APPLICATION OF RECOMBINANT DNA TECHNOLOGY IN FRAGILE -X SYNDROME

THESIS SUBMITTED IN PARTIAL FULFILMENT FOR THE DEGREE OF M.D. HUMAN GENETICS

> ALICE K. TAMMAM ABD EL-ALEEM M.B., B.CH M.Sc. HUMAN GENETICS

5 3327

116.042

SUPERVISED BY

Pref.Dr. Mohamed A. Awadalia Prof. of Pediatrics & Genetics

Faculty of Medicine

Ain-Shams University

Prof.Dr. Samia A. El-Temtamy Prof. of Human Genetics

Human Genetics Departement National Research Center

Dr. Jörg Schmidtke Prof. of Human Genetics Institute of Human Genetics

Medizinische Hochschule Hannover, Germans

Prof. Dr. Moustafa K.El-Awady

Prof. of Biochemical & Molecular Genetics Human Genetics Departement National Research Center

Assistent Prof. Dr. Karam Abd El-Aleem

ssistent Prof. of Human Genetics Faculty of Medicine

Ain-Shams University

1995 (Q) 1915t

ACKNOWLEDGMENT

I would like to express my sincerest gratitude to Prof. Dr. Mohamed A. Awadallah, prof. of Pediatrics, Faculty of Medicine, Ain-Shams University for supervising this work and for his continuous kind guidance. I do want to thank him for the support he always offers.

I would like to express my recognition to Prof.Dr. Samia A. El-Temtamy, Prof. of Human Genetics, National Research Center for suggesting the point of the Thesis and for her enlightening supervision. I am deeply grateful to her for the generous cooperation she always offered.

I am grateful to Prof. Dr. Jörg Schmidtke, Prof. and Head of Institute of Humangenetics, MHH for the good chance he gave it to me to learn the basic techniques in molecular biology and to apply them in my work under his supervision I feel truly indebted to him because of the sound atmosphere he is creating in the institute, that the young researcher find the freedom in thinking and in the way of performing their individual work. He never said no for the application of a new technique or for the several times repetition of an unsuccessful experiment. I liked his way and I found it exceptional. I want to thank him for his instructions in rewiewing the Thesis.

I wish to thank Prof. Dr. Moustafa El-Awady, Prof. of biochemical and molecular genetics, National Research Center for his interest, valuable instructions and supervision of my Thesis.

Thanks to Dr. Karam Abd El- Aleem, Assistant Prof. of Human Genetics, Faculty of Medicine, Ain-Shams University for supervising my Thesis.

Thanks to Dr. Hanan Hussny, Lecturer of Human Genetics, National research Center for her help especially in the collection of some of the blood samples from Egyptian patients.



I wish to express my thanks to all my colleagues in the IHG, MHH especially Dr. Manfred Stuhrman who was my direct supervisor, he had helped me a lot and accepted my mistakes. Dr. Ingolf Böhm, the first one who learned me the ABC in molecular work and I did with him the first successful non-radioactive PCR experiment. Herrn Wolfgang Künau, he offered me a lot of his technical experience, his assessment was continuous during the two and half year that I spend it in the IHG MHH.

Many Thanks for Herrn Frank Schnieders, Frau Andria Weimann, Frau Tania Vogel,& Dr. Sybila Jakubiczki for their generous help and continuos support.

Contents

	page
- List of Intials and File Numbers for The Analysed Cases.	
- List of Tables.	
- List of Figures.	
- List of Abbreviations.	
1- Introduction	1
2- Aim of The Study	4
3- Review of Literature	. 5
3.1 Molecular Characterisation of The Fragile-X MR Syndrome	5
3.2 FRAXA / Clinical Overview	. 20
3.3 Cytogenetic Expression of Fragile-X Chromosome	23
3.4 Molecular Characterisation of FRAXE MR Syndrome	25
4- Material and Method	. 26
5- Results	60
6- Discussion	118
7- Summary and Recomendations	. 136
8- References	139
9- Appendix	151
Arabic Summary	

Names' intials & file number corresponding to the given case number

Group A:

Egyptian cases:

Family No.	Pedigree No.	Intials	File No.
1	П.2 Ш.4 П.3	mother of SF SF AF	3508
2	I.2 II.1 III.3 II.6	mother of AA AA MA Az A	4746 ၞ
3	П.8 П.2	HF TF	2689
4	I.2 II.3 II.2 II.1	RH AH HH YH	1750
5	II.2 III.3 III.2	mother of A El A El Y El	
6	I.2 II.5	mother of SS SS	4640
7	I.7 III.4	mother of A Eb A Eb	6597
8	П.1	Kh Ab	2750

German cases:

Family No.	Pedigree No.	File No.
9	II.2 III.3 III.2 II.3	922 923 924 1306
10	I.2 П.2 П.1	948 950 949
11	I.1 II.2 III.2 II.3 III.3 II.4	I II 0 840 838 833
12	L12 П.1 П.2	972 973 974
13	П.3 П.1 П.2	1196 Udo 1197
14	П.4 Ш.3 П.5	1121 1127 1109
15	H.2 Ш.2 Ш.1	976 975 9 8 8
16	III.3 IV.1 III.2	980 970 982
17	III.5 III.6 II.2 III.3 III.4 IV.1 IV.2 III.1	1824 1825 642 1822 1815 606 508 france

