

# **MOLECULAR DETERMINANTS OF LENS DISORDERS**



**NAMERAH AHMAD SABIR**

**CENTRE OF EXCELLENCE IN MOLECULAR  
BIOLOGY UNIVERSITY OF THE PUNJAB  
LAHORE PAKISTAN  
(2009)**

# **MOLECULAR DETERMINANTS OF LENS DISORDERS**

**A THESIS SUBMITTED TO  
UNIVERSITY OF THE PUNJAB  
IN PARTIAL FULFILLMENT OF THE  
REQUIREMENTS  
FOR THE DEGREE OF  
DOCTOR OF PHILOSOPHY IN  
MOLECULAR BIOLOGY**

**BY**

**NAMERAH AHMAD SABIR**

**SUPERVISOR:**

**DR. SHEIKH RIAZUDDIN**

**CENTRE OF EXCELLENCE IN MOLECULAR BIOLOGY  
UNIVERSITY OF THE PUNJAB LAHORE PAKISTAN**

**(2009)**



Our Lord! Let not our hearts deviate from the truth after You have guided us, and bestow upon us mercy from Your grace. Verily You are the Giver of bounties without measure.

(Quran 3:8)

## DEDICATED TO

My parents, my husband and specially to my little prince Rayyan

## CERTIFICATE

It is certified that the research work described in this thesis is the original work of the author **Namerah Ahmad Sabir** and has been carried out under our direct supervision. We have personally gone through all the data reported in the manuscript and certify their correctness/authenticity. It is further certified that the material included in this thesis have not been used in part or full in a manuscript already submitted or in the process of submission in partial/complete fulfillment of the award of any other degree from any other institution. It is also certified that the thesis has been prepared under our supervision according to the prescribed format and we endorse its evaluation for the award of Ph.D. degree through the official procedures of the University.

In accordance with the rules of the Centre, data books # **604, M-81** and **M-129** are declared as unexpendable document that will be kept in the registry of the Centre for a minimum of three years from the date of the thesis defense examination.

Signature of the Supervisor: 

Name: **Dr. S. Riazuddin**

Designation: Professor

# LIST OF CONTENTS

	Page
LIST OF FIGURES .....	xiii
LIST OF TABLES .....	x
SUMMARY .....	xi
ACKNOWLEDGMENTS .....	xiii
ABBREVIATIONS AND SYMBOLS .....	xiv
INTRODUCTION .....	1
<b><u>SECTION 1</u></b>	
<b>LITERATURE SURVEY .....</b>	<b>5-32</b>
<b>CHAPTER I</b>	
<b>THE EYE .....</b>	<b>6</b>
<b>OUTER STRUCTURES OF THE EYE .....</b>	<b>8</b>
Eye Socket or Orbit .....	8
Eyelashes and Eyebrows .....	8
Eyelids .....	8
Lacrimal Apparatus (Tear Duct) .....	8
Lacrimal Ducts .....	8
Conjunctiva .....	9
<b>STRUCTURES OF THE EYE BALL .....</b>	<b>9</b>
Outer Layer of the Eye .....	9
Middle Layer of the Eye .....	9
Inner Layer of the Eye .....	10
<b>CHAPTER 2</b>	
<b>LENS AND RELATED DISORDERS .....</b>	<b>11</b>
LENS OF THE EYE .....	12
ANATOMIC ASPECTS OF THE LENS .....	13

THE LENS CAPSULE.....	13
THE LENS EPITHELIUM.....	13
THE LENS CELLS OR FIBRES.....	13
COMPOSITION OF THE LENS.....	14
LENS TRANSPARENCY.....	14
EYE LENS PROTEOMICS.....	14
Water Soluble Protein Crystallins.....	14
Water-Insoluble Protein.....	15
LENS DISORDERS.....	15
CATARACT.....	15
CLASSIFICATION OF CATARACT.....	16
AGE OF ONSET.....	17
LOCATION OF OPACITY.....	17
Nuclear Cataract.....	18
Lamellar Cataract.....	18
Pulverulent Cataract.....	18
Total Cataract.....	18
Membranous Cataract .....	18
Cortical Cataract.....	18
Subcapsular Cataract.....	18
STAGES OF CATARACT.....	19
Immature.....	19
Mature.....	19
Hypermature.....	20
Morgagnian.....	20
ETOLOGICAL CLASSCIFICATION.....	20
Congenital or Developmental.....	20
Acquired cataract.....	20
SYMPTOMS AND TRAETMENT OF CATARACT.....	21
Phacoemulsification.....	21
Extracapsular Or Intracapsular Cataract Extraction.....	21



## CHAPTER 3

### LINKAGE ANALYSIS - A VALUABLE TOOL FOR MAPPING

DISEASE GENES.....	22
LINKAGE ANALYSIS.....	23
CROSSING OVER.....	23
GENETIC MAP UNIT .....	24
RECOMBINATION FREQUENCY .....	25
LOD SCORE .....	25
MULTIPOINT MAPPING .....	26

