

Genetic Tools In Management of Ophthalmological Disorders

Essay submitted
For
partial fulfillment of the Master Degree in Medical Genetics

Presented by

Marwa Ahmed Abd El-Fattah Khalil

*M.B.B.ch of Medicine
Faculty of Medicine
Ain Shams University
December 2006*

Under supervision of

Prof. Dr. Karam Abd ALAleem

*Professor of Human Medical Genetics
Faculty of Medicine
Ain Shams University*

Dr. Osama Kamal Zaki

*Assistant Consultant of Clinical Genetics
Director of Genetics Unit
Ain Shams University*

**Faculty of Medicine
Ain Shams University
2013**

Introduction:

Genetics, as its name implies, is involved in the organogenesis of each organ in the body. Therefore, disorders of each organ morphology have a definite relation to its genesis. This, in turn, highlights the role of genetics in dealing with such organ disorders.

Ophthalmology is an area of mainstream medicine where genetic testing for inherited eye disease is becoming increasingly important due to the advances in genetics and cell research, which gave hope to many inherited eye diseases, that lead to severe visual impairment in adults and in children and rendered them treatable or at least preventable through proper diagnosis, counseling and intervention [Besch et al., 2005].

There have been continuous researches to discover the underlying defects as well as to develop diagnostic and therapeutic tools for management of these disorders. To achieve these aims, early diagnosis is mandatory. Consequently, every ophthalmologist should be aware of the possibilities for preventing hereditary eye diseases. In the past, it was possible only to anticipate the risk of recurrence for a particular disorder. However, in the recent past some possible prevention could be done by minimal intervention in some cases as by diet restriction of certain products in some inborn errors of metabolism if early diagnosis was made [Moore et al., 2008].

The majority, if not all recent advances in ophthalmological disorders have proved that the actual perfection of management of these disorders can mostly be approached through genetic elements. Gene based therapies and stem cell therapies are actively being pursued to ameliorate ophthalmic genetic disorders that were once considered untreatable.

Patients with genetic eye disorders should have access to specialized care from teams with particular knowledge and experience in the diagnosis and management of these conditions. Such teams must combine specialists in ophthalmology, genetics, genetic counseling, laboratory molecular genetics and electrophysiology [Moore et al., 2008].

1	Genetic Tools	9
1.1	Introduction	9
1.1.1	Genetic testing	9
1.1.2	Genetic Counselling	12
1.2	Treatment Tools	15
2	Metabolic Disorders	19
2.1	Introduction	19
2.2	Current Therapeutic Approaches to Inborn Errors of Metabolism	20
2.3	Disorders of Carbohydrate Metabolism	21
2.3.1	Diabetic Retinopathy	21
2.3.2	Disorders of Galactose Metabolism	22
2.4	Disorders of Amino Acid Metabolism and Transport . .	24
2.4.1	Disorders in Tyrosine metabolism	24
2.4.2	Disorders of Sulfur Amino Acid Metabolism . .	28
2.4.3	Disorders of Ornithine metabolism	29
2.5	Disorders of Metal Transport	30
2.5.1	Wilson Disease (Hepatolenticular Degeneration)	30
2.6	Lysosomal Storage Disorders	31
2.6.1	Mucopolysaccharidoses	31
2.6.2	Mucolipidoses	37
2.7	Disorders of Lipid Metabolism	37
2.7.1	Disorders of Sphingolipid metabolism	37
2.7.2	Disorders of Cholesterol Synthesis	45
2.7.3	Peroxisomal Disorders (Disorders of Very Long Chain Fatty Acids)	47
2.7.4	Disorders of Bile Acid Synthesis	50
2.7.5	Disorders of Lipid and Bile Acid Metabolism . .	51
2.7.6	Disorders of Lipoproteins	53
3	Congenital Ocular Malformations	55
3.1	Teratogens and Ocular Anomalies:	55
3.1.1	Infectious agents:	55
3.2	Malformations Of The Ocular Adenexae	58

