ OMIM#	Protein Name	Function	Frequency
LCA1	17p13.1	GUCY2D or retGC-1 204000	Retinal guanylyl cyclase 1	Guanylate cyclase enzyme is responsible for the generation of cGMP	6%
LCA10	12q21.32	CEP290	Centrosomal protein Cep290	Unknown	Unknown
LCA2	1p31	RPE65 180069	Retinal pigment epithelium-specific 65 kDa protein	Protein necessary for the production of I I-cis-vitamin A	7-16%
LCA3	14q24.1	RDH12	Retinol dehydrogenase 12	Protein has a dual specificity for all trans- and cis-etinlols	unknown
	14q24	Unknown	Unknown	Unknown	Homozygosity
LCA4	17p13.1	AIPL1 604392	Aryl-hydrocarbon interacting protein-like 1	Protein involved in nuclear transport and chaperone activity during development	10%
LCA5	6q11-q16	Unknown	Unknown	Unknown	Limited number of consanguineous families
LCA6	14q11	RPGRIP1 605446	X-linked retinitis pigmentosa GTPase regulator-interacting protein 1	Protein interacts with RPGR and localizes to ciliary structure between outer and inner photoreceptor segment	6%
LCA7	1q31-q32.1	CRB1 600105	Crumbs homolog	Protein involved cell-cell interaction and in	9- 13%

				cell polarity	
LCA8	19q13.3	CRX 120970	Cone-rod homeobox	A transcription factor that interacts with NRL	3%
LCA9	1p36	Unknown	Unknown	Unknown	One Consang-uineous Pakistani family.

(RetNet)

MATERIALS AND METHODS

Familial study

In the current study, two families were sampled for the gene identification, mapping and characterization of autosomal recessive retinal dystrophies; one family of arRP disorder and other of congenital blindness (LCA). Blood samples of LCA family were collected from Attock while that of RP were collected from Eidgah, CMH road Jehlum. These families were visited at their residential places. A certain criteria was observed including; family at least consists of 4 generations, family should be cooperative in behaviour, at least 5 members should be affected in the family to get better results and it should not be sampled earlier by some one else. The information collected include the type of marriage, exact relationship between husband and wife, their family history including information about number and sex of offsprings, normal and diseased individuals in the family, and age of onset was also recorded. Pedigrees of the families were drawn using a standard method (Bennett *et al.*, 1995) and software package, Cyrillic.210. Families were processed at the Institute of Biomedical and Genetics Engineering (IBGE), KRL hospital, Islamabad. In the pedigrees males were symbolized by squares and females by circles. The normal individuals were designated with unfilled, while the affected with filled symbols (circles or squares). Each generation was denoted by Roman numerals, while individuals within a generation were denoted by Arabic numerals. Double lines between the partners showed cousin marriages. Mode of inheritance was deduced through pedigree analysis.

Blood sampling

Peripheral blood samples were collected by veni puncture technique from both normal and affected individuals including their parents. For DNA extraction, 8-10 ml of whole blood was collected from each individual. To preserve, the blood samples were collected in vaccutainer tubes containing ACD (Acid Citrate Dextrose) and potassium EDTA anticoagulant that inhibit the clotting of blood by removing calcium ions from blood. Each blood sample was assigned unique sample number. Before the DNA extraction was started the blood samples were stored at 4°C.

Genomic DNA Extraction

Genomic DNA was prepared using modified genomic extraction method (Maniatis *et al.*, 1982). Before the genomic DNA extraction from blood samples was started, a log sheet having columns for family ID, sample ID, individual names, age, sex, sample OD and DNA concentration was created. DNA extraction was carried out in thermo control centrifuge by using standard Phenol Chloroform DNA Extraction protocol, explicated as under:

Day 1

- Blood sample from each tube was transferred to the appropriately labeled falcon tube.
- Freshly prepared cell lysis buffer (KHCO3, NH4Cl, and 0.5 M EDTA) was added to each blood sample in 3:1 ratio. Then sample tubes were placed on ice for 30min.
- Samples were centrifuged at 3200 rpm at 4°C for time of 20 minutes.
- The pellet was re-suspended after discarding the supernatant; if the pellet was reddish then 10ml of cell lysis buffer was added. Again samples were centrifuged for 20 minutes at 2000 rpm at 4°C.
- The pellet was re-suspended after discarding the supernatant
- To the re-suspended, 4.75ml of STE (Saline Tris EDTA) was added and then 250µl of 10%SDS (Sodium Dodecile Sulphate) was added drop wise while vortex the mixture.
- To this mixture, 10µl of proteinase K (20mg/ml) was added. Sample tubes were placed in water bath at 55°C for overnight incubation.

Day 2

- Samples were extracted with 5ml (equal quantity) of equilibrated phenol; samples were kept on ice for 10min and gentle shaking with intervals. After that centrifugation was performed for 30min at 4°C using the speed of 3200 rpm. The aqueous layer from each sample tube was removed with cut tips in to new appropriately labeled tubes.

- To the supernatant removed, 5ml of chilled chloroform-isoamylalcohol (24:1) was added. Sample tubes were shaked for 10 minutes on ice. After that centrifugation was performed for 30min at 4°C using the speed of 3200 rpm. By using cut-tips, aqueous layer was removed from each tube in to new appropriately labeled tubes.
- To this aqueous layer, 500µl of 10M ammonium acetate and 5ml of chilled isopropanol (or 10ml of chilled absolute ethanol) was added and gently shaked until DNA precipitated as visible white threads. Precipitated DNA was placed overnight at -20°C (or for 15min at -70°C).

Day 3

- Precipitated DNA samples were centrifuged at 3200 rpm for 60min at the temperature of 4°C and then the pellet was re-suspended after discarding the supernatant.
- DNA was washed with 5ml of chilled 70 % ethanol for 40 minutes at 3200rpm at 4°C.
- Then the pellet was dried after discarding the supernatant and samples were re-suspended in 10mM Tris-HCL (pH 8.0, volume of Tris added according to the size of pellet).
- Re-suspended samples were transferred into appropriately labeled eppendorf tubes. DNA samples were stored at 4°C as Stock.

DNA Dilution

Optical Densities (ODs) of stock DNA were analyzed on spectrophotometer and DNA concentrations were calculated for each stock sample. The dilution of 100 ng/µl of each stock sample was prepared for PCR amplification.

Polymerase Chain Reaction (PCR)

Genotyping was performed by PCR (polymerase chain reaction), followed by resolving the alleles on 8% non-denaturing polyacrylamide gel by electrophoresis. The PCR reaction was carried out in a total 10 µl volume with 2 µl template DNA sample from DNA dilutions, 0.3 µl of each primer (20 µM stock), 1 µl 10X PCR buffer with $MgCl_2$, 0.8 µl dNTPs (2.5mM stock) and 0.3µl of Taq DNA Polymerase (3U/µl) (BIOMATIK) in 5.3 µl PCR water. The master mix was centrifuged for 10 seconds for thorough

mixing. PCR was carried out in Gene Amp PCR system 9700 (Applied Biosystems, USA).

Thermal cycling conditions

Step 1 (1 cycle)			
Initial denaturation	95°C	×	5min
Step 2 (35 cycles)			
Denaturation	95°C	×	45sec
Annealing	55°C	×	1min
Extension	72°C	×	1min
Step 3 (1 cycle)			
Final extension	72°C	×	10min

Polyacrylamide Gel Electrophoresis (PAGE)

1. Inner plate of the gel assembly was siliconized by siliconization solution (Sigmacote).
2. Front plate was wiped with 70% ethanol.
3. After siliconization, glass plates were assembled with (0.75mm) spacers and clamps.
4. Assembled plates were fixed into base of the apparatus.
5. Gel solution (8%) recipe

De-ionized water	175 ml
40% acrylamide solution	62 ml
10X TBE buffer	25 ml

6. From the above solution, 50ml was transferred into a cylinder and 300µl of APS (Ammonium Per Sulphate) and 300µl of TEMED were added.
7. Solutions were mixed and immediately poured into the base.
8. To the remaining 200ml of solutions, 850µl of APS and 150µl of TEMED were added and mixed.

9. As the gel in the base was polymerized, plates were laid down against a Styrofoam rack in a tilled position and 200ml gel solution was poured into plate (inner) slowly and continuously avoiding air bubble formation.
10. After pouring the gel solution comb was inserted between two plates carefully not to allow any air bubble trapped under teeth.
11. Gel was allowed to polymerize overnight.
12. Next day gel plates were taken out from the base and were placed in electrophoretic tank.
13. Electrophoretic tank was filled with 1X TBE buffer and gel was allowed to pre-run at 100W for about 10-15 minutes.
14. Comb was removed from the polymerized gel.
15. To the PCR products, 5µl of loading dye was added, mixed and whole mixture were loaded into each wells.
16. In the first well DNA size ladder/marker was loaded.
17. The gel was allowed to run at 100W for about 4-5 hr depending on size of the PCR Products that were tracked by the dye.
18. After electrophoresis, gel was stained for about 1 minute in a tray containing ethidium bromide staining solution.
19. Gels were analyzed in UV-illumination for the presence / absence of alleles and then photographed and saved.

Reagents used in gel solution

1- 10X TBE

Tris Boric Acid EDTA (TBE) buffer: pH 8.2

	Mol.wt	gm/L	Final Conc
Tris (Trizma Base)	121	108	0.89 M
Borate	61.83	55	0.889 M
EDTA	372.2	7.44	0.02 M

1. 108gm Tris, 55gm Boric Acid and 7.44gm EDTA were weighted and transferred into a beaker.

2. De-ionized water was added in a beaker up to 0.8 L and dissolved on hot stirrer.
3. Volume of solution was raised up to 1 liter in a cylinder.
4. The pH of solution was adjusted at 8.03.
5. Filter and transferred to labeled bottles and stored at room temperature.

2- APS 25% (w/v)

25 grams of ammonium per sulphate was dissolved in the distilled water of amount 100ml, labeled and then stored in refrigerator.

Genotyping and Primer Database Analysis

Microsatellite markers mapped by Cooperative Human Linkage Center (CHLC) were obtained from Research Genetics, Inc. (USA). The cytogenetic locations of these markers, their heterozygosity as well as the sizes of the amplified products were obtained from LDB Genetic Map (H.H. Stassen, Psychiatric University Hospital Zurich, Switzerland).

Linkage Studies

In the current study several candidate loci were tested by typing microsatellite markers linked to these loci. The autosomal recessive RP and LCA loci initially investigated and the microsatellite markers for RP and LCA with their cytogenetic location are shown in the table 2.1 and 2.2 respectively.

Linkage to Known LCA Loci and Known RP Loci

Over 19 loci/genes are reported to be associated with arRP, and 5 loci have been reported to contain the unidentified genes affecting the individuals (den Hollander *et al.*, 2007) and 10 autosomal recessive LCA loci are mapped. To exclude all the known autosomal recessive RP and LCA loci, an initial search for linkage was carried out by using polymorphic markers mapped within these loci. Table 2.1 summarizes microsatellite markers located in the region of known RP loci, which were used as first pass analysis for genetic linkage in families with LCA. Table 2.2 summarizes microsatellite markers located in the region of known RP loci.

Linkage to RP12 Locus (*CRB1*)

For the exclusion of RP12 locus from linkage, following approach was followed:

Linkage of the arRP family to RP12 locus was investigated by typing the microsatellite markers D1S518, D1S1660 and D1S1678 of chromosome 1, mapped in the linkage interval of the locus.

Linkage to LCA4 Locus (*AIPL1*)

Linkage of the LCA family to LCA4 locus was investigated by typing the microsatellite markers D17S1308, D17S1298 and D17S974 of chromosome 17, mapped in the linkage interval of the locus.

