# MICROARRAY-BASED PHARMACOGENETICS TESTING AND HAPLOTYPE/PREDICTED PHENOTYPES DETERMINATION OF *CYP2D6* AND *CYP2C19* CORRELATION WITH TAMOXIFEN RESPONSE IN THAI PATIENTS IN ADJUVANT TREATMENT OF BREAST CANCER

**MONTRI CHAMNANPHON**

**A THESIS SUBMITTED IN PARTIAL FULFILLMENT**
**OF THE REQUIREMENTS FOR**
**THE DEGREE OF MASTER OF SCIENCE**
**(CLINICAL PATHOLOGY)**
**FACULTY OF GRADUATE STUDIES**
**MAHIDOL UNIVERSITY**
**2013**

Thesis
entitled

# MICROARRAY-BASED PHARMACOGENETICS TESTING AND HAPLOTYPE/PREDICTED PHENOTYPES DETERMINATION OF *CYP2D6* AND *CYP2C19* CORRELATION WITH TAMOXIFEN RESPONSE IN THAI PATIENTS IN ADJUVANT TREATMENT OF BREAST CANCER


……..
Mr.Montri Chamnanphon
Candidate

..……..
Asst. Prof. Chonlaphat Sukasem,
Ph.D.
Major-advisor

……..
Prof. Wasun Chantratita,
Ph.D.
Co-advisor

……..
Assoc. Prof. Wilai Noonpakdee,
Ph.D.
Co-advisor

……..
Mr.Ekawat Pasomsub,
Ph.D.
Co-advisor

……
Prof. Banchong Mahaisavariya,
M.D., Dip. Thai Board of Orthopedics
Dean
Faculty of Graduate Studies
Mahidol University

……..
Asst. Prof. Pitak Santanirand,
Ph.D.
Program Director
Master of Science Program
in Clinical Pathology
Faculty of Medicine
Ramathibodi Hospital
Mahidol University

Thesis
entitled

# MICROARRAY-BASED PHARMACOGENETICS TESTING AND HAPLOTYPE/PREDICTED PHENOTYPES DETERMINATION OF *CYP2D6* AND *CYP2C19* CORRELATION WITH TAMOXIFEN RESPONSE IN THAI PATIENTS IN ADJUVANT TREATMENT OF BREAST CANCER

was submitted to the Faculty of Graduate Studies, Mahidol University
for the degree of Master of Science (Clinical Pathology)
on
February 14, 2013

……..
Mr.Montri Chamnanphon
Candidate

...……...
Assoc. Prof. Wichittra Tassaneeyakul,
Ph.D.
Chair

…….
Asst. Prof. Chonlaphat Sukasem,
Ph.D.
Member

…….
Prof. Wasun Chantratita,
Ph.D.
Member

…….
Assoc. Prof. Wilai Noonpakdee,
Ph.D.
Member

…….
Mr.Ekawat Pasomsub,
Ph.D.
Member

…….
Prof. Banchong Mahaisavariya,
M.D., Dip. Thai Board of Orthopedics
Dean
Faculty of Graduate Studies
Mahidol University

……..
Prof. Winit Phuapradit,
M.D., M.P.H.
Dean
Faculty of Medicine
Ramathibodi Hospital
Mahidol University

# ACKNOWLEDGEMENTS

This thesis could not be possibly completed without the extensive support and assistance from my major advisor, Asst. Prof. Chonlaphat Sukasem and my co-advisor, Prof. Wasun Chantratita, Assoc. Prof. Wilai Noonpakdee and Dr. Ekawat Pasomsub. I deeply thank them for their helpful comments, guidance, valuable advice, supervision and encouragement throughout the course of this thesis. They were never lacking in kindness and support.

I also would like to sincerely thank Assoc. Prof. Wichittra Tassaneeyakul for serving as my external examiner of thesis defense.

I would like to special thank for all of staffs at the Division of Pharmacogenomics and Personalize Medicine and Division of Virology laboratory in Department of Pathology, Faculty of Medicine at Ramathibodi Hospital, Mahidol University, for their helpful in providing facilities and materials for my thesis experiment.

I would like to special thank for Thailand Center of Excellent Life Science (TCELS) for the grant.

Finally, my graduation would not be achieved without the best wish from my parents, Mr.Noo and Mrs.Buddee Chamnanphon, who help me for everything and always gives me greatest love, willpower and financial support until this study completion and the last gratefully special thanks to my relation and my friends for their help and encouragement.