4	Genetic Disorders of the Cornea	65
4.1	Congenital Corneal Dystrophies	65
4.1.1	Anterior Corneal Dystrophies	67
4.1.2	Stromal Dystrophies	68
4.1.3	Endothelial Dystrophies	69
4.2	Corneal Clouding Associated with Inherited Systemic Disorders	70
5	Genetic Disorders of the Lens	73
5.1	Noncataractous Anomalies	73
5.1.1	Lenticonus	73
5.1.2	Microspherophakia	74
5.1.3	Ectopia Lentis	75
5.2	Cataracts	78
5.2.1	Morphological Classification of Cataract:	79
5.2.2	Genes involved in congenital cataract	80
5.2.3	Etiological Classification of Cataract:	80
5.3	Workup of Congenital and Pediatric Cataracts:	89
6	Genetic Disorders of the Retina	93
6.1	Hereditary Retinal and Choroidal Degeneration	93
6.1.1	Retinitis Pigmentosa	95
6.1.2	Lebers Congenital Amaurosis (Congenital Retinitis Pigmentosa)	98
6.1.3	Age Related Macular Degeneration	98
6.1.4	Generalized Choroidal Dystrophies	100
6.1.5	Congenital Stationary Night Blindness	100
6.1.6	Usher Syndrome	101
6.2	Macular Dystrophies	102
6.2.1	Stargardt Macular Dystrophy	102
6.2.2	Best Macular Dystrophy	103
6.2.3	Juvenile X-Linked Retinoschisis	103
6.3	Vitreo Retinal Degenerations	103
6.3.1	Stickler Syndrome	103
6.3.2	Wagner Disease	104

6.3.3	Familial Exudative Vitreoretinopathy (FEVR)	105
6.3.4	Incontinentia Pigmenti (IP)	106
6.3.5	Norrie Disease	106
6.4	Color Vision Defects	106
6.4.1	Red-Green Color Vision Defects	107
6.4.2	Blue-Yellow (Tritan) Color Vision Defects	107
6.4.3	The Achromatopsias	107
7	Inherited Disorders Of The Optic Nerve	109
7.1	Optic Atrophy	109
7.1.1	Isolated Optic Atrophies	109
7.2	Optic Nerve Anomalies	111
7.2.1	Optic Nerve Hypoplasia	111
7.2.2	Optic Disc Cloboma	111
7.2.3	Optic Nerve Dysplasia and Renal Coloboma Syn- drome	112
8	Glaucoma	113
8.1	Primary Glaucomas	114
8.2	Congenital Glaucomas	114
8.3	Secondary Glaucomas	115
9	Genetic Disorders Of The Mitochondria	119
9.1	Inheritance and Genetics Of Mitochondrial Disease	119
9.2	Optic Neuropathy	120
9.2.1	Leber Hereditary Optic Neuropathy (LHON)	120
9.2.2	Dominant Optic Atrophy (DOA)	121
9.2.3	Other Mitochondrial Optic Neuropathies	122
9.3	Chronic Progressive External Ophthalmoplegia	124
9.4	Pigmentary Retinopathy	125
9.4.1	Neurogenic Muscle Weakness, Ataxia, And Re- tinitis Pigmentosa (NARP) And Maternally In- herited Leigh Syndrome	125
9.5	Retrochiasmal Visual Loss	125
9.5.1	Mitochondrial Encephalomyopathy With Lactic Acidosis And Stroke-Like Episodes (MELAS)	126

9.6	Myoclonic Epilepsy and Ragged Red Fibers Syndrome (MERRF)	126
9.7	Management of Mitochondrial Disorders	126
10	Retinoblastoma	131
10.1	Genetics of retinoblastoma	131
10.2	Genetic Testing and Counseling	132
10.3	Prenatal Assessment and Diagnosis	133
10.4	Therapeutic Strategies	134
11	Chromosomal Disorders and the Eye	137
11.1	Genetic Counselling and Prevention for Chromosomal Disorders	137
11.2	Trisomy Syndromes	138
11.3	Deletion Syndromes	141
11.4	Duplication Syndromes	144
11.5	Sex-Determining Chromosomes	144
	Bibliography	146