Bioinformatics Approach

Bioinformatics approach was used to establish the record of powerful candidate genes encoded by genomic regions that showed linkage to numerous retinal dystrophies. The list of most current information and all the loci which are identified so far worldwide for the various autosomal recessive RP and LCA was obtained from RetNet and survey of latest literature (RetNet). List for arRP and LCA with unknown specific genes for autosomal recessive forms identified in the world was assembled using this web based source again. NCBI and Ensemble databases were used to assemble all the required identified genes lying with in all these listed genomic intervals, bordered by two genetic markers. List of the genes for all the specific loci acquired by using NCBI and Ensemble databases were searched for retinal gene-expression in GeneCards database. All consequent genes were checked for their expression in the retina of eye. The genes showing nil retinal expression were eliminated from consideration while the genes showing retinal expression were further analyzed by knowing their function. A gene lying with in the range of specific locus has to be involved with in the function related to retina, in order to it being considered as the candidate gene. Following this criterion we compiled the table for all the candidate genes for different loci for RP as well as LCA.

Table 2.1: Microsatellite Markers and Cytogenetic Location for Autosomal Recessive RP (arRP) Loci

Locus	Cytogenetics Location	Markers	Distance (cM)	Type	Product Size
RP20/L CA2	1p31	D1S1665	90.8	4	227
RP19	1p21-p13	D1S1588	102.2	3	130
		D1S1631	107.0	3	144.5
		D1S206	106.3	2	212
		D1S3723	107.2	4	177
RP12	1q31-q32.1	D1S518	194.8	4	208
		D1S1660	204.6	4	234
		D1S1678	211.8	4	305
	1q41	D1S1663	223.9	4	417
		D1S549	239.5	4	179
		D1S213	243.0	2	114
		D1S3462	246.2	3	258.5
RP28	2p11.2-p15	D2S2739	62.6	4	305
		D2S1394	72.7	4	168
	2q14.1	D2S436	105.8	4	193
		D2S1790	96.7	4	302
		D2S410	125.3	4	170
		D2S1328	141.4	4	164
		D2S286	78.6	2	147
RP26	2q31.2-q32.3	D2S1776	168.1	4	298
		D2S364	191.7	2	327
	2q37.1	D2S427	247.9	4	253
		D2S396	245.8	2	237
RP4	3q21-q24	D3S2460	136.6	4	159
		D3S1292	144.3	2	154
		D3S1764	150.2	4	241
CSNB3	4p16.3	D4S412	4.1	2	243
		D4S2366	7.5	4	132
		D4S403	19.5	2	244

	4p12-cen	D4S1627	47.2	4	185
		D4S3248	61.7	4	245
RP29	4q32-q34	D4S1625	144.1	4	190
		D4S1629	148.0	4	149
		D4S2368	150.9	4	316
		D4S2431	159.5	4	246
	4q31	SAME AS ABOVE			
	5q31.2-q34	D5S1480	151.3	3	228.5
		D5S820	166.5	4	198
RP14	6p21.3	GGAA15	38.4	4	211
		B08	39.1	4	234
		D6S1019			
RP25	6q14-q21	D6S240	71.9	2	155
		D6S1053	67.0	4	308
		D6S1031	70.9	3	258.5
	10q23	D10S2327	82.7	4	212
		D10S677	96.1	4	206
RP27	14q11.1-q11.2	D14S608	31.7	4	206
		D14S599	41.0	3	91.5
		D14S742	26.2	4	405
		D14S1280	31.0	4	295
	15q23	D15S643	59.6	4	209
		D15S211	86.4	2	234
	15q26	D15S655	94.3	3	243
		D15S652	100.6	3	295.5
		D15S816	104.0	4	138
		D15S657	104.6	4	346
		D15S642	106.0	4	203
RP22	16p12.3-p12.1	D16S764	13.4	4	106
		D16S403	30.3	2	143
	16q13	D16S3253	65.0	4	183
		D16S2620	69.4	4	274

Table 2.2: Microsatellite Markers and Cytogenetic Location for LCA Loci.

Locus	Cytogenetics Location	Markers	Distance (cM)	Type	Product Size
LCA1	17p13.1	D17S1298	6.2	4	252
		D17S974	12.3	4	205
		D17S1303	12.7	4	235
		D17S938	8.4	2	173
LCA10	12q21.32	D12S1052	87.7	4	157
		D12S1064	94.6	4	185
		D12S351	95.0	2	160
		D12S375	82.7	4	176
		D12S1300	102.0	4	125
		D12S326	88.9	2	249
LCA2	1p31	D1S1665	90.8	4	227
LCA3	14q24	D14S592	63.5	3	231
		D14S588	71.0	4	129
LCA4	17p13.1	D17S1308	2.1	4	306
		D17S1298	6.2	4	252
		D17S974	12.3	4	205
LCA5	6q11-q16	D6S1053	67.0	4	308
		D6S1031	70.9	3	258.5
LCA6	14q11	D14S742	26.2	4	405
		D14S1280	31.0	4	295
		D14S608	31.7	4	206
LCA7	1q31-q32.1	D1S518	194.8	4	238
		D1S1660	204.6	4	234
		D1S1668	245.3	4	179
		D1S413	204.1	2	254
		D1S1656	245.0	4	145
		D1S1678	211.8	4	305
		D1S238	195.5	2	287
LCA8	19q13.3	D19S178	48.5	2	167
		D19S246	57.8	2	207
		D19S589	59.6	4	173
		D19S433	37.8	4	207

		D19S418	64.2	2	87
		D19S420	48.8	2	259
		D19S220	45.1	2	274
LCA9	1p36	D1S468	2.5	2	182
		D1S1612	4.7	4	112
		D1S1597	9.1	4	169
		D1S3669(G	16.1	4	195
		ATA29A06)			
		D1S552			
			22.6	4	
	14q23.3	D14S592	63.5	3	231
		D14S588	71.0	4	129

RESULTS

Inherited retinal degenerations show genetic as well as clinical heterogeneity. The premature death of the rod and cone photoreceptors causes the loss of vision in various diseases in this class (Rivolta *et al.,* 2002). Showing an estimated occurrence of about 1 in 3000 to 1 in 5000, these represent the most common forms of human visual mutilation collectively (Bunker *et al.,* 1984). Over 40 loci for human hereditary retinal degenerations involving the photoreceptors and retinal pigment epithelium are known (Morimura *et al.,* 1998). Among the peripheral retinal dystrophies, RP is the most prevalent kind of hereditary retinal dystrophies. LCA, showing an autosomal recessive inheritance form, is the harshest type of retinal dystrophies (Alstrom CH, 1957). In this case both rods and cones are dead at birth or early in infancy (Francois J, 1968).

In the consanguineous families affected with autosomal recessive RP, the affected individuals must show homozygous pattern for the regions linked to disease causing loci (kondo *et al.*, 2004). Recessive RP is the most important form of RP as it accounts for approximately 70% of all RP cases (Jay M, 1982). There are no distinguished clinical differences between arRP, adRP or xlRP (X-linked RP). The arRP usually shows clinical similarities with other forms of retinal degenerations like early-onset RP and LCA (Lebers congenital amaurosis). Autosomal recessive RP also shows extensive genetic heterogeneity. Over 19 loci/genes are reported to be associated with arRP. Among these loci 5 are reported to contain the unidentified genes affecting the individuals (den Hollander *et al.*, 2007). The most genes for arRP cause 1% cases or even less than that, so considered as rare, however retinal pigment epithelium-specific 65kD protein (*RPE65*), rod cGMP phosphodiestrase alpha and beta subunit (*PDE6A and PDE6B*) are considered as the common genes as these cover 2-5% cases of all the cases for arRP. *USH2A* gene is the gene causing both Usher syndrome and arRP. About 5% of arRP cases are accounted by this gene. There are few examples for genes' mutations being the frequent cause of autosomal recessive RP such as, *RP25* is involved in 10-20% of arRP in Spain and rarely associated with the disease anywhere else in the world. Similarly, mutations in nuclear receptor subfamily 2 group E3 (*NR2E3*) are only reported in Sephardic Jews in Portugal (Bader *et al.*, 2003).

LCA is collection of such states which leads to early onset retinal dystrophy, which over time may be accompanied by pigmentary changes in the retina (Hooser *et al.*, 2002). It represents the most frequently occurring genetic causes of congenital visual impairment in children along with infants. The mutations with in the gene that encodes *RPE65* are the cause of about 10% cases of the all LCA cases (Pang *et al.*, 2005). LCA patients are diagnosed usually at the time of birth or during initial months of life, with extremely damaged eye vision or the complete blindness. The pathophysiology of the disease LCA is little known. It is considered as a result of either defect in the photoreceptor cells' development or deterioration of normally developed cells at very initial stage (Perrault *et al.*, 1999). LCA accounts for about 5% of all inherited retinopathies and responsible for *10-18%* of congenital blindness (Graw J, 2003). LCA phenotype is also reported to be associated with many syndromic anomalies of the renal, cardiac, skeletal and central nervous system. Among the associated symptoms, mental retardation seems to be an important feature. It is found in around 20% of the LCA population (Fazzi *et al.*, 2003).

Autosomal recessive retinal dystrophies are relatively more common in those geographical areas where strong caste system acts as strong social bonds and produced solidarity in such society with high consanguinity, which makes genetic linkage analysis, the method of choice for gene identification. Homozygosity mapping is based on the assumption that a mutation is inherited from both parents because of recessive mode of inheritance. This method efficiency maps recessive conditions in consanguineous families. Pakistani population is an interesting one because of close relative marriage and particularly in first cousin marriages, which are preferred here.

Description of the Families Studied

Family A

Family A affected with LCA was located in Attock. After a careful investigation about the history of the family, a genetic pedigree was drawn. The family history presented in the pedigree (Figure 3.1) indicates four generations consisting of 35 members out of whom six are affected. These affected members developed the disease symptoms few years after birth that is why LCA is called congenital blindness. According to pedigree analysis disease appeared in fourth generation through parents and grand parents that

appeared phenotypically normal, suggests that the trait is transmitted in autosomal recessive manner. Through detailed discussion relating to medical history of the individuals and family relationships a conclusion was made that LCA is not caused because of the environmental factors and infections. The samples of blood were taken from all the participating family individuals.

Family B

Family B was located in Jehlum and was affected with autosomal recessive RP. After a careful investigation about the history of the family, a genetic pedigree was drawn. The family history presented in the pedigree (Figure 3.33) indicates four generations consisting of twenty four members out of whom five are affected. According to pedigree analysis disease appeared in third generation through parents that appeared phenotypically normal, suggests that the trait is transmitted in autosomal recessive manner. Through detailed discussion relating to medical history of the individuals and family relationships it was concluded that there was no possibility of the environmental factors and infections as a cause of arRP. The affected persons are blind since the age of about 11 to 12 years. The samples of blood were taken from all the participating family individuals.

Linkage Studies

Already known autosomal recessive RP and LCA loci mentioned in table 2.1 and table 2.2, were tested through homozygosity mapping. For this purpose microsatellite markers were used in the candidate intervals. Information about genetic location of recessive retinal dystrophies was obtained from RetNet database of human eye disorders (RetNet). Lists for microsatellite markers used for exclusion mapping are shown in table 2.1 and 2.2. The microsatellite markers were analysed using a model PCR reaction and electrophoresis on the 8% non-denaturing polyacrylamide gel as discussed in Materials and Methods. This gel was stained with ethidium bromide in order to visualize the amplified PCR products, and then the close visual examination was performed to assign genotypes.

