CONTENTS

	Page
Contents	(viii)
List of Tables	(xii)
List of Figures	(xiii)
List of Abbreviations and symbols	(xv)
Chapter	
1 Introduction	1
2 Literature review	4
2.1 Characteristics of breast cancer	4
2.1.1 Breast anatomy and physiology	4
2.1.2 Breast cancer	4
2.1.3 Classification of the breast cancer	6
2.1.3.1 Invasive carcinoma	6
2.1.3.2 Carcinoma in situ	7
2.1.3.3 Cancer of the male breast	8
2.2 Risk factors	9
2.2.1 Age	9
2.2.2 Family history	10
2.2.3 Hormonal and reproductive factors	10
2.2.4 Race / Ethnicity	12
2.2.5 Smoking	13
2.2.6 Breastfeeding	14
2.2.7 Food	14
2.2.8 Benign breast disease	15
2.2.9 Environmental factor	15
2.2.10 Genetic factor	15
2.3 Breast cancer susceptibility gene 1 or BRCA1	17
2.4 Structure and function of the BRCA1 protein	18
2.5 Pathobiology of hereditary breast cancer	20

CONTENTS (Continued)

			Page
	2.6	Mutation spectrum of BRCA1	20
	2.7	Genetic testing for the BRCA1 gene	24
	2.8	The screening techniques	26
		2.8.1 Single-stranded conformation polymorphism (SSCP)	28
		2.8.2 Heteroduplex analysis (HA)	28
		2.8.3 Protein truncation test (PTT)	29
		2.8.4 Direct sequencing (DS)	30
	2.9	Recommendations of high risk women	30
		2.9.1 Breast self-examination (BSE)	31
		2.9.2 Clinical breast examination (CBE)	31
		- Mammography	31
		- Ultrasound	32
		- Magnetic resonance imaging (MRI)	32
		2.9.3 Prevention surgery	32
		2.9.4 Chemoprevention	33
3	Ma	terials and Methods	34
	3.1	Materials	34
		3.1.1 Clinical specimens	34
		3.1.2 Materials for genomic DNA	34
		3.1.3 Materials for polymerase chain reaction (PCR)	35
		3.1.4 Materials for agarose gel electrophoresis	35
		3.1.5 Materials for single-stranded conformation polymorphism	35
		3.1.6 Materials for DNA sequencing	36
		3.1.7 PCR primers specific for <i>BRCA1</i> gene	36
	3.2	Instruments	38
	3.3	Methods	38
		3.3.1 Genomic DNA extraction	38
		3.3.1.1 Collection of blood samples	38
		3.3.1.2 Separation of white blood cells	39

CONTENTS (Continued)

			Page
		3.3.1.3 DNA extraction from white blood cells	39
		3.3.2 The purity and concentration of DNA	39
		3.3.3 Screening for <i>BRCA1</i> mutations	40
		3.3.3.1 DNA amplification	40
		3.3.3.2 Analysis of the exon-intron 7 boundary sequence of	40
		Patient ID 17 and the patient's daughter	
		3.3.3.3 Agarose gel electrophoresis	42
		3.3.3.4 Single-Stranded Conformation Polymorphism	43
	3.4	Data analysis	44
4	Res	ults	45
	4.1	Clinical / pathological characteristics of the patients	45
	4.2	Purity and concentrations of the genomic DNA	48
	4.3	PCR optimization for the amplification of BRCA1 exons	49
		4.3.1 Magnesium concentration	51
		4.3.2 Primer concentration	51
		4.3.3 Template concentration	52
		4.3.4 PCR programs	52
		4.3.5 Annealing temperature	52
	4.4	Single-Stranded Conformation Polymorphism analysis of	55
		BRCA1 exons	
	4.5	Direct sequencing of BRCA1 exons	73
	4.6	Single-Stranded Conformation Polymorphism (SSCP) analysis of	75
		patient's healthy daughter (ID18)	
	4.7	Sequence analysis of the exon-intron 7 boundary of patient ID 17	78
		and the patient's daughter (ID 18)	
	4.8	Pfu DNA polymerase on patients whose exons exhibiting changes	78
		in SSCP analysis	
5	Disc	cussion	82

