شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

بسم الله الرحمن الرحيم

HANAA ALY

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جامعة عين شمس

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قسم

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HANAA ALY

ENVIRONMENTAL AND GENETIC EVALUATION IN 46, XY DISORDERS OF SEX DEVELOPMENT

Submitted By

Shereen Adel Abdelkader Sayed

M.B.B.Ch., Faculty of Medicine, Cairo University, 2004

Master in Clinical & Chemical Pathology, Faculty of Medicine, Cairo University, 2015

A Thesis Submitted in Partial Fulfillment

Of

The Requirement for the Doctor of Philosophy Degree

In

Environmental Sciences

Department of Environmental Medical Sciences

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Ain Shams University

2021

APPROVAL SHEET

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Abstract

Background: Disorders/Differences of Sex Development (DSD) are congenital medical conditions in which the external appearance of the individual does not coincide with the chromosomal constitution or the gonadal sex. 46,XY disorders of sex development (46,XY DSD) are clinically and genetically heterogeneous group that lead to genital abnormalities at birth, during puberty and in adulthood. Genetic and environmental factors play an important role in development of 46,XY DSD. The phenotype of patients with (DSD) is usually presented by a broad clinical spectrum ranging from genital ambiguity to phenotypes that appears normal, it can be diagnosed at wide range of ages, from neonatal period to late adulthood. The aim of the study is to evaluate the role of Multiplex Ligation dependent Probe Amplification (MLPA) technique in diagnosis of 46,XY DSD patients and to correlate genotypic abnormalities with the clinical phenotype and study possible correlation of paternal and maternal exposure to environmental risk factors to the causation of their phenotype.

Methods: The study was carried out on 35 patients selected from a total of 225 DSD patients referred to the Endocrinology and DSD Clinic at National Research Centre over a period of 3 years. Patients presented with variable presentations of disorders of sex development presenting with ambiguous genitalia, hypospadias, or with female phenotype with primary amenorrhea. Clinical personal, family history and thorough clinical examination were done with special emphasis on the appearance of external genitalia. Parents were asked to fill a questionnaire about frequency of dealing with some environmental factors including smoking, drugs and some other materials having estrogenic effect as plastics, insecticides, and others. Conventional cytogenetics studies and

fluorescence in situ hybridization (FISH) on peripheral blood as well as DNA extraction and Multiplex Ligation-dependent Probe Amplification (MLPA) were done using two kits including specific sex determination genes and genes for gonadal development disorder.

Results: In the current study parental consanguinity was detected in 60% of patients. Associated congenital abnormalities were detected in 14% of patients. Sixty nine percent of patients were in neonatal and childhood period, while 31% of patients were in pubertal and adult stage. Maternal exposure during pregnancy and paternal exposure to various Endocrine Disrupting Chemicals (EDCs) were insignificantly associated with DSD compared to control cases. Only 46,XY DSD cases were included in this study, however, 3 patients showed structural chromosomal abnormalities. MLPA analysis showed a clinically significance CNVs in 11% in the form of deletion in *SOX9 gene* in 2 patients, deletion in *DMRT1 gene* in 1 patient and duplication in *HSD17B3 gene* of unknown significant in 1 patient.

Conclusion: This study demonstrates the importance of proper detection of copy number variation (CNV) areas in DSD patients. It was concluded that using MLPA is recommended for DSD cases but it should be complemented with another high-resolution techniques as array comparative genomic hybridization or whole exome panel for DSD sequencing for whole genome coverage to detect unidentified genomic abnormalities.

Keywords: Disorders of Sex Development, Multiplex Ligation-dependent Probe Amplification, Copy Number Variation.

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List of Abbreviations

AMH	Anti-Mullerian hormone
ATX	Alpha Thalasemia X chromosome
AZF	Azoospermia factor of the human male Y chromosome
BMP15	Bone morphogenetic protein 15 gene
CAIS	Complete androgen insensitivity syndrome
CDY	Chromatin structure changing
CMPD	Campomelic dysplasia
CT	Computerized topography
DAX1	Dosage-sensitive sex reversal, adrenal hypoplasia critical region on chromosome X *gene* 1
DDS	Dose dependent sex reversal
DHT	Dihydrotestosterone
DIAPH	Homolog of drosophila Diaphanous gene
DMRT	Doublesex and Mab-3-related transcription factor
DSD	Disorders of sex development
FISH	Fluorescence insitu hybridization
FOXL	Forkhead box L gene
GB	Gonadoblastoma
GCT	Germ cell tumour
GD	Gonadal dysgenesis
GTG	Giemsa trypsin banding
HCG	Human chorionic gonadotropin
HSFY	Heat shock transcription factor Y
LH	Lutenizing hormone
MIS	Mullerian inhibitory substance

MRI	Magnetic resonance imaging
MSY	Male specific region of the Y chromosome
NR0B1	Nuclear receptor super family
NRY	Non-recombining Y region
PAR	Pseudo-autosomal region
PAIS	Partial androgen insensitivity syndrome
POF	Premature ovarian failure
RBMY	RNA binding motif Y chromosome
SCMY	Selected mouse cDNAinAZFb region on Y-chromosome
SF-1	Streroidogenic factor 1
SHOX	Short stature homeoboxgene
SOX9	SRY-box containing gene 9
SRY	Sex determining region of Y chromosome
SRA	Autosomal *sex reversal*
TSPY	Testis-specific protein Y-encoded
TS	Turner syndrome
TDF	Testis determining factor
UTY	Ubiquitous tetracopeptide Y
WT1	Wilm'stumour gene
XIC	X inactivation center
XIST	X- inactive specific transcript gene
ZFY	Zinc finger Y

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Introduction

Differences/ Disorders of sex development (DSD) is a revised term ***(Johnson et al., 2017)*** as disorders of sex development; it is a group of heterogeneous conditions with diverse pathophysiology. They are generally characterized by an abnormality of the chromosomal, gonadal or phenotypic features that typically define sex development. Such conditions usually present with atypical genitalia in the newborn period or as delayed puberty in an adolescent or present later in life as infertility ***(Hughes et al., 2008).***

A number of genes contribute both early and late to the process of sex determination and differentiation. *SRY*, located on the Y short arm proved to be the testes determining gene; however, several autosomal genes act in association with *SRY* contribute to normal development of male phenotype as *SOX9*, in which its expression is augmented by the action of *SRY* and NR5A1 together. Then, during testicular development anti-Mullerian hormone (AMH) production is regulated with help of *DMRT1* to repress genes such *as WNT4 and FOXL2* which are involved in ovarian development ***(Wilhelm et al., 2007).***

DSD affection ranges from neonatal period to adulthood with various phenotypes and severity ranging from atypical genitalia to male or female normal external genitalia. Yet, it is often difficult to provide patients with DSD with a definite molecular diagnosis due to great clinical heterogeneousity. Genetic testing is recognized as a key element in the investigation of individuals with a suspected DSD. The first line investigation of a possible DSD includes karyotype and FISH analysis, which is crucial when there is uncertainty about sex assignment, and important guide for management ***(Schober et al, 2012).*** Second line of investigation includes newer technologies as Multiplex Ligation-dependent Probe Amplification (MLPA) technique and array comparative