In family A (Figure 3.1), eight DNA samples including four affected and four normal individuals were used for genotyping. Several LCA loci were tested for linkage. Analysis of genotyping results indicates that family A was not linked to any locus and thus family A was excluded.

In family B (Figure 3.33), six DNA samples including two affected and four normal were used for genotyping. Several arRP loci were tested for linkage. Analysis of genotyping results showed that family B was linked to locus RP19. DNA analysis with polymorphic microsatellite markers linked to RP19 candidate linkage interval revealed that markers D1S3723 (Figure 3.34), D1S1631 (Figure 3.35) and D1S206 (Figure 3.35) were homozygous in all affected individuals but heterozygous in normal, thus establishing convincing linkage of family B to RP19 locus containing *ABCA4* on chromosome 1p21-p13.

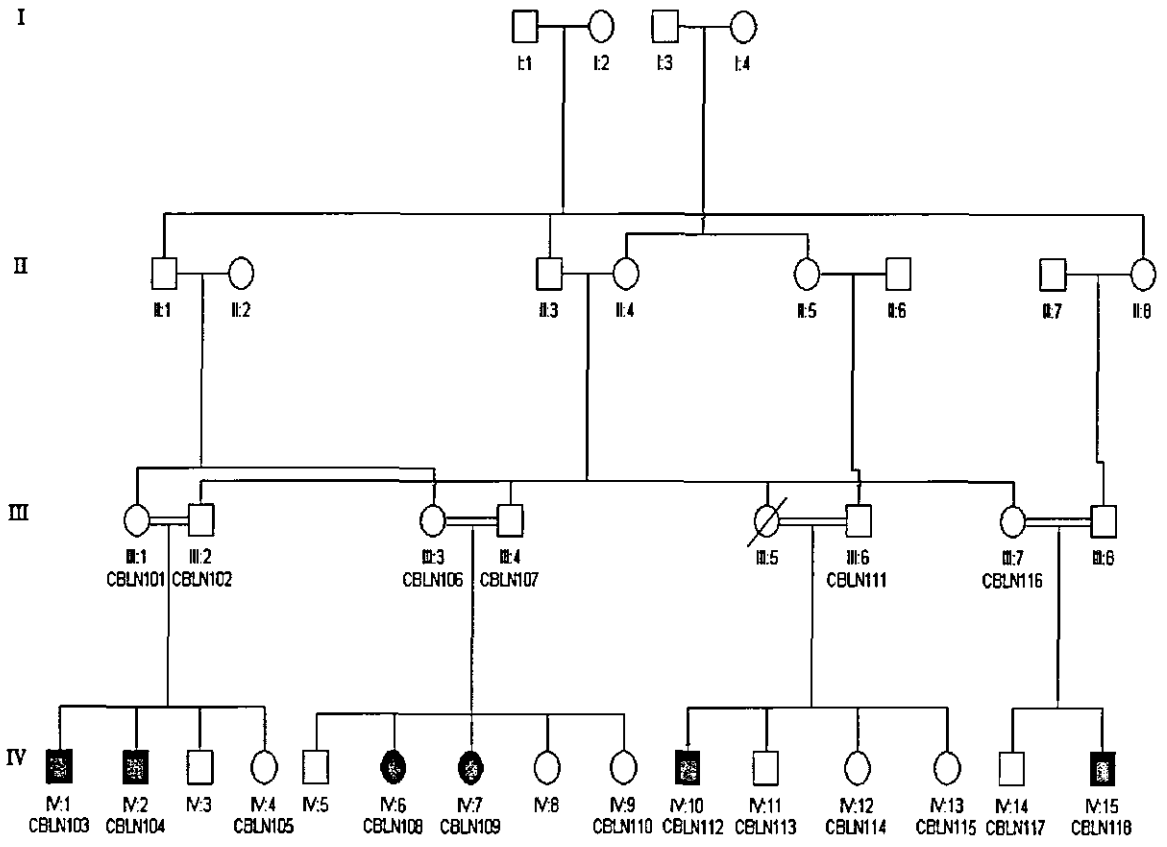
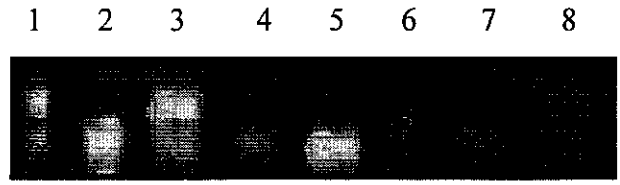


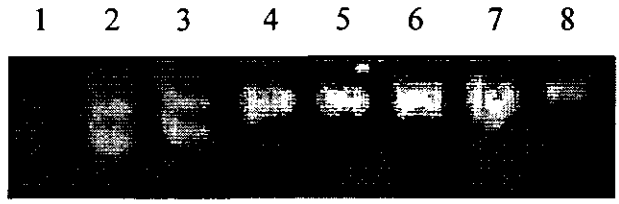
Figure 3.1: Pedigree of family A with autosomal recessive congenital blindness (CBLN). Circles and squares represent females and males, respectively. Filled circles and squares represent affected individuals. Double lines indicate cousin marriages.

Family A



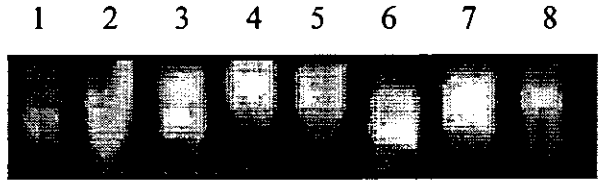
Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.2: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D17S974 at 12.3 cM on chromosome 17p13.1 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



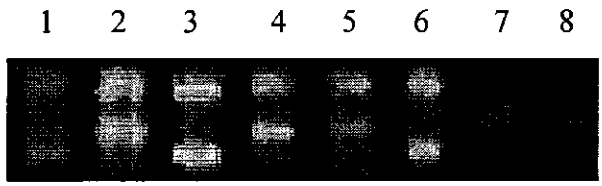
Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.3: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D17S1303 at 12.7 cM on chromosome 17p13.1 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



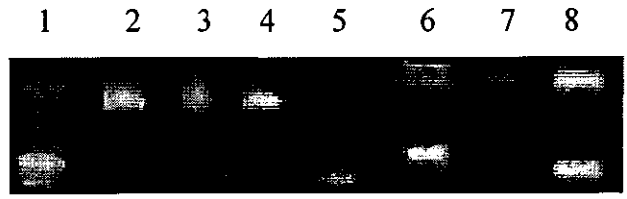
Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.4: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D1S1665 at 90.8 cM on chromosome 1p31 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



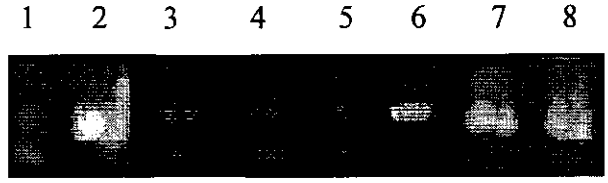
Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.5: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D14S592 at 63.5 cM on chromosome 14q24 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



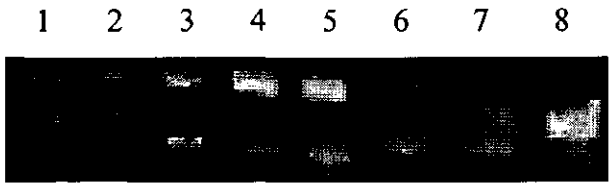
Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.6: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D17S974 at 12.3 cM on chromosome 17p13.1 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



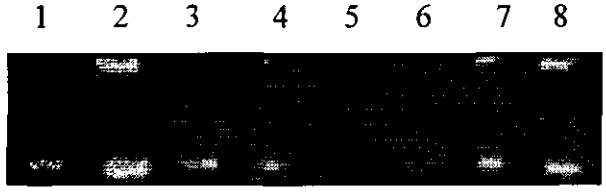
Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.7: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D17S1298 at 6.2 cM on chromosome 17p13.1 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



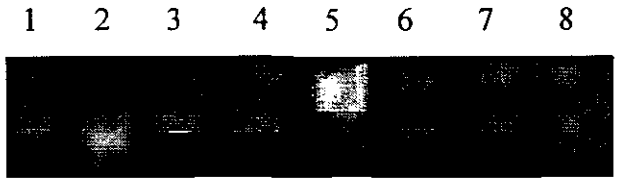
Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.8: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D17S1308 at 2.1 cM on chromosome 17p13.1 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



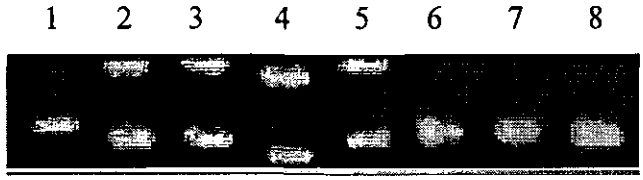
Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.9: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D6S1053 at 67.0 cM on chromosome 6q11-16 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



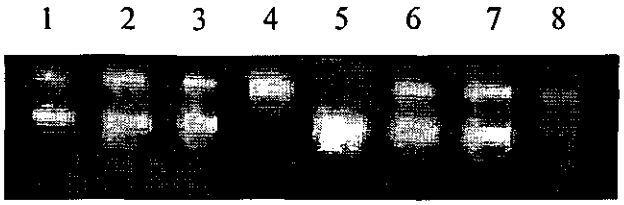
Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.10: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D6S1031 at 70.9 cM on chromosome 6q11-16 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



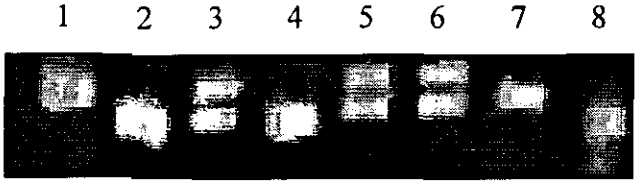
Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.11: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D14S742 at 26.2 cM on chromosome 14q11 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



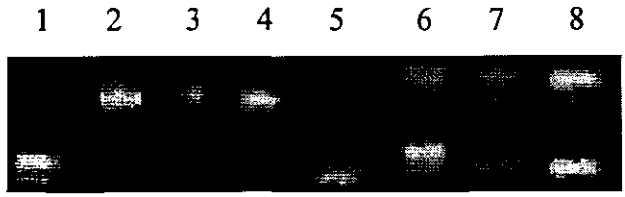
Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.12: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D14S608 at 31.7 cM on chromosome 14q11 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



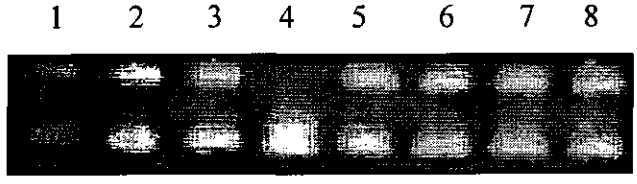
Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.13: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D1S1660 at 204.6 cM on chromosome 1q31-32.1 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



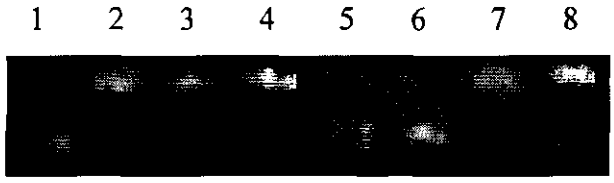
Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.14: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D1S238 at 195.5 cM on chromosome 1q31-32.1 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