Montri Chamnanphon

MICROARRAY-BASED PHARMACOGENETICS TESTING AND HAPLOTYPE/PREDICTED PHENOTYPES DETERMINATION OF *CYP2D6* AND *CYP2C19* CORRELATION WITH TAMOXIFEN RESPONSE IN THAI PATIENTS IN ADJUVANT TREATMENT OF BREAST CANCER

MONTRI CHAMNANPHON 5336203 RACP/M

M.Sc. (CLINICAL PATHOLOGY)

THESIS ADVISORY COMMITTEE: CHONLAPHAT SUKASEM, Ph.D., WASUN CHANTRATITA, Ph.D., WILAI NOONPAKDEE, Ph.D., EKAWAT PASOMSUB, Ph.D.

ABSTRACT

The cytochrome P450 (CYP) 2D6 and 2C19 enzymes had an influence in the clinical outcomes of breast cancer patients treated with tamoxifen. Forty-eight Thai breast cancer patients receiving tamoxifen therapy were recruited to investigate the impact of *CYP2D6* and *CYP2C19* polymorphisms in predicting tamoxifen efficacy and clinical outcomes in Thai breast cancer patients. The study was designed by matching 24 cases and 24 controls. The *CYP2D6* and *CYP2C19* genotypes were determined using the microarray-based technique. The association of polymorphisms in *CYP2D6* and *CYP2C19* on disease free survival (DFS) was evaluated using the Kaplan-Meier method and Cox regression analysis.

Based on the genotype data, five *CYP2D6* predicted phenotype groups were identified in this study including EM/EM (11 of 48, 22.9%), EM/IM (20 of 48, 41.7%), EM/PM (2 of 48, 4.2%), IM/IM (12 of 48, 25%), IM/PM (3 of 48, 6.2%) and three *CYP2C19* phenotype groups including EM/EM (21 of 48, 43.8%), EM/IM (24 of 48, 50%) and PM/PM (3 of 48, 6.2%). The *CYP2D6* variant alleles were **10* (44 of 96, 45.9%), **5* (4 of 96, 4.2%), **41* (2 of 96, 2.1%), **4* (1 of 96, 1%), **36* (1 of 96, 1%) and *CYP2C19* variant alleles were **2* (24 of 96, 25%), **3* (6 of 96, 6.2%). Kaplan-Meier estimates showed significant shorter disease free survival in patients with homozygous *TT* compared to those with heterozygous *CT* or homozygous *CC* at nucleotides 100C>T and 1039C>T in post-menopausal (Log-rank test, $P = 0.046$), and had an increased risk of recurrence of breast cancer, but no statistically significant association was observed (HR, 2.01; 95% CI, 0.95-4.28; $P = 0.068$).

The *CYP2D6* and *CYP2C19* polymorphisms were not involved in tamoxifen efficacy, except in post-menopausal subgroup. As the number of breast cancer patients was relatively small in this study, the results should be confirmed in a larger group of prospective patients.

KEY WORDS: *CYP2D6*/ *CYP2C19*/ POLYMORPHISMS/ BREAST CANCER/ PHARMACOGENETICS

142 pages

การตรวจวินิจฉัยทางเภสัชพันธุศาสตร์และการแปลผลแบบ haplotype/predicted phenotypes ของยีน *CYP2D6* และ *CYP2C19* ในผู้ป่วยมะเร็งเต้านมกับผลการตอบสนองต่อการรักษาเสริมของยา tamoxifen ด้วยเทคนิคไมโครอาเรย์

MICROARRAY-BASED PHARMACOGENETICS TESTING AND HAPLOTYPE/PREDICTED PHENOTYPES DETERMINATION OF *CYP2D6* AND *CYP2C19* CORRELATION WITH TAMOXIFEN RESPONSE IN THAI PATIENTS IN ADJUVANT TREATMENT OF BREAST CANCER

มนตรี ชำนาญพล 5336203 RACP/M

วท.ม. (พยาธิวิทยาคลินิก)

คณะกรรมการที่ปรึกษาวิทยานิพนธ์: ชลภัทร สุขเกษม, Ph.D., วสันต์ จันทราทิตย์, Ph.D., วิไล หนุนภักดี, Ph.D., เอกวัฒน์ ผสมทรัพย์, Ph.D.