Family No.	Pedigree No.	File No.
18	No Pedigree	1028 1029
19	П.1 I.2	764 765
20	I.2 П.1	7 6
21	Ш.2 IV.2	17/88 f. 17/88 m.
22	I.2 II.2 III.1 III.2	18 19 22 23
23	II.2 III.3 III.2	12 5 4
24	I.2 II.1	9 8
25	I.2 II.2 III.2 IV.1 IV.2 III.3	8796 8797 8545 8546 8748 8798
26	П.6 П.5	9484 9339
27	Ш.3	9425

Group B:

Egyptian Cases:

Case No.	Intials	File No.
1	AR _.	5669
2 3	AEb I Ab	949
4 5	RM NM	2711
6 7	Ksh NEl	7027
8 9	AA TEI	6582
10 11	AS FAb	6549
12 13	MEb FAb	6856
14 15	FN AN	886
16&17 18&19 20&21 22&23		6800 6801 6603 5948

German cases:

Case No.	File No.
1	22/92
	115
2 3	273/92
4	269/92
5	301/92
6	348/92
7	151
8	Hŏpack
9	311
10	431
11	700
12	719
13	695
14	704
15	455
16	457
17	456
18	968
19	969
20	918
21	919
22	920
23	20
24	21
25	1036
26	1016
27	1124
28	1152
29	1061
30	913
31	641
32	771
33	776
34	759

List of Tables

		page
Table (1a)	Genetic, Cytogenetic and Main Clinical Features in 11 Egyptian	
	Affected Individuals (9 males & 2 females) with The Fra-X Full	
	Mutation	67
Table (1b)	: Expression of Fragility and Main Clinical Features in 11 Egyptian	
	Individuals With The Fra-X Full Mutation.	69
Table (2):	Genetic, Cytogenetic and Main Clinical Features in The Two	
	Egyptian Individuals Who Were Fra-X positive on Cytogenetic	
	Analysis and Showed Neither FRAXA Nor FRAXE Mutation	70
Table (3a)	: Genetic, Cytogenetic and Main Clinical Features in 26 German	
	Patients (20 males & 6 females) With Fra-X Full Mutation	71
Table (3b)	: Expression of Fragility and Main Clinical Features in 26 German	
	Individuals With The Fra-X Full Mutation.	76
Table (4):	Genetic, Cytogenetic and Main Clinical Features in Cases of	
	Group B, Whose Cytogenetic Results Were Negative or Not Done	
	and Proved to Carry the Typical Fra-X Mutation. (one Egyptian and	
	6 German Cases).	77
Table (5):	Genetic, Cytogenetic and Main Clinical Features in Two German	
	Male Carriers of Premutation Who Belong to Group B	79
Table (6):	Genetic, Cytogenetic and Main Clinical Features in 12 Egyptian	
	Probands of Group B in Whom the Typical Fra-X Mutation Was	
	Not Found.	80
Table (7):	Percentage of Cytogenetic Expression and Range of Full Mutation	
. ,	in 10 Egyptian Patients With Fra-X Mutation.	83

		Page
Table (8):	Percentage of Cytogenetic Expression and Range of Full Mutation	
	in 13 German Patients With Fra-X Mutation.	84

List of Figures

	Pa	ge
Figure (1):	Intron / exon distances of FMR1.	7
Figure (2):	Analysis of FMR1 protein in lymphoblastoid cell lines of males carrying normal or mutated alleles.	11
Figure (3):	Separation of StB12xx (1.7 kb) from the plasmid (2.9 kb) , after XhoI digestion , through 1 % LMP agarose.	44
Figure (4):	Dig-labeled genomic probes StB12.3 & oxE 20 via PCR amplification	44
Figure (5a)	: Electrophoretic separation of well digested genomic samples	47
Figure (5b)	Electrophoretic separation of incompletely digested genomic samples	47
Figure (6):	Diagrammatic representation of the Southern blotting technique	48
• .,	Diagrammatic representation showing the principle of non-radioactive chemiluminescence detection. (from Boehringer Mannheim)	50
- '	Schematic representation of normal & mutated restriction fragments detected by probe StB12.3	61
	Map of restriction sites & probes used in the direct DNA diagnosis of the fragile X syndrome.	61
Figure (10)	Restricion map across the FRAXE region.	65
Figure (11)	: Pedigree symbols	85
Figures (12	-49): Families Pedigrees	105

Figures (50 & 51):	Non - radioactive detection of normal, premuta full mutated	1
	using the PCR agarose assay and genomic Southern blot	
	with probe StB12.3, EcoRI/EagI double digest	108. 11

List of Abbreviations

APS Ammoniumperoxodisulfate

ATP Adenosine triphosphate

bp base pair C Cytosine

cDNA complementary DNA CTP Cytosine triphosphate

CGG Cytosine Guanine Guanine
C.V.S. Chorionic Villus Sample

d ATP deoxy Adenosine triphosphate
d CTP deoxy Cytosine triphosphate
d GTP deoxy Guanosine triphosphate
d TTP deoxy Thymidine triphosphate
d UTP deoxy Uridine triphosphate
DNA deoxy ribonucleic acid

Dig. Digoxigenin

DMSO Dimethylsulfoxide

EDTA Ethylendiamintetraacetat

g gram H2O Water

HGD Human Genetics Departement

hn RNA heterogeneous nuclear ribonucleic acid

IHG Institute of Human Genetics

Kb Kilobase
KD Kilodalton

LMP-agarose Low melting point agarose

L/V/I-G Leucine/valine/Isoleucine-Glycine

mRNA messenger RNA

M-B Martin-Bell syndrome

MCS Multiclonal sites (in plasmid vector)

MR Mental Retardation

MHH Medizinische Hochschule Hannover

NFM Normal Transmitting Male