## CHAPTER 4

MOLECULAR DETERMINANTS OF LENS DISORDERS.....	27
GENETICS OF LENS DISORDERS.....	28
MOLECULAR GENETICS OF CONGENITAL CATARACT .....	28
NON SYNDROMIC/ISOLATED CATARACTS.....	29
GENES/LOCI IMPLICATED IN AUTOSOMAL RECESSIVE CATARACT.....	29
SYNDROMES ASSOCIATED WITH LENTICULAR DISORDERS.....	31
WEILL-MARCHESANI SYNDROME (WMS).....	32
MARFAN SYNDROME.....	32
PETERS ANOMALY.....	32
MARINESCO-SJOGREN SYNDROME (MSS).....	32

## SECTION 2

MATERIALS & METHODS.....	33-62
PHASES OF WORK .....	34
FIELD WORK.....	34
IDENTIFICATION AND ENROLLMENT OF FAMILIES.....	34
DOCUMENTATION AND MAINTENANCE OF THE FAMILY DATA .....	35
CLINICAL TESTS .....	35
OPHTHALMOSCOPY.....	36

VISUAL ACUITY TEST .....	36
CONTRAST SENSITIVITY TEST .....	36
GLARE SENSITIVITY TEST.....	36
TONOMETRY .....	36
POTENTIAL ACUITY TEST.....	36
BENCH WORK AT CEMB LABS.....	37
DNA EXTRACTION .....	37
FROM BLOOD SAMPLES .....	37
FROM BUCCAL SWABS .....	38
QUANTIFICATION OF DNA.....	39
PREPARATION OF PLATE MAP AND REPLICA PLATES.....	40
LINKAGE ANALYSIS FOR ALREADY REPORTED CONGENITAL CATARACT LOCI40	
TYPING STR MARKERS BY POLYMERASE CHAIN REACTION.....	43
PCR CYCLE FOR EXCLUSION STUDIES .....	43
SAMPLE PREPARATION FOR ABI 3730 GENETIC ANALYZER.....	44
HAPLOTYPE ANALYSIS.....	45
DATA ORGANIZATION AND ANALYSIS.....	46
LOD SCORE CALCULATION .....	49
GENOME WIDE SCAN .....	50
STANDARDIZATION OF MULTIPLEX PCR.....	55
MULTIPLEX PCR PROTOCOL .....	55
ORGANIZATION AND ANALYSIS OF GENOME SCAN DATA.....	56
LOD SCORE CALCULATIONS.....	58
SEQUENCING.....	58
Amplification of PCR Fragments .....	58
Agarose Gel Electrophoresis, Purification And Ethanol Precipitation Of PCR Product	59
Sequencing Reaction.....	60
Preparing A Product For Sequencing On Abi Prism® 3730 Genetic Analyzer	61
Analysis of DNA Sequences.....	61

## **SECTION 3**

<b>RESULTS AND DISCUSSION .....</b>	<b>63-117</b>
<b>CHAPTER 1 .....</b>	<b>64</b>
<b>RESULTS.....</b>	<b>64</b>
<b>OVERVIEW OF WORK DONE.....</b>	<b>65</b>
<b>PART I</b>	
<b>LINKAGE ANALYSIS OF REPORTED CONGENITAL</b>	
<b>CATARACT LOCI.....</b>	<b>66</b>
<b>LINKAGE TO SIL1.....</b>	<b>68</b>
<b>PKCC148 .....</b>	<b>68</b>
<b>CLINICAL EVALUATION .....</b>	<b>68</b>
<b>LINKAGE ANALYSIS.....</b>	<b>69</b>
<b>MUTATIONAL ANALYSIS .....</b>	<b>69</b>
<b>LINKAGE TO BFSP1.....</b>	<b>73</b>
<b>PKCC126.....</b>	<b>73</b>
<b>CLINICAL EVALUATION .....</b>	<b>73</b>
<b>LINKAGE ANALYSIS.....</b>	<b>73</b>
<b>MUTATIONAL ANALYSIS.....</b>	<b>73</b>
<b>PART 2</b>	
<b>GENOME WIDE SCAN FOR THE SEARCH OF A NEW</b>	
<b>LINKAGE.....</b>	<b>76</b>
<b>MAPPING OF A NEW REGION OF AUTOSOMAL RECESSIVE CONGENITAL</b>	
<b>CATARACT ON CHROMOSOME 3q.....</b>	<b>80</b>
<b>PKCC144 .....</b>	<b>81</b>
<b>CLINICAL EVALUATION .....</b>	<b>81</b>
<b>LINKAGE ANALYSIS.....</b>	<b>81</b>
<b>Exclusion Studies.....</b>	<b>81</b>
<b>Genome-Wide Linkage Analysis For Mapping A New Locus.....</b>	<b>81</b>