CONTENTS (Continued)

	Page
5.1 Pathological background of the patients	82
5.2 PCR-SSCP analysis	83
5.3 BRCA1 mutations in Thai breast cancer patients	86
5 Conclusion	102
Bibliography	
Appendix	127
Vitae	135

LIST OF TABLES

Tak	ole	Page
2.1	Percentage of breast tumors classified by locations	6
2.2	Cumulative risks (standard error) of breast cancer among relatives	16
	with BRCA1 mutation	
2.3	Estimated cancer risks in BRCA1 mutation carriers	16
2.4	Total number of mutation, polymorphism and variants of BRCA1	21
	from Breast Cancer Information Core (BIC) database	
2.5	Frequencies of BRCA1 mutation types	22
2.6	The advantages and disadvantages of each method used in routine	27
	diagnosis	
3.1	Oligodeoxyribonucleotide primers used for PCR amplification and	36
	nucleotide sequences	
3.2	The conditions of thermal cycle	40
3.3	Oligodeoxyribonucleotide primers used for PCR amplification of	42
	exon-intron 7 boundary and nucleotide sequences	
3.4	Preparation of 10% non-denaturing polyacrylamide gel	43
4.1	Percentage of the patients classified by clinical/pathological	45
	characteristics	
4.2	Summary of purity and concentrations of the genomic DNA	48
4.3	Summary of optimal conditions for PCR amplification of BRCA1	49
	exons	
4.4	Summary of selected patients whose exons exhibiting changes in	55
	electrophoretic mobility on 10% non-denaturing polyacrylamide gel	
5.1	Examples of disorders of trinucleotide repeat expansions and deletion	95
5.2	DNA repeats, symmetry elements and alternative DNA structures	99
7	The family history information and clinical/pathological	128
	characteristics of 17 patients	

LIST OF FIGURES

Fig	ure	Page
2.1	Schematic illustration of breast anatomy, lymphatic drainage of the	5
	breast and breast position	
2.2	Normal breast tissue	8
2.3	Cancerous breast tissue (ductal carcinoma)	8
2.4	Cancerous breast tissue (lobular carcinoma)	9
2.5	Age-standardized incidence and mortality rates for breast cancer	13
2.6	Two-hit hypothesis of tumor suppressor gene	17
2.7	Schematic diagram of the BRCA1 protein and sites of its interaction	19
	with other proteins	
2.8	Suggested algorithm for genetic screening in women with family	25
	history of breast cancer	
3.1	The exon-intron 7 boundary sequences in the BRCA1 gene	41
4.1	The pedigrees of 5 patients with a family history of cancer	46
4.2	The effect of primer concentration on PCR amplification of	51
	BRCA1 gene using BRCF3 and BRCR3 primers	
4.3	A-E The pattern of PCR products amplified with each pair of primer	52
	at designated annealing temperature of BRCA1 exons	
4.4	4.19 The typical SSCP results for each exon of <i>BRCA1</i> of 31	56
	Thai patients	
4.20	SSCP analysis of the PCR fragment of <i>BRCA1</i> exons of patient ID 17	72
4.2	Genomic sequences of the <i>BRCA1</i> IVS7+34_47	73
	del TTCTTTTTTT deletion	
4.22	2 Pedigree of patient ID 17	74
4.23	3 SSCP analysis of the PCR fragment of BRCA1 exons of ID 18	76
4.24	4 Genomic sequences of the <i>BRCA1</i> exon 7 - intron 7 boundary in	77
	the sense and antisense strands of the patient ID 17's healthy daughter	
4.23	5 Single-stranded conformation polymorphism of PCR products with	78
	silver staining	

LIST OF FIGURES (Continued)