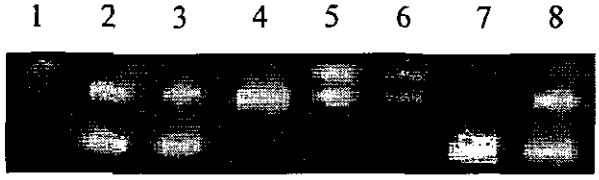
Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.15: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D1S413 at 204.1 cM on chromosome 1q31-32.1 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



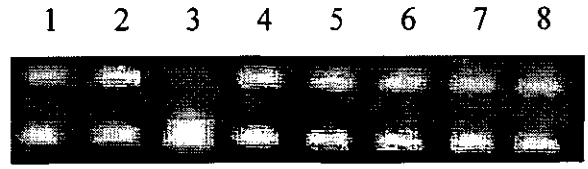
Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.16: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D1S1678 at 211.8 cM on chromosome 1q31-32.1 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



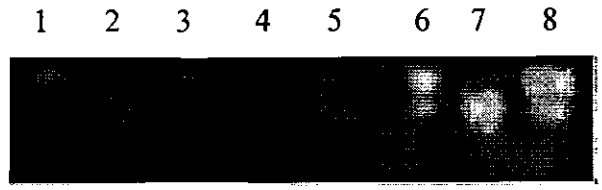
Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.17: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D19S178 at 48.5 cM on chromosome 19q13.3 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



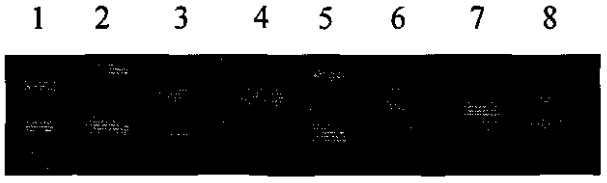
Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.18: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D19S246 at 57.8 cM on chromosome 19q13.3 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



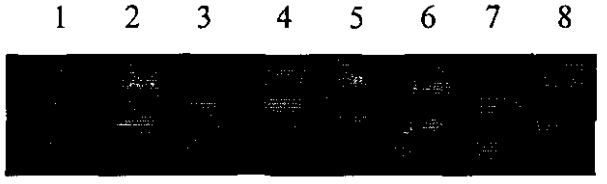
Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.19: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D19S418 at 64.2 cM on chromosome 19q13.3 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



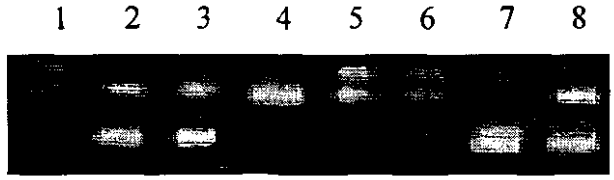
Lane 1. III-1 Lane 5. III-3
Lane 2. IV-1 Lane 6. IV-6
Lane 3. IV-2 Lane 7. IV-7
Lane 4. IV-3 Lane 8. IV-8

Figure 3.20: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D19S433 at 37.8 cM on chromosome 19q13.3 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



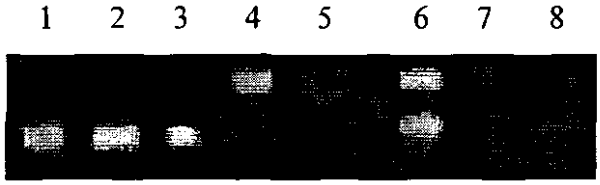
Lane 1. III-1 Lane 5. III-3
Lane 2. IV-1 Lane 6. IV-6
Lane 3. IV-2 Lane 7. IV-7
Lane 4. IV-3 Lane 8. IV-8

Figure 3.21: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D19S220 at 45.1 cM on chromosome 19q13.3 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



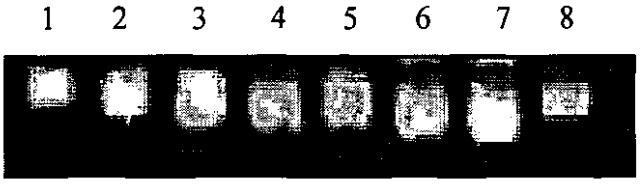
Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.22: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D19S420 at 48.8 cM on chromosome 19q13.3 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.23: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D1S1612 at 4.7 cM on chromosome 1p36 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.

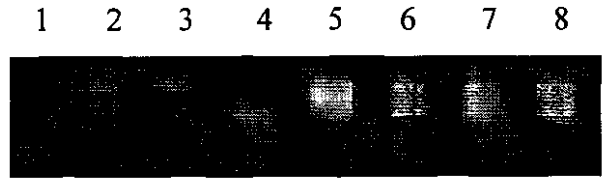


Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.24: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D1S3669 at 16.1 cM on chromosome 1p36 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.

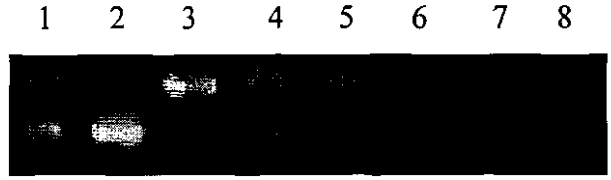
Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.25: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D1S1597 at 9.1 cM on chromosome 1p36 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



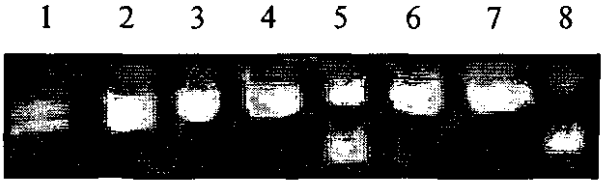
Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.26: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D1S552 at 22.6 cM on chromosome 1p36 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



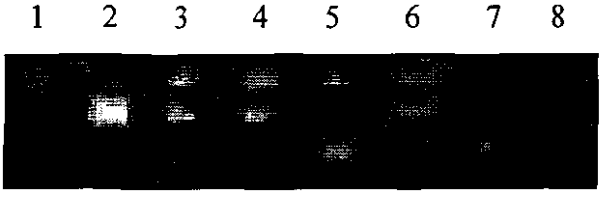
Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.27: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D12S1052 at 87.7 cM on chromosome 12q21.32 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



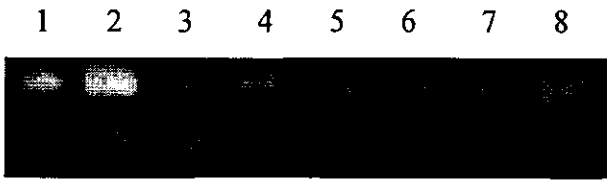
Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.28: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D12S1300 at 102.0 cM on chromosome 12q21.32 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



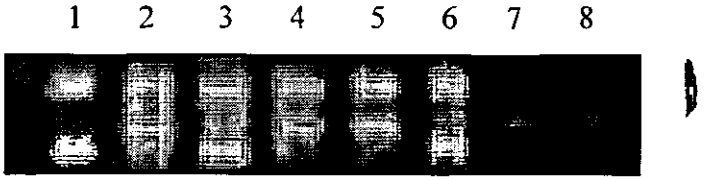
Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.29: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D12S375 at 82.7 cM on chromosome 12q21.32 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



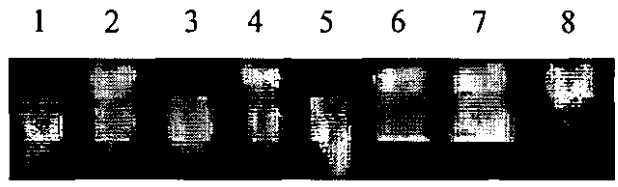
Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.30: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D12S326 at 88.9 cM on chromosome 12q21.32 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.31: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D12S351 at 95.0 cM on chromosome 12q21.32 showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



Lane 1. III-1
Lane 2. IV-1
Lane 3. IV-2
Lane 4. IV-3
Lane 5. III-3
Lane 6. IV-6
Lane 7. IV-7
Lane 8. IV-8

Figure 3.32: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D14S588 at 71.0 cM on 14q23.3 chromosome showing heterozygosity among the affected individuals (IV-1, IV-2, IV-6, IV-7) and normal individuals (III-1, III-3, IV-3, IV-8) of family A. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.

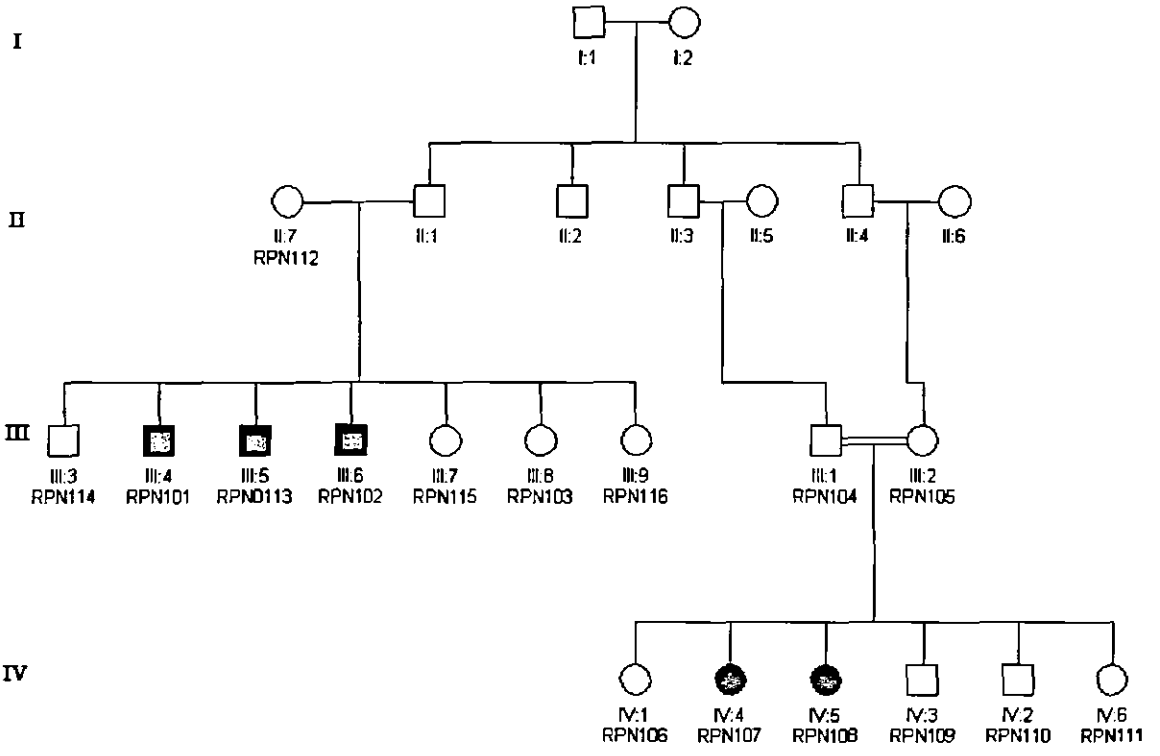
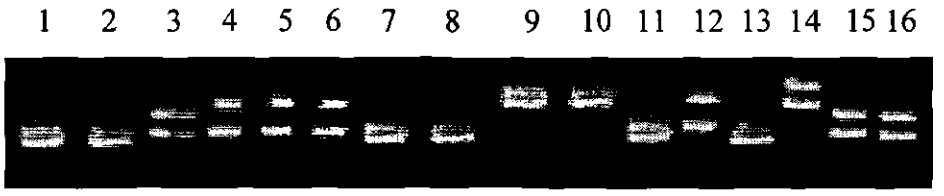


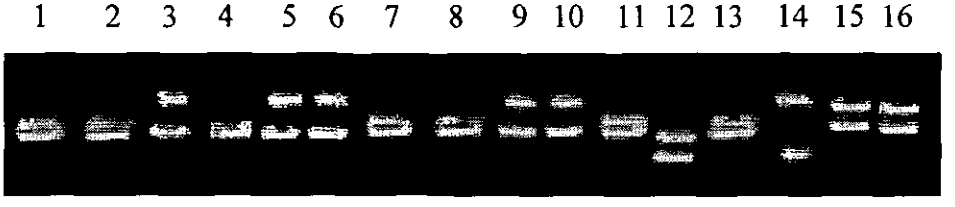
Figure 3.33: Pedigree of family B with autosomal recessive retinitis pigmentosa. Circles and squares represent females and males, respectively. Filled circles and squares represent affected individuals. Double lines indicate cousin marriages.

