STUDY OF CYSTIC FIBROSIS TRANSMEMBRANE CONDUCTANCE REGULATOR (CFTR) GENE IN AZOOSPERMIC PATIENTS

THESIS SUBMITTED FOR PARTIAL FULLFILMENT OF MASTER DEGREE IN DERMATOLOGY AND VENEROLOGY

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بسم الله الرحمن الرحيم

لله ما في السموات والأرض
يخلق مايشاء يهب لمن يشاء
اناثا ويهب لمن يشاء الذكور *
او يزوجهم ذكرانا واناثا
ويجعل من يشاء عقيما انه
عليم قدير *

صدق الله العظيم

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LIST OF ABBREVIATIONS

µg	Microgram
µl	Microlitre
AD	Autosomal Dominant
Ad	Dark Type A Spermatogonia
Ap	Pale Type A Spermatogonia
AR	Androgen Receptor
AVD	Absent Vas deferens
AZF	Azoospermic Factor
B	Type B spermatogonia
bp	Base pair
cAMP	Cyclic Adenosine monophosphate
CBAVD	Congenital Bilateral Absent Vas deferens
CF	Cystic Fibrosis
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
CUAVD	Congenital Unilateral Absent Vas deferens
DAZ	Deleted in azoospermia
FSH	Follicular Stimulating Hormone

g/l	gram per litre
GnRH	Gonadotrophine Releasing Hormone
ICSI	Intracytoplasmic Sperm Injection
IVF	In Vitro Fertilization
L	Leptotene primary spermatocyte
ml	Millilitre
n mol	Nano mole
NOAZ	Non Obstructive Azoospermia
OAZ	Obstructive Azoospermia
P	Pachytene primary spermatocyte
P.aeruginosa	Pseudomonas Aeruginosa
P mol	Pico mole
R	Preleptotene primary spermatocyte
R domain	Regulatory Domain
SCO	Sertoli Cell only syndrome
Taq	Taq polymerase
TRUS	Transrectal Ultrasonography
URA	Unilateral Renal Agenesis
WHO	World Health Organization
Z	Zygotene primary spermatocyte

Introduction

Infertility has been reported in 97 % to 98 % of male adults with cystic fibrosis. This condition is associated with azoospermia and defects in the anatomic transport of sperm cells, due mainly to congenital bilateral absence of vas deferens ***(Gaillard et al., 1997).***

Congenital bilateral absence of the vas deferens (CBAVD) is a rare cause of male infertility. The condition was found in 2 % of patients with obstructive azoospermia in the Edinburgh infertility clinic and obstructive azoospermia accounts for 0.9 % of male infertility worldwide (***Donat et al., 1997).***

The cloning of the cystic fibrosis transmembrane conductance regulator (CFTR) gene and the cooperative research activity in mutation analysis have led to the establishment of genotype and phenotype correlations. The Δ F 508 + / + mutation was shown to be the most common genotype and usually associated with severe cystic fibrosis diseases, with lung and pancreatic failure and male infertility ***(Tsui, 1992).***

A strong association between CBAVD and mutations of the cystic fibrosis transmembrane conductance regulator (CFTR) gene has been identified recently ***(Donat et al., 1997).***

Microdeletions affecting various parts of the long arm of the Y chromosome in approximately 10% of men with non obstructive azoospermia and severe oligozoospermia but not in a

fertile comparison population have been reported ***(Hargreave et al., 1996).***

In the majority of cases, CBAVD can now be considered as a genital form of cystic fibrosis, presenting without the other clinical features of cystic fibrosis. Nevertheless, in about 20% of patients, CBAVD is associated with urinary tract malformations and in these cases the etiology of CBAVD is not related to defects in the CFTR gene ***(Augarten et al., 1994 ,Dumur et al., 1995, Casals et al., 1995 ,Mercier et al.,1995, Rave-Har et al.,1995).***

Similarly, ***McCallum T and colleagues*** postulated that unilateral renal agenesis (URA) and CBAVD may have a non-CF mutation-mediated genetic basis that leads to abnormal development of the entire mesonephric duct at a very early stage in embryo development (< or = 7 weeks) ***(McCallum, et al., 2001).***

Aim of Work

This work aims to investigate the cystic fibrosis transmembrane conductance regulator (CFTR) gene in patients with azoospermia. Radiological imaging will check associated anomalies in urinary tract.

