

CONTENTS

	Page
Contents	viii
List of Tables	ix
List of Figures	x
List of Abbreviations and Symbols	xi
Chapter	
1. Introduction	1
Background and Rationale	1
Review of Literatures	2
Objectives	11
2. Research Methodology	12
Materials	12
Methods	17
3. Results	29
4. Discussion	59
5. Conclusion	81
Bibliography	82
Appendix	95
Vitae	133

LIST OF TABLES

Table	Page
1. The subgroups and numbers of DNA samples	13
2. The oligonucleotide sequences of primers	14
3. The microsatellite and SNP markers	18
4. The protocol for silver staining of nucleic acids in polyacrylamide gels	22
5. The conditions and product sizes of PCR	26
6. Allele distributions and frequencies of the microsatellite and SNP markers in normal controls and FXS patients	38
7. Frequencies of each haplotype set in normal and FXS groups	41
8. Common haplotypes among normal and FXS subjects using seven polymorphic markers	42
9. Haplotype associations in subgroups of normal CGG repeats	44
10. AGG Interruption patterns in each subgroup of control samples	47
11. Comparison of DXS548 markers among different populations	63
12. Comparison of FRAXAC1 markers among different populations	65
13. Comparison of SNP markers among different populations	67
14. Comparison of haplotype (DXS548-FRAXAC1) among different populations	69
15. Distribution of FMR1 flanking haplotypes (FRAXAC1-ATL1-IVS10) in four Asian populations	73
16. Comparison of the AGG interspersion patterns among different populations	76
17. Comparative analysis of haplotype (FRAXAC1-ATL1) and AGG configuration among different populations	78
18. The raw data for the relationship among CGG repeat numbers, haplotype and AGG interruption patterns	96
19. SNPs map in the <i>FMR1</i> gene	99
20. Allele nomenclature of DXS548	103
21. Allele nomenclature of FRAXAC1	103
22. The raw data of AGG interspersion patterns within nine ethnic groups	104
23. Relationship among CGG repeat numbers, haplotype (FRAXAC1-ATL1) and AGG interruption patterns in six different ethnic groups	114

LIST OF FIGURES

Figure	Page
1. The locations of microsatellite and SNP markers	19
2. Schematic of the tetra-primer in Biallelic-ARMS PCR method	21
3. Schematic of PCR-RFLP for rs25731	25
4.1. The representative polyacrylamide gel for DXS548	29
4.2. The representative polyacrylamide gel for FRAXAC1	30
4.3. The representative agarose gel for WEX5	31
4.4. The representative agarose gel for ATL1	32
4.5. The representative agarose gel for rs25731	33
4.6. The representative agarose gel for IVS10	34
4.7. The representative agarose gel for rs25702	35
4.8. The representative agarose gel for rs25723	36
5. The alignment of CGG repeat regions	46
6. AGG Interspersion patterns of 95 CGG alleles	49
7.1. The pedigree of affected family 4 and 16	50
7.2. The pedigree of affected family 20	51
8.1. The alignment of AC repeat regions for FRAXAC1	52
8.2. The alignment of WEX5	53
8.3. The alignment of ATL1	54
8.4. The alignment of rs25731	55
8.5. The alignment of IVS10	56
8.6. The alignment of rs25702	57
8.7. The alignment of rs25723	58
9. A model suggesting the causes of repeat instability	80
10. The CGG allele distribution in normal Thai males	122
11.1. The electropherograms of the CGG-21 allele	123
11.2. The electropherograms of the CGG-29 allele	124
11.3. The electropherograms of the CGG-30 allele	125
11.4. The electropherograms of the CGG-33 allele	126
11.5. The electropherograms of the CGG-36 allele	127
11.6. The electropherograms of the CGG-43 allele	128

LIST OF ABBREVIATIONS AND SYMBOLS

AC	=	adenosine-cytosine
APS	=	ammonium persulfate
ARMS	=	amplification refractory mutation system
AGG	=	adenosine-adenosine-guanine
bp	=	base pair(s)
BSA	=	bovine serum albumin
cm	=	centimeter
°C	=	degree Celsius
CGG	=	cytosine-guanine-guanine
CNS	=	central nervous system
CpG	=	cytosine preceding guanine
dATP	=	deoxyadenosine-5'-triphosphate
dCTP	=	deoxycytidine-5'-triphosphate
dGTP	=	deoxyguanosine-5'-triphosphate
7-deaza-dGTP	=	7-deaza- deoxyguanosine-5'-triphosphate
dTTP	=	deoxythymidine-5'-triphosphate
dNTPs	=	dATP+ dCTP+ dGTP+ dTTP
DMSO	=	dimethylsulfoxide
DNA	=	deoxyribonucleic acid
<i>Dra</i>	=	<i>Deinococcus radiophilus</i>
EDTA	=	ethylenediamine tetra-acetic acid, disodium salt
et al.	=	et ali (Latin) and others
EtBr	=	ethidium bromide
FM	=	full mutation
<i>FMR1</i>	=	<i>fragile X mental retardation 1</i>
FMRP	=	fragile X mental retardation protein
FXS	=	fragile X syndrome
Hap	=	haplotype
Het	=	heterozygosity
i.e.	=	id est, for example
IQ	=	intelligence quotient
Kb	=	kilobase pair (s)

LIST OF ABBREVIATIONS AND SYMBOLS (CONTINUED)

M	=	molar
mg	=	milligram
min	=	minute (s)
μl	=	microliter
ml	=	milliliter
μM	=	micromolar
mM	=	millimolar
MR	=	mental retardation
mRNA	=	messenger ribonucleic acid
ng	=	nanogram
OD	=	optical density
PCR	=	polymerase chain reaction
PM	=	premutation
q-arm	=	long arm of chromosome
RE	=	restriction enzyme
RNA	=	ribonucleic acid
rpm	=	revolution per minute
sec	=	second (s)
SDS	=	sodium dodecyl sulfate
SNP	=	single nucleotide polymorphism
TAE	=	tris acetate, EDTA
Taq	=	thermus aquaticus
TBE	=	tris borate, EDTA
TEMED	=	N, N, N', N'-tetramethyl-ethylenediamine
Tris-HCl	=	Tris-(hydroxymethyl)-aminoethane hydrochloride acid
U	=	unit (s)
UTR	=	untranslated region
UV	=	ultraviolet