Family B



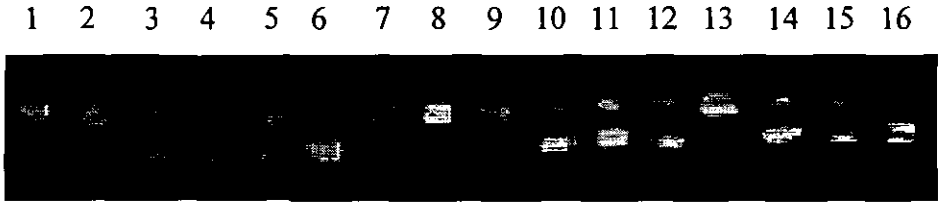
Lane 1. III-2	Lane 5. III-9	Lane 9. IV-4	Lane 13. III-3
Lane 2. III-4	Lane 6. IV-1	Lane 10. IV-5	Lane 14. III-1
Lane 3. III-6	Lane 7. IV-2	Lane 11. IV-6	Lane 15. III-5
Lane 4. III-8	Lane 8. IV-3	Lane 12. II-1	Lane 16. III-7

Figure 3.34: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D1S3723 at 107.2 cM on chromosome 1p21-p13 showing homozygosity among the affected individuals (III-2, III-3, III-4, IV-2, IV-3) and normal individuals (II-1, III-1, III-5, III-6, III-7, III-8, III-9, IV-1, IV-4, IV-5, IV-6) of family B. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



Lane 1. III-2	Lane 5. III-9	Lane 9. IV-4	Lane 13. III-3
Lane 2. III-4	Lane 6. IV-1	Lane 10. IV-5	Lane 14. III-1
Lane 3. III-6	Lane 7. IV-2	Lane 11. IV-6	Lane 15. III-5
Lane 4. III-8	Lane 8. IV-3	Lane 12. II-1	Lane 16. III-7

Figure 3.35: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D1S1631 at 107.0 cM on chromosome 1p21-p13 showing homozygosity among the affected individuals (III-2, III-3, III-4, IV-2, IV-3) and normal individuals (II-1, III-1, III-5, III-6, III-7, III-8, III-9, IV-1, IV-4, IV-5, IV-6) of family B. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.



Lane 1. III-2	Lane 5. III-9	Lane 9. IV-4	Lane 13. III-3
Lane 2. III-4	Lane 6. IV-1	Lane 10. IV-5	Lane 14. III-1
Lane 3. III-6	Lane 7. IV-2	Lane 11. IV-6	Lane 15. III-5
Lane 4. III-8	Lane 8. IV-3	Lane 12. II-1	Lane 16. III-7

Figure 3.36: Gel Electropherogram of ethidium bromide stained 8% non-denaturing polyacrylamide gel for marker D1S206 at 106.3 cM on chromosome 1p21-p13 showing homozygosity among the affected individuals (III-2, III-3, III-4, IV-2, IV-3) and normal individuals (II-1, III-1, III-5, III-6, III-7, III-8, III-9, IV-1, IV-4, IV-5, IV-6) of family B. The Roman numerals indicate generation of the individuals within pedigree while Arabic numerals indicate their positions within generation.

Results of Bioinformatics Approach

The candidate genes for the autosomal recessive retinitis pigmentosa as well as LCA phenotypes, worldwide, were established following bioinformatics scheme that showed series of results as follow.

All loci with unknown specific genes for autosomal recessive RP and LCA forms found worldwide were identified using RetNet database of inherited retinal diseases (Table 3.2). All genes within each locus were identified using NCBI and Ensemble. After it we used the human database of gene expression, GeneCards database to observe the status of genes' retinal expression for the first locus (Table 3.3). Then certain genes having nil retinal expression at the same locus were eliminated from the candidate list and the genes showing retinal expression were analysed for their aliases, functions and product or cellular localization (Table 3.4). After that all remaining loci were analysed following the same approach (Table 3.5-3.10).

After characterization on the basis of gene's retinal expression and the function involved in retina of the eye, it has been concluded that *VAX2* (OMIM# 604295) located at 2p13, *LRAT* (OMIM# 6048663) located at 4q31, *NR2E1* (OMIM# 603849) located at 6q21 and ELOVL4 (OMIM# 605512) located at 6q14 are the candidate genes having the disorder of early-onset severe retinal dystrophy. The detail of these candidate genes is also mentioned (Table 3.11). Then homologous species and the percent similarity to these human genes were identified (Table 3.12). The percent similarity is followed either by (n) where the comparison was based on nucleic acid or (a) for amino acid based comparison. Finally the candidate genes are graphically represented, showing their intensity of retinal expression (Figure 3.37). Exactly the same process was performed for LCA loci and genes; resultantly one candidate gene from the input list was established.

Table 3.1: List of Autosomal Recessive RP and LCA Loci

Locus Name	Location	Locus Name	Location	Locus Name	Location
RP28	2p15-p11	RP25	6q14-q21	RP29	4q32-q34
RP22	16p12.3-p12.1	---	---	---	---
LCA3	14q24	LCA5	6q11-q16	LCA9	1p36

Table 3.2: List of Genes Located at RP28 (2p15-p11), Present in GeneCards Database, Showing their Status of Retinal Expression

Gene/ Symbol	Retinal Expression	Gene/ Symbol	Retinal Expression
ANKRD53	Yes	FLJ43987	Unknown
BMP10	Nil	IGKV1-27	Nil
C2orf13	Nil	IGKV1-33	Nil
C2orf7	Yes	IGKV1-35	Nil
CEP68	Yes	IGKV1-37	Nil
COMMD1	Yes	IGKV1-39	Nil
DCTN1	Yes	IGKV1-6	Nil
IGKV1D-13	Nil	IGKV6-21	Nil
IGKV2-18	Nil	LOC51057	Yes
IGKV2D-10	Nil	LOC642678	Nil
IGKV2D-19	Nil	LOC644838	Nil
IGKV3-31	Nil	LOC647112	Nil
IGKV3D-34	Nil	LOC654342	Yes
IGKV6-21	Nil	LOC729854	Nil
LOC100129991	Nil	LOC730198	Nil
LOC100130782	Nil	LOC732417	Nil
LOC100131592	Nil	MDH1	Yes
LOC100131818	Nil	NAT8B	Yes
LOC100132300	Nil	OR7E62P	Nil
LOC100132331	Nil	PLGLB2	Yes
LOC129293	Yes	POLE4	Nil
LOC388955	Nil	PPP3R1	Yes
LOC402076	Nil	PROKR1	Nil
IGKV1D-13	Nil	RGPD1	Yes
IGKV2-18	Nil	ST3GAL5	Yes
IGKV2D-10	Nil	TLX2	Yes
IGKV2D-19	Nil	VAMP8	Yes
IGKV3-31	Nil	VAX2	Yes
IGKV3D-34	Nil	ZNF638	Yes

Table 3.3: List of Genes Located at RP28 (2p15-p11) with their Aliases, Functions and Product/ Localization in the Cell Showing Retinal Expression

Gene/ Symbol	Aliases	Function	Product/ Cellular Localization
ANKRD53	FLJ12056 FLJ36160	Unknown	unknown
C2orf7	MGC13004 PAP21	Unknown	unknown
CEP68	FLJ25920 FLJ36750	Unknown	unknown
COMMD1	C2orf5 MGC27155 MURR1	Inhibits TNF-induced NFKB1 activation. May function to facilitate biliary copper excretion within hepatocytes	unknown
DCTN1	DAP-150 DP-150 HMN7B P135	Necessary for dynein-driven retrograde translocation of organelles and vesicles along the microtubules, with in the cytoplasm	Unknown
LOC129293	FLJ46467	Unknown	Unknown
LOC51057	DKFZp686C1 2204	Unknown	Unknown
LOC654342	Nil	Unknown	Unknown
MDH1	EC 1.1.1.37 MDH-s MGC:1375 MOR2	It catalytically oxidises malate to oxaloacetate reversibly using system of NAD/NADH cofactor in the citric acid cycle in the cell	Cytoplasm
NAT8B	CML2 Hcml2 NAT8BP	May play a role in regulation of gastrulation	Unknown

PLGLB2	PLGL PLGP1 PRGB	It attaches to the lysine binding sites noncovalently located with in the plasminogen and may result in the localization of action at those places which areessential for destruction of the extracellular matrix	Outside the cell membrane
RGPD1	RANBP2L6 RGP1	Unknown	Unknown
ST3GAL5	EC 2.4.99.9 SIAT9 SIATGM3S	Catalyzes the formation of ganglioside GM3	Golgi apparatus
TLX2	Enx HOX11L1 NCX	Unknown	Unknown
VAMP8	EDB Endobrevin VAMP-8 endobrevin	It focuses the movement of vesicles to their target membrane with the secretion of dense granules in the thrombocytes. It is essential for pancreatic cells' secretion of various enzymes	Perinuclear vesicular structures
VAX2	DRES93	This gene is crucially important for the development of eye; especially it specifies the ventral optic vesicle with in the human eye. The gene may be a regulator of axial polarization in the retina of the eye. In mouse studies, this gene was found to be required for the correct formation of the optic fissure and other aspects of retinal development.	This gene encodes a homeobox protein and shows the absolute expression in the retina's ventral part while eye is being developed
ZNF638	MGC26130 NP220 ZFML Zfp638	Binds to cytidine clusters in double-stranded DNA	Nucleoplasm

Table 3.4: List of Genes Located at RP29 (4q32-q34), Present in GeneCards Database, Showing their Status of Retinal Expression

Gene/ Symbol	Retinal Expression	Gene/ Symbol	Retinal Expression
AADAT	Yes	LOC338095	Unknown
ACCN5	Nil	LOC391707	Nil
ADAM29	Nil	LOC391710	Nil
AGA	Yes	LOC391711	Nil
ANXA10	Yes	LOC391713	Nil
ASB5	Nil	LOC391718	Nil
C4orf18	Yes	LOC391719	Nil
C4orf27	Yes	LOC402192	Nil
C4orf39	Yes	LOC402193	Nil
CBR4	Yes	LOC441050	Nil
CLCN3	Yes	LOC441052	Nil
CPE	Yes	LOC642544	Nil
CTSO	Yes	LOC646890	Nil
DCHS2	Nil	LOC646954	Nil
DDX60L	Nil	LOC646966	Nil
FBXO8	Yes	LOC646995	Nil
FTHP2	Nil	LOC653794	Nil
GALNT7	Yes	LOC727835	Nil
GK3P	Nil	LOC727929	Nil
GLRA3	Nil	LOC728081	Nil
GPM6A	Yes	LOC729725	Nil
GRIA2	Yes	LOC729743	Nil
GUCY1B3	Yes	LOC729902	Nil
HADHAP	Nil	LOC92345	Unknown
HAND2	Yes	LRAT	Yes
HMGB2	Yes	MAP9	Yes
HPGD	Nil	MFAP3L	Yes
HSP90AA6P	Nil	MIRN578	Unknown
KIAA1450	Unknown	MORF4	Yes
KIAA1712	Yes	NACA3P	Nil
KLHL2	Yes	NBLA00301	Nil
LOC100128050	Nil	NEIL3	Nil
LOC100128186	Nil	NEK1	Yes
LOC100128187	Nil	NPY1R	Yes
LOC100128266	Nil	PALLD	Yes
LOC100128700	Nil	PLRG1	Yes
LOC100128824	Nil	RAPGEF2	Yes
LOC100129857	Nil	RBM46	Yes
LOC100129957	Nil	RXFP1	Nil
LOC100130244	Nil	SAP30	Yes
LOC100131135	Nil	SC4MOL	Nil
LOC100131276	Nil	SCRG1	Yes
LOC100131470	Nil	SH3RF1	Yes
LOC100131553	Nil	SPATA4	Yes
LOC100132013	Nil	SPCS3	Nil
LOC100132326	Nil	TDO2	Nil