Autosomal Recessive Disorders

Of all the recognized single gene traits, more than half are inherited in an autosomal dominant pattern, one third are inherited in an autosomal recessive pattern, and the remainder are x-linked ***(Thompson et al. ,1991).***

Autosomal recessive inheritance is the single largest category of mendelian disorders. Because autosomal recessive disorders result only when both alleles at a given loci are mutants, such disorders are characterized by the following features: -

1) The trait does not usually affect the parents, but siblings may show the disease.
2) Siblings have one chance in four of being affected (i.e., the recurrence risk is 25% for each birth).Fig. (1).
3) If the mutant gene occurs with a low frequency in the population, there is a strong likelihood that the proband is the product of a consanguineous marriage (***Cotran, 1999).***

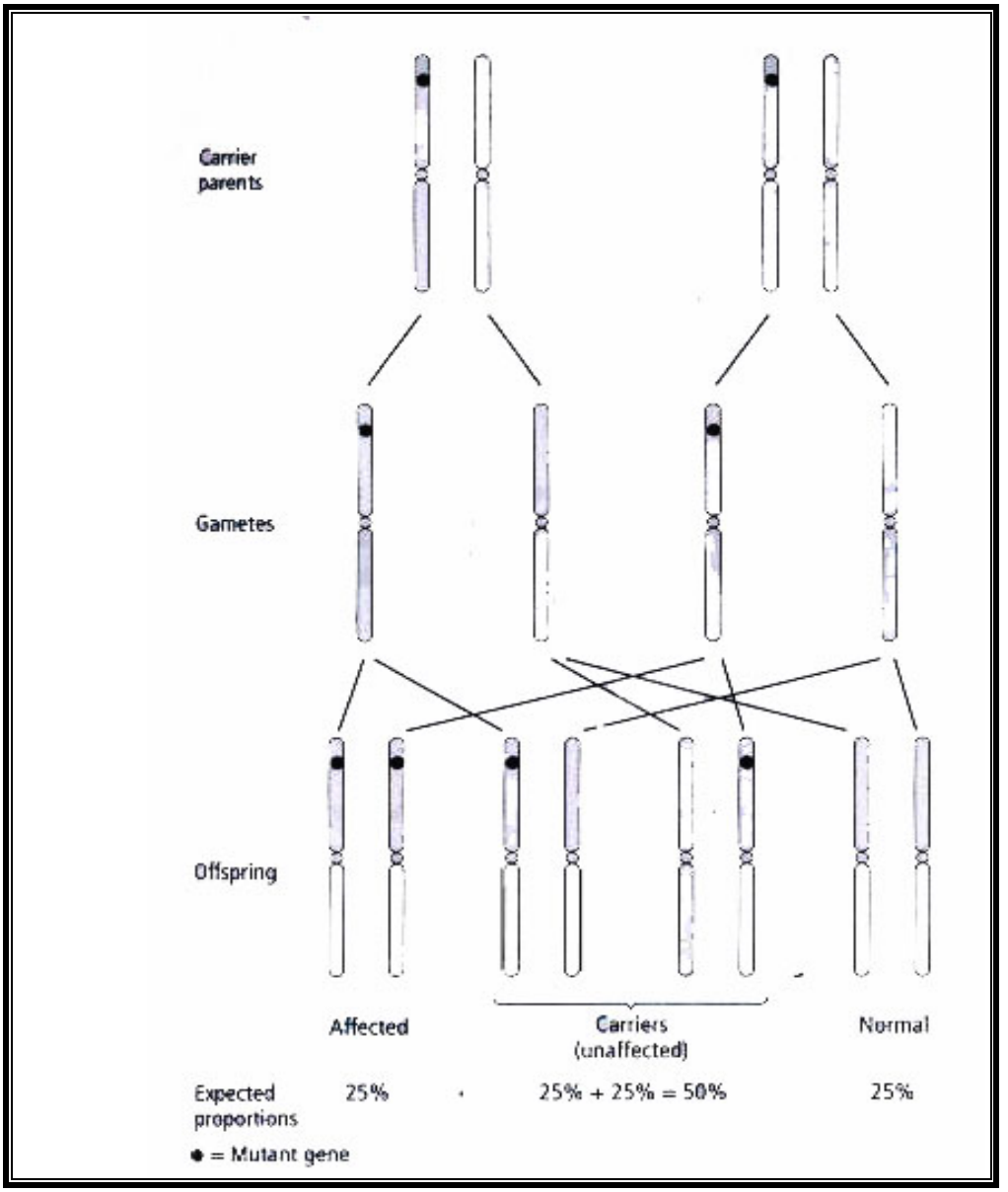

Fig. (1) Diagram of autosomal recessive inheritance ***(Connor, 1997).***

In autosomal recessive conditions, both unaffected parents are carriers and each has one mutant copy and one normal copy of the gene. When both parents are carriers, they have a 25% chance with each pregnancy of having an affected child who inherits both copies of the mutant gene (***Roche and Kuller, 1996).***

In contrast to those of autosomal dominant diseases, the following features generally apply to most autosomal recessive disorders: -

1) The expression of the defect tends to be more uniform than in autosomal dominant disorders.
2) Complete pentrance is common.
3) Onset is frequently early in life.
4) Although new mutations for recessive disorders do occur, they are rarely detected clinically. Since the individual with a new mutation is an asymptomatic heterozygote, several generations may pass before the descendants of such a person mate with other heterozygotes and produce affected offspring.
5) In many cases, enzyme proteins are affected by a loss of function. In heterozygotes, equal amounts of normal and defective enzyme are synthesized. Usually the natural "margin of the safety" ensures that cells with half their usual complement of the enzyme will function normally **(*Cotran, 1999*)**.