SCREENING OF FAMILIES FOR CHROMOSOME 3 LOCUS .....	82
<b>IDENTIFICATION OF A NEW REGION OF AUTOSOMAL RECESSIVE CONGENITAL CATARACT ON CHROMOSOME 8p.....</b>	<b>85</b>
PKCC146.....	86
CLINICAL EVALUATION .....	86
LINKAGE ANALYSIS.....	86
Exclusion Studies.....	86
Genome-Wide Linkage Analysis For Mapping A New Locus.....	86
SCREENING OF FAMILIES FOR CHROMOSOME 8 LOCUS .....	87
<b>A NEW LOCUS FOR PETER’S ANOMALY ON CHROMOSOME 1p.....</b>	<b>91</b>
PKCC139.....	93
CLINICAL EVALUATION .....	93
LINKAGE ANALYSIS.....	93
Genome-Wide Linkage Analysis For Mapping A New Locus.....	93
CANDIDATE GENE SCREENING .....	94
 <b>CHAPTER 2</b>	
<b>DISCUSSION.....</b>	<b>101-116</b>
 <b><u>SECTION 4</u></b>	
<b>REFERENCES.....</b>	<b>117-125</b>

## LIST OF FIGURES

		<b>Page</b>
Fig 1.1	Cross sectional view of an eye .....	7
Fig 1.2	Lacrimal Apparatus and Lacrimal Gland.....	9
Fig 1.3	The Crystalline Lens.....	12
Fig 1.4	Vision Through a Normal and a Cataractous Lens.....	16
Fig 1.5	Flow Chart Representing the Classification of Cataract.....	17
Fig 1.6	Types of Cataract according to Morphology.....	19
Fig 1.7	Phacoemulsification.....	21
Fig 1.8	Recombination event .....	24
Fig 2.1	Snellen Eye Chart.....	36
Fig 2.2	Dilution for DNA .....	39
Fig 2.3	Thermocycling profiles for amplification of STR markers.....	44
Fig 2.4	Electropherogram representing alleles.....	47
Fig 2.5	Procedure to Run Macros.....	48
Fig 2.6	Thermocycling profiles for amplification of Panel markers (multiplex PCR)..	55
Fig 2.7	Thermocycling profile for exon amplification .....	60
Fig 3.1	Pedigree Drawing of PKCC148 .....	70
Fig 3.2	Pedigree Drawing of PKCC126.....	74
Fig 3.3	Diagrammatic representation of the ABI PRISM® Linkage Mapping Set.....	78
Fig 3.4	Pedigree Drawing Of PKCC144 .....	83
Fig 3.5	Pedigree Drawing Of PKC146.....	88
Fig 3.6	Photographs showing clinical phenotypes PKCC146.....	89
Fig 3.7	Pedigree drawing of PKCC139.....	95
Fig 3.8	Phenotypic representation of PKCC139.....	96
Fig 3.9	Schematic representation of exons of SIL1 gene.....	104
Fig 3.10	Genes in the Linkage interval defined by PKCC144.....	106
Fig 3.11	Linkage interval defined by PKCC144 .....	108
Fig 3.12	Genes in the linkage interval defined by PKCC146.....	110
Fig 3.13	Genes in the Linkage interval defined by PKCC146.....	111
Fig 3.14	Role of FOXE3 in lens .....	113
Fig 3.15	Linkage interval defined by PKCC139.....	114

## LIST OF TABLES

	<b>Page</b>
Table 1.1 Reported autosomal recessive congenital cataract loci /genes .....	30
Table 2.1 STR markers used for linkage analysis of known loci/genes.....	41
Table 2.2 Reaction mixture for amplification of STR marker for genotyping .....	43
Table 2.3 Sets of multiplex PCR for HD10 genome wide panel .....	50
Table 2.4 Reaction mixture for amplification of PCR Fragments .....	59
Table 2.5 Reaction mixture for sequencing reaction.....	60
Table 3.1 Two point lod score obtained for PKCC148 .....	71
Table 3.2 Primers used for sequencing of exons of SIL1 gene.....	72
Table 3.3 Primers used for sequencing of exons of BFSP1 gene .....	75
Table 3.4 Two point lod score obtained for PKCC144 .....	84
Table 3.5 Candidate genes and their putative role .....	84
Table 3.6 Two point lod score of PKCC146 .....	90
Table 3.7 Candidate genes and their putative role .....	90
Table 3.8 Two point lod score of PKCC139 .....	97
Table 3.9 Clinical findings of affected individuals of PKCC139 .....	97
Table 3.10 Results of a comprehensive ocular examination of PKCC139.....	98
Table 3.11 Candidate genes and their putative role .....	99
Table 3.12 Primers for sequencing of exons of FOXE3 gene .....	100
Table 3.13. Primers for sequencing of exons of CYP4B1 gene .....	100

## SUMMARY

The last decade witnessed rapid progress in studies of the genetics of congenital disorders of the lens. This present study is designed to investigate the molecular and genetic basis of hereditary disorders of lens in Pakistani population. Among the disorders of the lens, cataract remains the most frequent disorders resulting in visual impairment and is responsible for approximately 48% of world blindness.