LOC100132746	Nil	TMEM192	Nil
LOC100132889	Nil	TRIM60	Yes
LOC100132922	Nil	TRIM61	Nil
LOC100133261	Nil	TRIM75	Nil
LOC133332	Nil	VEGFC	Yes
LOC201725	Unknown	WDR17	Yes
LOC285501	Nil		

Table 3.5: List of Genes Located at RP29 (4q32-q34) with their Aliases, Functions and Product/ Localization in the Cell Showing Retinal Expression

Gene/ Symbol	Aliases	Function	Product/ Cellular Localization
AADAT	AadAT EC2.6.1.7 KAT2 KATII	It controls the activity of tryptophan, hydroxykinurenine and aspartate	Unknown
AGA	AGU ASRG EC 3.5.1.26 GA	Involved in the catabolism of N-linked oligosaccharides of glycoproteins. It cleaves asparagine from N-acetylglucosamines as one of the final steps in the lysosomal breakdown of glycoproteins	Lysosome
ANXA10	ANX14 Annexin-10 Annexin-14	Unknown	A member of the annexin family. Members of this calcium-dependent phospholipid-binding protein family play a role in the regulation of cellular growth and in signal transduction pathways
C4orf18	AD021 AD036 FLJ23966	Unknown	Unknown
C4orf27	FLJ20534 FLJ33423 FLJ42042	Unknown	Unknown

C4orf39	FLJ31659	Unknown	Unknown
CBR4	EC 1.1.1 FLJ14431 SDR45C1	Unknown	Unknown
CLCN3	CLC3 ClC-3	Regulation of cell volume; membrane potential stabilization, signal transduction and transepithelial transport. help neuronal cells to establish short-term memory	Unknown
CPE	CPH EC 3.4.17.10	Removes residual C-terminal Arg or Lys remaining after initial endoprotease cleavage during prohormone processing. Processes proinsulin	Peripheral membrane protein
CTSO	CTSO1 EC 3.4.22.42	Proteolytic enzyme possibly involved in normal cellular protein degradation and turnover	Cysteine proteinase
FBXO8	DC10 FBS FBX8	May promote guanine-nucleotide exchange on an ARF. Promotes the activation of ARF through replacement of GDP with GTP	A member of the F-box protein family
FTHP2	Unknown	Unknown	Unknown
GALNT7	EC 2.4.1 GALNAC-T7 GalNAc-T7 GalNAcT7	Involved in O-linked oligosac-charide biosynthesis, which catalyzes the transfer of an N-acetyl-D-galactosamine residue to an already glycosylated peptide	Type II transmembrane protein (enzyme)
GK3P	Unknown	Unknown	Unknown
GLRA3	Unknown	Unknown	Unknown
GPM6A	M6A M6a	Unknown	unknown
GRIA2	GLUR2 GLURB GluR-2 GluR-K2	L-glutamate induces a conformation change, leading to the opening of the cation channel, and thereby converts the chemical signal to an electrical	Glutamate receptors, mammalian brain

	HBGR2	impulse. The receptor then desensitizes rapidly and enters a transient inactive state	
GUCY1B3	EC 4.6.1.2 GC-S-beta-1 GC-SB3 GCS-beta-3 GUC1B3 GUCB3 GUCSB3 GUCY1B1	Unknown	Soluble guanylate cyclase (sGC), a heterodimeric protein consisting of an alpha subunit and a beta subunit
HAND2	DHAND DHAND2 FLJ16260 Hed MGC125303 Thing2	Essential for cardiac morphogenesis, particularly for the formation of the right ventricle and of the aortic arch arteries	HAND proteins, are asymmetri-cally expressed in the developing ventricular chambers
KIAA1450	Unknown	Unknown	Unknown
KIAA1712	PS1TP3	Unknown	Unknown
KLHL2	ABP-KELCH MAV MAYVEN	May play a role in organizing the actin cytoskeleton of the brain cells	unknown
LRAT	EC 2.3.1.135 MGC33103	Plays a critical role in vision. It provides the all-trans retinyl ester substrates for the isomerohydrolase which processes the esters into 11-cis-retinol in the retinal pigment epithelium; due to a membrane-associated alcohol dehydrogenase, 11 cis-retinol is oxidized and converted into 11-cis-retinaldehyde which is the chromophore for rhodopsin and the cone photopigments	Microsomal enzyme that catalyzes the esterification of all-trans-retinol into all-trans-retinyl ester, an essential reaction for the retinoid cycle in visual system and vitamin A status in liver.

			Mutations in this gene have been associated with early-onset severe retinal dystrophy
MAP9	ASAP FLJ21159 OTTHUMP0 0000196622	Involved in organization of the bipolar mitotic spindle. Required for bipolar spindle assembly, mitosis progression and cytokinesis. May act by stabilizing interphase microtubules	Microtubule-associated protein
MFAP3L	KIAA0626 NYD-sp9	Unknown	unknown
MIRN578	Unknown	Unknown	Unknown
MORF4	CSR CSRB MRG15 SEN SEN1	Component of the NuA4 histone acetyltransferase (HAT) complex which is involved in transcriptional activation of select genes principally by acetylation of nucleosomal histone H4 and H2A	Unknown
NEK1	EC 2.7.11.1 KIAA1901 MGC138800 NY-REN-55	Phosphorylates serines and threonines, but also appears to possess tyrosine kinase activity. Implicated in the control of meiosis	unknown
NPY1R	NPY1-R NPYR NPYY1	Psychomotor activity, food intake, regulation of central endocrine secretion, and potent vasoactive effects on the cardiovascular system	One of the most abundant neuropeptides in the mammalian nervous system
PALLD	CGI-151 FLJ22190 KIAA0992 PNCA1 SIH002	Roles in establishing cell morphology, motility, cell adhesion and cell-extracellular matrix interactions in a variety of cell types	Palladin, a component of actin-containing microfilaments
PLRG1	MGC110980 PRL1	Necessary for spliceosome assembly and for pre-mRNA splicing	Unknown

RAPGEF2	CNrasGEF KIAA0313 NRAPGEP PDZ-GEF1 RA-GEF	Serve as RAS activators by promoting acquisition of GTP to maintain the active GTP-bound state and are the key link between cell surface receptors and RAS activation	Member of the RAS subfamily of GTPases
RBM46	CT68 MGC27016	Unknown	Unknown
SAP30	Nil	Involved in the functional recruitment of the Sin3-histone deacetylase complex (HDAC) to a specific subset of N-CoR corepressor complexes	A component of the histone deacetylase complex, which includes SIN3, SAP18, HDAC1, HDAC2, RbAp46, RbAp48, and other polypeptides
SCRG1	MGC26468 SCRG-1 ScRG-1	Associated with neurodegenerative changes observed in transmissible spongiform encephalopathies	The scrapie responsive protein 1 may be partly included in the membrane or secreted by the cells due to its hydrophobic N-terminus
SH3RF1	EC 6.3.2 FLJ21602 KIAA1494 POSH RNF142 SH3MD2	Might act as an E3 ubiquitin-protein ligase, or as part of E3 complex, which accepts ubiquitin from specific E2 ubiquitin-conjugating enzymes such as UBE2D1 or UBE2N and then transfers it to substrates	Contains an N-terminus RING-finger, four SH3 domains, and a region implicated in binding of the Rho GTPase Rac. Golgi

			apparatus
SPATA4	MGC33432 SPEF1B TSARG2	Unknown	Unknown
TRIM60	FLJ35882 MGC119325 RNF129 RNF33	Unknown	The protein contains a RING finger domain, a motif present in a variety of functionally distinct proteins
VEGFC	Flt4-L VEGF-C VRP	Growth factor active in angiogenesis, and endothelial cell growth, stimulating their proliferation and migration and also has effects on the permeability of blood vessels	A member of the platelet-derived growth factor/vascular endothelial growth factor (PDGF/VEGF) family
WDR17	FLJ26618	Unknown	A WD repeat-containing protein. It is abundantly expressed in retina and testis

Table 3.6: List of Genes Located at RP25 (6q14-q21), Present in GeneCards Database Showing their Status of Retinal Expression

Gene/ Symbol	Retinal Expression	Gene/ Symbol	Retinal Expression
C6orf167	Nil	LOC100131959	Nil
C6orf182	Yes	LOC100132988	Nil
C6orf184	Nil	LOC442249	Nil
C6orf186	Nil	LOC643749	Yes
CASP8AP2	Yes	LOC643884	Nil
CCNC	Yes	LOC728012	Yes
CDC40	Yes	LOC728562	Nil
FBXL4	Nil	MAP3K7	Nil
FLJ42177	Yes	MICAL1	Yes
GABRR1	Nil	NR2E1	Yes
GABRR2	Nil	NT5E	Yes
GAPDHL5	Nil	PDSS2	Nil
GAPDHL8	Nil	POPDC3	Nil
GPR6	Nil	REV3L	Yes
HEY2	Yes	RTN4IP1	Yes
HTR1E	Yes	SLC16A10	Yes
LIN28B	Yes	SNAP91	Yes
LOC100127905	Nil	SNX14	Yes
LOC100127917	Nil	TBX18	Nil
LOC100128159	Nil	TMEM30A	Yes
LOC100129847	Nil	TRAF3IP2	Yes
LOC100129852	Unknown	TUBE1	Yes

Table: 3.7: List of Genes Located at RP25 (6q14-q21) with their Aliases, Functions and Product/ Localization in the Cell Showing Retinal Expression