Fig	ure	Page
4.26	6 Genomic sequence of the <i>BRCA1</i> IVS7+50_63del	80
	TTCTTTTTTTTT deletion using Pfu DNA polymerase in the sense	
	And antisense strand sequences of the patient ID 17	
4.27	Genomic sequence of the <i>BRCA1</i> exon-intron 7 boundary using <i>Pfu</i>	81
	DNA polymerase in the sense and antisense strand sequences of the	
	healthy patient's daughter	
5.1	Deduced normal nucleotide sequence containing the (TTC) ₇ .(GAA) ₇	91
	repeat tract in intron	
5.2	Proposed looped structure involved in	92
	IVS7+34_47delTTCTTTTTTT	
5.3	Location of repeat tract in triplex repeat disease genes	93
5.4	Location of repeat tract in triplex repeat of BRCA1 gene	94
5.5	Non-B DNA conformations involved in genomic rearrangements	96
5.6	Proposed intramolecular triplex DNA structures and looped structure	97
	For IVS7+34_47delTTCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	
5.7	Proposed looped structure involved in	98
	IVS7+50_63 delTTCTTTTTTTTTT in the sense strand and in	
	IVS7+34_47 delAAGAAAAG AAAAA in the antisense strand of	
	the proband's daughter	
5.7	A-C Proposed intramolecular triplex DNA structures and looped	100
	structure formed for the IVS7+50_63delTTCTTTTTTTTT and	
	IVS7+34_47 delAAGAAAAG AAAAA of the proband's daughter	

LIST OF ABBREVIATIONS AND SYMBOLS

 $AgNO_3$ = silver nitrate

ASO = allele-specific oligonucleotide hybridization

ASR = age-standardized incidence rates

BAP1 = BRCA1-associated proteins

BARD = BRCA1-associated RING-domain protein

BIC = breast information core

bp = base pair

BRCA1 = breast cancer susceptibility gene 1 / breast cancer suppressor

gene 1

BRCT = BRCA1 *C*-terminal domain

BSE = breast self-examination

CBE = clinical breast examination

CCM = chemical cleavage mismatch

°C = degrees celsius

dATP = deoxyadenosine triphosphate

DCIS = ductal carcinoma *in situ*

dCTP = deoxycytidine triphosphate

DDF = dideoxy fingerprinting assay

DDT = dichlorodiphenyl trichloroethane

DGGE = denaturing gradient gel electrophoresis

dGTP = deoxyguanosine triphosphate

dHPLC = denaturing high performance liquid chromatography

DNA = deoxyribonucleic acid

dNTP = deoxynucleotide triphosphate

DS = direct sequencing

dTTP = deoxythymidine triphosphate

EDTA = ethylenediaminetetraacetate

ERE = estrogen responsive element

 $ER-\alpha$ = estrogen receptor alpha

LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

Fig = figure

HA = heteroduplex analysis

hr = hour

HRT = hormone replacement therapy

ID = identity

IDC = invasive ductal carcinoma

ILC = invasive lobular carcinoma

IVS = noncoding intervening sequence

KCl = potassium chloride

LCIS = lobular carcinoma *in situ*

LIQ = lower inner quadrant
LOH = loss of heterozygosity
LOQ = lower outer quadrant

LOQ lower outer qua

mg = milligram

 $MgCl_2$ = magnesium chloride

ml = millilitre mM = millimolar

MRI = magnetic resonance imaging
mRNA = messenger ribonucleic acid

ng = nanogram

NLS = nuclear localization signal domain

OCP = oral contraceptive

 OD_{260} = optical density at 260 nm OD_{280} = optical density at 280 nm PCBs = polychlorinated biphenyls PCR = polymerase chain reaction

PTT = protein truncation test

Rb = retinoblastoma gene

rpm = revolutions per minute

LIST OF ABBREVIATIONS AND SYMBOLS (Continued)

SDS = sodium dodecyl sulphate

SSCP = single-stranded conformation polymorphism

TAE = tris-acetate- ethylenediaminetetraacetate buffer

TE = tris-ethylenediaminetetraacetate buffer

TEMED = N, N, N', N'-tetramethylethylenediamine

Tris = tris (hydroxymethyl) aminomethane

 T_m = melting temperature UIQ = upper inner quadrant UOQ = upper outer quadrant

UV = ultraviolet

WBC = white blood cell

 $\mu M = micromolar$

 $\mu g = microgram$