List of Figures

2.1	Oculocutaneous Albinism Type 1a	25
2.2	Chediak Higashi Syndrome	28
2.3	Inferonasal Dislocated Lens in Homocystinuria	29
2.4	Gyrate Atrophy of The Retina	30
2.5	Kayser-Fleischer Ring In Wilson Disease	31
2.6	Corneal Clouding in MPS	32
2.7	Pseudoexophthalmos in MPS	33
2.8	Cherry red spot in The Retina	38
2.9	Whorl Like Corneal Pattern in Fabry Disease	43
2.10	Smith Lemli Opitz Syndrome	47
2.11	Rhizomelic Chondrodysplasia Punctata	49
2.12	Cerebrotendinous Xanthomatosis	51
3.1	congenital toxoplasmosis	57
3.2	Waardenburg Syndrome	59
3.3	Fraser Syndrome	60
3.4	Anophthalmic socket (left eye)	61
3.5	Aniridia	63
5.1	Weil Marchesani Syndrome	75
5.2	Subluxated Lens in Marfan Syndrome	79
6.1	Stickler Syndrome	105
10.1	13q deletion syndrome	134
11.1	Down Syndrome	140
11.2	Edward Syndrome	141
11.3	Patau Syndrome	142
11.4	Wolfhirschhorn Syndrome 4p-	143

List of Tables

2.1	Enzyme Defect, Glycosaminoglycan Deposited, Gene Locus and Inheritance Pattern of the Mucopolysaccharidoses (Ashworth et al., 2006).	33
2.2	Clinical Features of the Mucopolysaccharidoses (Ashworth et al., 2006).	34
5.1	Aetiological Classification of Ectopia Lentis (Ahram et al., 2009)	77
5.2	The Morphological Appearance and Onset of Cataract in Genetic Syndromes	91
5.3	Investigations Done for Bilateral Cataract Cases: . . .	91

1.1 Introduction

The eye has played a major role in human genomics including gene therapy. It is the fourth most common organ system to be involved in genetic disorders. The eye is involved in single gene disorders and those caused by multifactorial etiology. The first autosomal dominant, autosomal recessive, X-linked recessive, X-linked dominant, digenic, triallelic, mitochondrial, and 2-hit disorders all had eye manifestations or were primarily eye diseases. We can actually see the lyonization taking place in the retina of a female carrier of ocular albinism or Lowe syndrome. As a result of this advantage, we now have wide diagnostic opportunities to find the genetic basis of a patient's eye disease. Genetic tools and their application to clinical ophthalmological practice are very beneficial and allows better, earlier diagnosis, prevention or treatment of the disease and genetic counselling (Sadagopan et al., 2012).

1.1.1 Genetic testing

Genetic testing provides opportunity to gain information regarding diagnosis, prognosis, the need to screen other organ systems, surveillance, therapy, counseling and research. Testing may involve cytogenetic analysis and molecular genetic analysis as shown below (Sadagopan et al., 2012).

- **Cytogenetic Investigations** Completion of the Human Genome Project stimulated development of ancillary technologies that continue to revolutionize medical sciences and diagnostic techniques.

Current conventional cytogenetic analysis include:

- **G-banded karyotype**, Which shows the number and shape of chromosomes. This analysis can be undertaken on patients, when the patient has multiple congenital anomalies, or prenatally on fetal cells obtained through such techniques as amniocentesis and CVS, if a parent has had multiple miscarriages, or if a family history of chromosomal aberration is present. It can detect unbalanced structural rearrangements and numeric abnormalities, as well as apparently balanced rearrangements within the limits of resolution of the technique. The resolution of the current conventional cytogenetic analyses lies in the range of 310 Mb (1 Mb=1 million base pairs) and requires dividing cells. Therefore, chromosomal microdeletions or microduplications (those smaller than 3 Mb) will go undetected with conventional cytogenetic analyses. These submicroscopic rearrangements may account for a sizable portion of the human genetic disease burden, with some estimates as high as 15% (Abotalib, 2013).
 - **Fluorescent in situ hybridization (FISH)** FISH is a cytogenetic technique that is used to detect and localize the presence or absence of specific DNA sequences on chromosomes. FISH uses fluorescent probes that bind to only those parts of the chromosome with which they show a high degree of sequence complementarity. This technology can be used to detect chromosomal abnormalities smaller than 3 Mb, but because of technical limitations, it can only screen for a limited number of chromosomal abnormalities at one time, one must have a specific syndromic suspicion to select the desired probe.
- Multiplex in situ hybridization (M-FISH)** banding probe sets are a variety of the FISH technique using a 24-color karyotyping technique so each human chromosome can be identified by a characteristic color using whole-chromosome probe mixtures and a variety of ratios of colors which can be used to create secondary colors, that may help in the detection of

translocations. It is the method of choice for studying complex interchromosomal rearrangements (Abotalib, 2013).