Gene/ Symbol	Aliases	Function	Product/ Cellular Localization
C6orf182	MGC21731 MGC70837	unknown	unknown
CASP8AP2	CED-4 FLASH FLJ11208 KIAA1315 RIP25	Participates in TNF-alpha-induced blockade of glucocorticoid receptor (GR) transactivation at the nuclear receptor coactivator level, upstream and independently of NF-kappa-B	This protein is highly similar to FLASH, a mouse apoptotic protein identified by its interaction with the death-effector domain (DED) of caspase 8
CCNC	CycC hSRB11	Involved in regulated gene transcription of nearly all RNA polymerase II-dependent genes	A member of the cyclin family of proteins
CDC40	EHB3 Ehb3 FLJ10564 PRP17	Associates with the spliceosome late in the splicing pathway and may function in the second step of pre-mRNA splicing	It has a sequence similarity to yeast Prp17 protein. Spliceosome
FLJ42177	Nil	unknown	Unknown
HEY2	CHF1 GRL HERP1 HESR2 HRT2 MGC10720 bHLHb32 hCHF1 hHRT2	Downstream effector of Notch signalling which may be required for Cardiovascular development. Transcriptional repressor which binds preferentially to the canonical E box sequence5'-CACGTG-3'. Represses Transcription by the cardiac transcriptional activators GATA4 and GATA6	A member of the hairy and enhancer of split-related (HESR) family of basic helix-loop-helix (bHLH)-type transcription factors
HTR1E	5-HT-1E 5-HT1E S31	Receptors for 5-hydroxytryptamine (serotonin), a biogenic hormone that functions as a neurotransmitter, a	unknown

		hormone, and a mitogen	
LIN28B	CSDD2 FLJ16517 Lin-28.2	When overexpressed, isoform 1 stimulates growth of the breast adenocarcinoma cell line MCF-7. Isoform 2 has no effect on cell growth	unknown
LOC100129852	Unknown	Unknown	Unknown
LOC643749	Unknown	Unknown	unknown
LOC728012	Unknown	Unknown	unknown
MICAL1	FLJ11937 MICAL NICAL	May be a cytoskeletal regulator that connects NEDD9 to intermediate Filaments	unknown
NR2E1	TLL TLX XTLL	May be required for brain development. May be involved in the regulation of retinal development	unknown
NT5E	5'-NT CD73 E5NT EC 3.1.3.5 NT NTE	Hydrolyzes extracellular nucleotides into membrane permeable nucleosides	Ecto-5-prime nucleotidase enzyme. Plasma membrane, cytoplasm, lysosome
REV3L	POLZ hREV3	Unknown	unknown
RTN4IP1	MGC12934 NIMP	Appears to be a potent inhibitor of regeneration following spinal cord Injury	A novel mitochondrial protein
SLC16A10	MCT10 PRO0813 TAT1	Sodium-independent transporter that mediates the update of aromatic acid	A member of a family of plasma membrane amino acid transporters
SNX14	MGC13217 RGS-PX2	May be involved in several stages of intracellular trafficking	A member of the sorting nexin family
TMEM30A	C6orf67 CDC50A	Unknown	unknown

	FLJ10856		
TRAF3IP2	ACT1 C6orf2 C6orf4 C6orf5 C6orf6 CIKS MGC3581	Could be involved in theactivation of both NF-kappa-B via a NF-kappa-B inhibitor kinase (IKK)-dependent mechanism and stress-activated protein kinase (SAPK)/JNK	A protein involved in regulating responses to cytokines by members of the Rel/NF-kappaB transcription factor family
TUBE1	FLJ22589 TUBE dJ142L7.2	Involved in cell cycle	Unknown. Centrosome and centriole

Table 3.8: List of Genes Located at RP22 (16p12.3-p12.1), Present in GeneCards Database, Showing their Status of Retinal Expression

Gene/ Symbol	Retinal Expression	Gene/ Symbol	Retinal Expression
ABCC6P1	Nil	LOC342293	Yes
ACSM1	Nil	LOC440345	Nil
ACSM2A	Nil	LOC441750	Nil
ACSM3	Nil	LOC554206	Yes
AQP8	Nil	LOC641298	Nil
ATP2A1	Yes	LOC728222	Nil
C16orf62	Yes	LOC728276	Nil
CDR2	Yes	LOC728423	Nil
COG7	Yes	LOC730094	Unknown
COQ7	Yes	MIR16	Yes
DCTN5	Yes	NDUFAB1	Yes
EARS2	Yes	PALB2	Yes
EEF2K	Yes	PKD1P4	Nil
ERN2	Nil	PKD1P5	Nil
FLJ41766	Yes	PLK1	Yes
GGA2	Yes	POLR3E	Yes
GPR139	Yes	RPS15A	Yes
GPRC5B	Yes	RUNDC2C	Nil
HMGN2P3	Unknown	SCNN1B	Yes
IGSF6	Yes	SCNN1G	Nil
IQCK	Yes	SLC5A11	Nil
JMJD5	Yes	SMG1	Yes
KIAA0556	Yes	THUMPD1	Yes
LOC100129329	Nil	TMC7	Nil
LOC100131998	Nil	TMEM159	Yes
LOC100132620	Nil	UQCRC2	Yes
LOC146110	Nil	USP31	Yes
LOC162073	Unknown	XYLT1	Yes
LOC23117	Nil	ZKSCAN2	Yes

Table: 3.9: List of Genes Located at RP22 (16p12.3-p12.1) with their Aliases, Functions and Product/ Localization in the Cell Showing Retinal Expression

Gene/ Symbol	Aliases	Function	Product/ Cellular Localization
ATP2A1	ATP2A EC 3.6.3.8 SERCA1	The hydrolysis of ATP coupled with the translocation of calcium from the cytosol to the sarcoplasmic reticulum lumen	One of the SERCA Ca(2+)-ATPases, which are intracellular pumps located in the sarcoplasmic or endoplasmic reticula of muscle cells
C16orf62	FLJ21040 MGC16824	unknown	Unknown
CDR2	CDR62 PCD17	unknown	Unknown
COG7	CDG2E	Required for normal golgi function	Multiprotein complexes, key determinants of Golgi apparatus structure
COQ7	CAT5 CLK-1 CLK1	Potential central metabolic regulator	Protein is similar to a mitochondrial di-iron containing hydroxylase
DCTN5	MGC3248 p25	unknown	Unknown
EARS2	GluRS MSE1	unknown	Unknown
EEF2K	EC 2.7.11.20 HSU93850 MGC45041	Phosphorylates eukaryotic elongation factor-2. Binds calmodulin	A highly conserved protein kinase in the calmodulin-mediated signalling pathway
FLJ41766	Nil	unknown	Unknown
GGA2	FLJ20966 KIAA1080 VEAR Vear	Plays a role in protein sorting and trafficking between the trans-Golgi network (TGN) and endosomes	A member of the Golgi-localized, gamma adaptin ear-containing, ARF-binding (GGA) family

GPR139	GPRG1 GPRg1 PGR3	Orphan receptor. Seems to act through a G(q/11) -mediated pathway	Unknown
GPRC5B	A-69G12.1 RAIG-2 RAIG2	Unknown	A member of the type 3 G protein-coupled receptor family
HMGN2P3	Nil	Unknown	Unknown
IGSF6	DORA	Unknown	Unknown
IQCK	FLJ20115 MGC35048	Unknown	unknown
JMJD5	FLJ13798	Unknown	unknown
KIAA0556	KIAA0556	Unknown	unknown
LOC162073	Nil	Unknown	Unknown
LOC342293	Nil	Unknown	Unknown
LOC554206	MGC39831	Unknown	Unknown
LOC730094	Nil	Unknown	Unknown
MIR16	Nil	Unknown	Unknown
NDUFAB1	ACP CI-SDAP FASN2A SDAP	Carrier of the growing fatty acid chain in fatty acid biosynthesis in mitochondria	unknown
PALB2	FANCN FLJ21816	Essential partner of BRCA2 that promotes the localization and stability of BRCA2	This protein binds to and colocalizes with the breast cancer 2 early onset protein (BRCA2) in nuclear foci and likely permits the stable intranuclear localization and accumulation of BRCA2
PLK1	PLK PLK-1 STPK13	Regulation of centrosome maturation and spindle assembly, the removal of cohesins from chromosome arms	Unknown
POLR3E	FLJ10509 KIAA1452	Catalyzes the transcription of DNA	Unknown

	RPC5 SIN	into RNA using the fourribonucleoside triphosphates as substrates	
RPS15A	FLJ27457 MGC11120 8 S15a	Unknown	The protein belongs to the S8P family of ribosomal proteins. It is located in the cytoplasm.
SCNN1B	Beta-ENaC Beta-NaCH ENaCB ENaCb ENaCbeta SCNEB	Unknown	Mediates the electro-diffusion of the luminal sodium (and water, which follows osmotically) through the apical membrane of epithelial cells
SMG1	61E3.4 ATX EC 2.7.11.1 KIAA0421 LIP	Involved in both mRNA surveillance and genotoxic stress response pathways	A protein involved in nonsense-mediated mRNA decay (NMD) as part of the mRNA surveillance complex
THUMPD1	FLJ20274	Unknown	Unknown
TMEM159	Promethin	Unknown	Unknown
UQCRC2	QCR2	Unknown	A component of the ubiquinol-cytochrome c reductase complex, which is part of the mitochondrial respiratory chain
USP31	EC 3.1.2.15 KIAA1203	Unknown	May recognize and hydrolyze the peptide bond at the C-terminal Gly of ubiquitin
XYLT1	EC 2.4.2.26 XT1 XylT-I	Catalyzes the transfer of UDP-xylose to serine residues within XT recognition sequences	Unknown

		of target proteins	
ZKSCAN2	ZNF694 ZSCAN31	Unknown	May be involved in transcriptional regulation

Table 3.10: Detail of the Characterized Genes

Gene/ OMIM#	Chrom-osomal Location	Aliases	Function	Product/ Cellular Localization
VAX2 (604295)	2p13 (RP28)	DRES93	Plays a crucial role in eye development and, in particular, in the specification of the ventral optic vesicle. May be a regulator of axial polarization in the retina. In mouse studies, this gene was found to be required for the correct formation of the optic fissure and other aspects of retinal development	This gene encodes a homeobox protein and is almost Exclusively expressed in the ventral portion of the retina during Development./ Nucleus
LRAT (6048663)	4q31 (RP29)	EC 2.3.1.135 MGC33103	Plays a critical role in vision. It provides the all-trans retinyl ester substrates for the isomerohydrolase which processes the esters into 11-cis-retinol in the retinal pigment epithelium; due to a membrane-associated alcohol dehydrogenase, 11 cis-retinol is oxidized and converted into 11-cis-retinaldehyde which is the chromophore for rhodopsin and the cone Photopigments	Microsomal enzyme that catalyzes the esterification of all-trans-retinol into all-trans-retinyl ester, an essential reaction for retinoid cycle in visual system and vitamin A status in liver. Mutations associated with early-onset severe retinal dystrophy/ Endoplasmic reticulum membrane
NR2E1 (603849)	6q21 (RP25)	TLL TLX XTLL	May be required for brain development. May be involved in the regulation of retinal Development	Unknown/ Nucleus
ELOVL4 (605512)	6q14 (LCA5)	ADMD STGD2 STGD3	Consistent with the expression of the encoded protein in photoreceptor cells of the retina,	a membrane-bound protein which is a member of the ELO

			mutations and small deletions in this gene are associated with Stargardt-like macular dystrophy (STGD3) and autosomal dominant Stargardt-like macular dystrophy (ADMD), also referred to as autosomal dominant atrophic macular degeneration	family, proteins which participate in the biosynthesis of fatty acids./ Endoplasmic reticulum membrane

Table 3.11: Ortholog Organisms for Candidate Genes with the Percent Similarity to the Human Genes

Orthologs	Human Percent Similarity			
	VAX2	LRAT	NR2E1	ELOVL4
Chimpanzee *(Pan troglodytes)*	99.2 (n) 99.31 (a)	99.57 (n) 99.57 (a)	99.74 (n) 100 (a)	99.79(n) 99.68(a)
Cow *(Bos taurus)*	88.5 (n) 87.68 (a)	88.7 (n) 87.83 (a)	94.55 (n) 99.48 (a)	90.23(n) 91.72(a)
Mouse *(Mus musculus)*	86.04 (n) 88.24 (a)	81.14 (n) 80.26 (a)	91.86 (n) 98.96 (a)	84.29(n) 92.63(a)

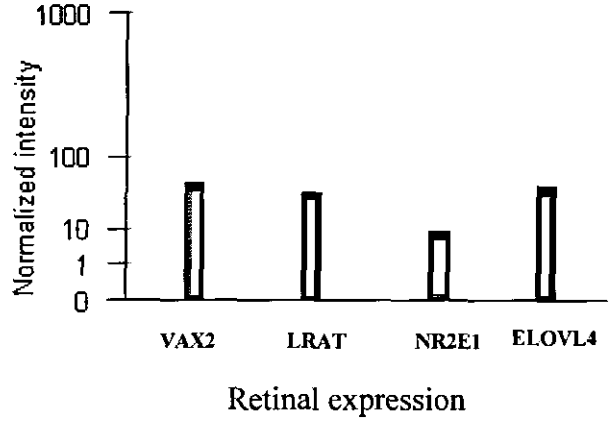


Figure 3.37: Graphical Representation of the Candidate Genes, showing Retinal Expression along X-axis and Normalized Expression Intensity along Y-axis

DISCUSSION

Recessively inherited diseases are more prevalent in populations where cousin marriages are common like Pakistan. The linkage analysis of retinal degenerations which are inherited in recessive manner like retinitis pigmentosa and congenital blindness is successfully be performed by these huge consanguineous families, which will be helpful in understanding the molecular and cellular biology of vision. The premature death of the rod and cone photoreceptors causes the loss of vision in various diseases in this class (Rivolta *et al.,* 2002). Showing an estimated occurrence of about 1 in 3000 to 1 in 5000, these represent the most common forms of human visual mutilation collectively (Bunker *et al.*, 1984). Over 40 loci for human hereditary retinal degenerations involving the photoreceptors and retinal pigment epithelium are known (Morimura *et al.,* 1998). Among the peripheral retinal dystrophies, RP is the most prevalent kind of hereditary retinal dystrophies. LCA, showing an autosomal recessive inheritance form, is the harshest type of retinal dystrophies (Alstrom CH, 1957). In this case both rods and cones are dead at birth or early in infancy (Francois J, 1968).