Here, in this dissertation, 39 consanguineous Pakistani families having a history of congenital disorders of the lens and an autosomal recessive pattern of inheritance have been reported. These families were identified through schools for blind children and ophthalmic clinics all over of Pakistan. Upon enrollment, a complete clinical history of the each family was obtained from family clinicians. All participating family members underwent a thorough clinical and a physical examination and denoted 8-10 cc blood samples that were used to extract genomic DNA. These genomic DNAs were initially subjected to exclusion analyses to check for reported loci for autosomal recessive congenital cataract. The families that were found associated with any of the known loci were excluded, whereas the rest of the unlinked families were interrogated in genome-wide scans to identify novel linkages and genes.

Genome wide linkage analysis was performed on 18 families and these efforts led to the identification of two novel non-syndromic autosomal recessive congenital cataract loci on chromosome 3q and chromosome 8p, respectively in two large families. On the 3p locus, a maximum two-point LOD score of 4.0 with marker D3S3609 was obtained for family PKCC144. Similarly maximum two-point LOD score of 3.17 with markers D8S550 was obtained for family PKCC146. Both these regions have not been associated with autosomal recessive congenital cataracts in any previous study.

Additionally, family PKCC139 manifested characteristics of Peter's anomaly, a rare form of anterior segment dysgenesis in which unusual cleavage of the anterior chamber occurs, which may involve the central or entire cornea. Genome wide scan was performed on family PKCC139 to determine the causative region segregating with the disease phenotype, which identified a region of significant linkage on chromosome 1p with two-point LOD score of

4.12 with marker DIS197. Additional markers were typed to refine the critical interval and defining the proximal and distal boundaries. This new reported region overlaps with the previous published region of congenital cataract but dissimilar clinical characteristics differentiate it as peters anomaly.

In short, this study reports the identification of two new loci for congenital cataracts on chromosomes 3q and 8p and one novel locus for syndromic cataract (peters anomaly) on chromosome 1p.



## ACKNOWLEDGMENTS

*All the praise to the Almighty Allah, the great benevolent and ever merciful, for filling me with courage and confidence to accomplish the desired task. Humble dedications are for the most perfect personality of the universe of all the times, Muhammad (P.B.U.H).*

*My warmest and heartiest obligations are to my supervisor Dr. S. Riazuddin, Professor Centre of Excellence in Molecular Biology, for his affectionate guidance and invaluable advice and also for providing me with world class laboratory facilities throughout my research work. I offer my heartfelt thanks to Dr. Shaheen. N. Khan, Professor CEMB, for her precious guidance and admirable supervision during my doctorate.*

*I greatly appreciate the contribution and help of all the staff of Lyton Rehmatullah Benevoalnt Trust Hospital (LRBT) for the identification and clinical tests of families .I am greatly indebted to Dr. Z. A. Qazi, Dr. M. Amer and Dr. Saleem Akhtar for their cooperation in basic clinical ophthalmology. I am particularly thankful to Dr. Shakeel Ahmad (Mayo Hospital) for his marvelous guidance and cooperation.*

*I am greatly thankful to all the members of enrolled families that made my studies possible.*

*I pay my utmost respect and gratitude to our collaborators at NEI, USA especially Dr. Amer Riazuddin for his innovative approach and assistance in interpretation of results and sequencing.*

*I wish to thank all my colleagues and lab fellows especially my husband Tariq, Haiba, Sabika, Amber, Muhammad Iqbal, Asif Naeem and Shahbaz, for their best wishes and support in my lab discussions. I am also grateful to members of deafness specially Shahid Yar Khan & Saima and to members of sequencing lab for their help in data analysis. Special thanks to all my friends for encouraging me and providing cheerful environment to learn and grow. I am thankful to all the scientific, Para scientific and administrative staff of CEMB as they were directly and indirectly instrumental in my research work.*

*I am deeply and forever indebted to all my family members especially my parents, brothers, my in laws for their affection, motivation and day and night prayers for my success specially to my little son , Rayyan whose arrival in this world became a blessing for me. No words can express the love, moral support and encouragement that my husband extended throughout the course of my research. It is all because of their prayers and affectionate attitude that enabled me to achieve my goal.*

*May Allah bestow His blessings to all my family members and friends and may they live long, enjoy healthy lives forever and brighten the path of my life with their never-ending guidance and love.*

*Namerah Ahmad Sabir*