– **Microarray comparative genomic hybridization (CGH)**

Genomic microarray-based technologies can theoretically detect human genomic DNA variation at virtually any site in the human genome. Genomic microarrays can detect both duplications and deletions, also referred to in the literature as genomic copy number variants. Which are deletions and duplications of DNA segments larger than 1,000 bases and up to several megabases in size. It can be used to perform karyotyping, it improves resolution over conventional G-banded karyotype in detecting chromosomal abnormalities smaller than 3 Mb. The two types of arrays currently available are targeted and genome-wide arrays. Targeted arrays are currently preferred in clinical genetic practice because they can detect chromosomal abnormalities for known genetic syndromes. This allows genetic counselling with more certainty regarding phenotype and long-term prognosis. While genome-wide arrays, however, are designed to cover a greater portion of the human genome than targeted arrays. Genome-wide arrays have been particularly useful in research efforts to discover new submicroscopic syndromes (Vialard et al., 2009).

- **Molecular Genetic Testing** Molecular genetic techniques are being used for prenatal diagnosis. DNA is extracted from amniocytes, chorionic villi, or fetal blood cells. Then, it is amplified by Polymerase chain reaction (PCR) and is used for the diagnosis of genetic mutations or deletions within a gene that causes a specific genetic disease. **Polymerase chain reaction (PCR)** is used for the diagnosis of single gene defects, including dominant and recessive disorders. PCR, sometimes called DNA amplification, is a technique in which a particular DNA sequence is copied many times in order to facilitate its analysis.

The following molecular biologic techniques can be used for prenatal diagnosis of different diseases:

- Restriction fragment length polymorphism
 - Single nucleotide polymorphisms
 - DNA chip
 - Dynamic allele-specific hybridization
- **Enzyme Assays** are laboratory methods for measuring enzymatic activity. Reaching a definitive diagnosis of many inherited metabolic disorders is not possible without demonstrating specific deficiency of the enzyme involved. The practical application of clinical diagnostic enzymology demands attention to a number of variables affecting the results of any particular assay. One important variable is the source of the enzyme to be assayed (tissue specificity). Many enzymes are tissue specific, and diagnostic analysis requires sampling of the relevant tissue (Fernandes et al., 2006).

1.1.2 Genetic Counselling

Genetic counseling is the process of providing individuals and families with information on the nature, inheritance, and implications of genetic disorders to help them make informed medical and personal decisions. It deals with genetic risk assessment and the use of family history and genetic testing to clarify genetic status for family members. It provides the patients with the preventive measures they can use for subsequent pregnancies and inform the patients with the most recent research results of available treatment options or clinical trials using gene therapy or other therapeutic strategies . Once a mutation or chromosomal aberration has been identified in the proband, targeted analysis can be performed for at risk family members. Prenatal and pre-implantation genetic diagnosis (PGD), may also be available for subsequent pregnancies. Thus preventing the birth of an affected child, or leading to early detection of several diseases and appropriate intervention that plays a key role in either preventing the genetic disease or reducing the severity of its clinical manifestations (Pagon et al., 2010).

This can be done through:

- Family history taking and pedigree construction to help know the inheritance patterns of diseases
- Clinical Examination
- Confirmatory diagnosis through history findings, clinical examination findings, radiological findings, laboratory parameter results, cytogenetic studies, and DNA studies results
- Calculation of recurrence risk
- Counseling and offering the available prevention or treatment options
- Follow-up

Premarital Examination should be applied to identify individuals with genetic predisposition to a disease, and to identify carriers for a particular gene defect. Subsequently, genetic counselling should be offered to prevent birth of an affected child or to prevent or delay disease progression.

Preimplantation Genetic Diagnosis Preimplantation genetic testing is a technique used to identify genetic defects in embryos created through in vitro fertilization (IVF) before pregnancy. Preimplantation genetic diagnosis (PGD) refers specifically to when one or both genetic parents has a known genetic abnormality and testing is performed on an embryo to determine if it also carries a genetic abnormality, so that only healthy and normal embryos are transferred into the mother's uterus, thus diminishing the risk of inheriting a genetic abnormality and late pregnancy termination. In order to have embryos to biopsy for PGD, patients must undergo in vitro fertilization (IVF). After fertilization of the egg with sperm, embryos are allowed to develop into cleavage-stage embryos. On day 3 after egg retrieval, a single blastomere is removed from the developing embryo for genetic