Recessive RP, the central RP kind, accounts near about 70% of all RP cases (Jay M, 1982). The arRP usually shows clinical similarities with other forms of retinal degenerations like early-onset RP and LCA (Lebers congenital amaurosis). Over 19 loci/genes are reported to be associated with arRP, and 5 loci have been reported for which the causative gene is still unknown (den Hollander *et al.*, 2007). The most genes for arRP cause 1% cases or even less than that, so considered a rare, however retinal pigment epithelium-specific 65kD protein (*RPE65*), rod cGMP phosphodiestrase alpha and beta subunit (*PDE6A and PDE6B*) are considered as the common genes as these cover 2-5% cases of all the cases for arRP. *USH2A* gene is the gene causing both Usher syndrome and arRP. About 5% of arRP cases are accounted by this gene. There are few examples for genes' mutations being the frequent cause of autosomal recessive RP such as, *RP25* is involved in 10-20% of arRP in Spain and rarely associated with the disease anywhere else in the world. Similarly, mutations in nuclear receptor subfamily 2 group E3 (*NR2E3*) are only reported in Sephardic Jews in Portugal (Bader *et al.,* 2003). LCA is collection of such states which leads to early onset retinal dystrophy, which over time

may be accompanied by pigmentary changes in the retina (Hooser *et al.*, 2002). It represents the most frequently occurring genetic causes of congenital visual impairment in children along with infants. The mutations with in the gene that encodes *RPE65* are the cause of about 10% cases of the all LCA cases (Pang *et al.*, 2005). LCA patients are diagnosed usually at the time of birth or during initial months of life, with extremely damaged eye vision or the complete blindness. The pathophysiology of the disease LCA is little known. It is considered as a result of either defect in the photoreceptor cells' development or deterioration of normally developed cells at very initial stage (Perrault *et al.*, 1999). LCA accounts for about 5% of all inherited retinopathies and responsible for *10-18%* of congenital blindness (Graw J, 2003). LCA phenotype is also reported to be associated with many syndromic anomalies of the renal, cardiac, skeletal and central nervous system. Among the associated symptoms, mental retardation seems to be an important feature. It is found in around 20% of the LCA population (Fazzi *et al.*, 2003).

In the present study, for searching a locus that contains the candidate gene, responsible for congenital blindness in family A and autosomal recessive RP in family B, linkage studies were performed by a method known as homozygosity mapping. In this method those specific genomic regions are identified which show autozygous pattern in the inherent patients of the particular disease. A LOD score for the examination through markers may well be calculated by making the comparison of the likelihood of being at the disease locus and the likelihood of being at a random genomic point, for the quantification of verification of linkage at that particular autozygous region (Lander and Botstein, 1987). This strategy of homozygosity mapping makes use of the reality that the human genome is rich in such polymorphic sequences which show variation among all the individuals (Shemer *et al.*, 2006). On average, $1/16^{th}$ part of the progeny genome with in the consanguineous marriages is supposed to show the homozygous pattern; which would follow arbitrary pattern among various individuals of the same cousin family, however the pattern of the disease locus which is common would be shared by the affected children.

For the present work, two families (A and B) with autosomal recessive LCA and RP were tested for linkage.

In family A, selected individuals were tested for the linkage to known LCA loci. Analysis of the results of the genotyped microsatellite markers (Figure 3.2-3.32) clearly identified that individuals having affected status follow heterozygous pattern by combining the alleles coming from parents in a different manner; thus excluding the family from linkage to these known genetic intervals.

In family B both normal and affected individuals were tested for linkage to all known arRP loci by using microsatellite markers from their candidate linkage intervals including RP18. Family B showed linkage at RP19 locus located on chromosome 1p13-21 containing *ABCA4* gene. Numerous types of the hereditary autosomal recessive retinal degenerations occur because of *ABCA4* gene (Gerber *et al.,* 1998). It encodes a protein which is a group member of the ABC, ATP-binding cassette transporters superfamily. The proteins of this family allow the transportation, from extracellular to intracellular membrane as well as form intracellular to the extracellular membrane, of numerous molecules. There are seven different subfamilies of the ABC genes which are MRP, ABC1, ALD, GCN20, MDR/TAP, White and OABP. ABC protein, a member of the ABC1 subfamily, is present with in the multicellular eukaryotes absolutely. It controls the transportation of a vital molecule across the cell membrane of the retinal photoreceptors, as this protein shows its expression with in the retinal photoreceptors completely (RefSeq). All the cases of autosomal recessive Stargardt disease, many cases of autosomal recessive cone–rod dystrophies and few cases of retinitis pigmentosa occur due to the mutations in this *ABCA4* gene which is retinal-specific (Ducroq *et al.,* 2002; Rozet *et al.,* 1999). Apart from it, the variants of *ABCA4* show the carrier frequency varying from 1/50 to 1/10 that is specifically high in the common population (Ducroq *et al.,* 2006).

In the present study Bioinformatics Approach is used to predict candidate genes for human retinal disorders. Initially all the loci containing those particular genes which are not known yet, for arRP kinds worldwide were identified using RetNet database of inherited retinal degenerations (RetNet). All the genes within each locus were identified using the databases of NCBI and Ensemble focusing on these genes with their transcripts localized with in the region edged by 2 genetic markers with in the genome (NCBI). After it those genes, which are lying in those specific loci, are analysed on the database of

the genes showing expression in human retina. The huge region of the human genome contains such a large number of genes that it is too difficult to perform gene characterization by analyzing the mutations of each alone gene at a time. So, a database of human retinal gene expression named as GeneCards was used to eliminate certain genes from the required genes which show no expression for this particular organ (GeneCards). The GeneCards database is a standard accessible and assimilated engine of genes present in humans which grants very useful information about all the predicted and known genes. It gives the brief genetic, functional, genomic, proteomic as well as transcriptomic analysis for the genes. Further more, this catalog confers information of mutations, expression of the genes, association of diseases, single nucleotide polymorphism markers, orthologs, gene physiology, protein-protein interactions, pathways, related compounds and drugs with accessible links to various tools such as antibodies, expression assays, recombinant proteins, RNAi reagents and clones.

All the genes were checked for their expression in the retina of eye. The genes showing 'Nil' retinal expression were eliminated from consideration while the genes showing retinal expression were further analyzed by knowing their functions and products form GeneCards as well as OMIM (NCBI/OMIM). A gene present in specific locus is considered as candidate gene if it would involve in retinal expression as well as function related to retina. Following this criterion we compiled the record of possible candidate genes for different RP loci. After characterization on the basis of gene's function involved in retina of the eye, it has been concluded that *VAX2*, *LRAT*, *NR2E1 and ELOVL4* are the candidate genes for retinal dystrophy. Then homologous species and the percent similarity to these human genes were identified.

VAX2, ventral anterior homeobox 2 shows an incredible expression in human, mouse as well as *Xenopus*, restricted to the neural retina's ventral portion. The development of an anomalous eye takes place along with the ventralizing effect of the developing eye, when *VAX2* within human embryos over-expresses. Due to this over-expression an expression domain of *Xpax2*, the transcription factor, shows extension towards retinal dorsal portion; however it usually restricts to ventral part of the developing retina. An optic stalk that is derived from the ventralmost area of the vesicle of the eye expands prominently on the over-expression of *VAX2*. In total, it signifies that this gene is responsible to develop eye

and specify the ventral optic vesicle (Barbieri *et al.*, 1999). The dynamic shuttling between the cytoplasm and nucleus, essentially involved in the differentiation of the retina, is performed by the *VAX2* while the eye is being developed (Kim and Lemke, 2006).

LRAT, phosphatidylcholine--retinol O-acyltransferase, plays a critical role in vision. It is situated largely with in liver and RPE cells and helps in the conversion of all-trans-retinol to all-trans-retinyl esters. A two cell cycling process, stocks up the rhodopsin's 11-cis-retinal chromophore in the retinal pigment epitheliam, so called the retinoid cycle. This process is performed by this particular LRAT gene, that's why a harsh retinal dystrophy LCA occurs in case of any abnormality or mutation in this gene (Batten *et al.*, 2003). It transfers the acyl group from the sn-1 position of phosphatidylcholine to all-trans retinol, producing all-trans retinyl esters. Retinyl esters are storage forms of vitamin A (Sweeney *et al.*, 2007).

The regulation of the determination of cell fate takes place due to numerous nuclear receptors. But these receptors are not present on the ligands in a significant amount, so the term 'orphan receptors' is used for these ligands (Benoit *et al.*, 2006). One of the orphan receptors is photoreceptor-specific nuclear receptor subfamily 2, group E, member 1 *NR2E1*, that is expressed almost solely in neural retina's layer of photoreceptor cells (McIlvain and Knox, 2007). Various retinal dystrophies are caused due ti its mutations. The comprehensive physiological analyses proposed that the development and maintenance of rod photoreceptors clearly depends on *NR2E1* (Oh *et al.*, 2008; McIlvain and Knox 2007; Chen *et al.*, 2005). *NR2E1* may be required for brain development (RefSeq).

ELOVL4 shows the particular elongase activity as it is a member of the fatty acid elongases family. A stargardt-like macular dystrophy occurs as a result of any mutation in this gene. Consistent with the expression of the encoded protein in photoreceptor cells of the retina, mutations and small deletions in this gene are associated with autosomal dominant Stargardt-like macular dystrophy (ADMD) and STGD3 which is a member of the ELO family, proteins which participate to synthesize fatty acids (Cameron *et al.*, 2007).

Although the specific genes responsible for all the phenotypes of arRP and LCA have not been identified, the map locations of a large number of these phenotypes are known (http://webh01.ua.ac.be/hhh/). It is necessary to perform the linkage analysis upon these genetically inherited phenotypes in order to refine the supposed genetic intervals to a region as tiny as possible. It is a labour-intensive task to perform the mutation analysis of all genes covering a huge area in the genome. For the reduction of the candidate genes to a limited number of mutation analyses, a bioinformatics approach is presented here.

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