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].

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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