บทคัดย่อ

ไซโทโครม พี 450 (CYP) 2D6 และ 2C19 เป็นเอนไซม์ที่มีอิทธิพลต่อผลทางด้านคลินิกของผู้ป่วยมะเร็งเต้านมซึ่งได้รับการรักษาเสริมด้วยยาทามอกซิเฟน ตัวอย่างผู้ป่วยมะเร็งเต้านมหญิงจำนวน 48 ราย ได้รับการคัดเลือกเพื่อนำมาศึกษา โดยแบ่งผู้ป่วยออกเป็น 2 กลุ่ม เท่ากัน คือ กลุ่มที่ไม่เกิดมะเร็งเต้านมกลับเป็นซ้ำ 24 ราย และกลุ่มที่เกิดกลับเป็นซ้ำ 24 ราย แล้วนำตัวอย่างที่ได้ไปศึกษาด้วยเทคนิคไมโครอาเรย์ เพื่อหาความสัมพันธ์ระหว่างความหลากหลายทางพันธุกรรมของยีน *CYP2D6* และ *CYP2C19* กับประสิทธิผลของยาทามอกซิเฟนและระยะเวลาปลอดการกลับเป็นซ้ำ โดยการวิเคราะห์ข้อมูลใช้สถิติ Kaplan-Meier method และ Cox regression

ผลการศึกษาพบว่า มีความถี่จีโนไทป์ของยีน *CYP2D6* 5 รูปแบบ ดังนี้ EM/EM (11 of 48, 22.9%), EM/IM (20 of 48, 41.7%), EM/PM (2 of 48, 4.2%), IM/IM (12 of 48, 25%), IM/PM (3 of 48, 6.2%) และความถี่จีโนไทป์ของยีน *CYP2C19* 3 รูปแบบ ดังนี้ EM/EM (21 of 48, 43.8%), EM/IM (24 of 48, 50%), PM/PM (3 of 48, 6.2%) ความถี่อัลลีลของยีน *CYP2D6* ดังนี้ **10* (44 of 96, 45.9%), **5* (4 of 96, 4.2%), **41* (2 of 96, 2.1%), **4* (1 of 96, 1%), **36* (1 of 96, 1%) และความถี่อัลลีลของยีน *CYP2C19* ดังนี้ **2* (24 of 96, 25%), **3* (6 of 96, 6.2%) โดยพบว่าลักษณะความหลากหลายทางพันธุกรรมของยีนทั้ง 2 ไม่มีความแตกต่างกันระหว่าง 2 กลุ่ม และจากการวิเคราะห์ระยะเวลาปลอดการกลับเป็นซ้ำของผู้ป่วยมะเร็งเต้านม พบว่า ผู้ป่วยกลุ่มวัยหมดประจำเดือนที่มีจีโนไทป์ของยีน *CYP2D6* สนิปส์ตำแหน่ง 100C>T และ 1039C>T แบบ *TT* มีระยะเวลาปลอดการกลับเป็นซ้ำที่สั้นกว่ากลุ่มที่มีจีโนไทป์แบบ *CT* และ/หรือ *CC* และพบว่ามีความเสี่ยงต่อการกลับเป็นซ้ำของมะเร็งเต้านมสูงมากกว่าผู้ที่ไม่มีจีโนไทป์แบบนี้

โดยสรุป คือ ลักษณะความหลากหลายทางพันธุกรรมของยีน *CYP2D6 และ CYP2C19* อาจจะมีอิทธิพลต่อระยะเวลาปลอดการกลับเป็นซ้ำในผู้ป่วยมะเร็งเต้านมกลุ่มวัยหมดประจำเดือน แต่เนื่องจากข้อมูลจำนวนกลุ่มตัวอย่างผู้ป่วยที่ใช้ในการศึกษานี้มีจำนวนน้อย ดังนั้น จึงจำเป็นต้องมีการศึกษาเพิ่มเติมในอนาคต

142 หน้า

# CONTENTS

# CONTENTS (cont.)

# LIST OF TABLES

# LIST OF TABLES (cont.)

# LIST OF FIGURES

# LIST OF FIGURES (cont.)

# LIST OF FIGURES (cont.)

# LIST OF FIGURES (cont.)

# LIST OF FIGURES (cont.)

# LIST OF ABBREVIATIONS

| | |
|---|---|
| A | Adenine |
| ABCB1 | ATP-binding cassette sub-family B member 1 |
| ABCC2 | ATP-binding cassette sub-family C member 2 |
| AIs | Aromatase inhibitors |
| BCSS | Breast cancer specific survival |
| C | Cytosine |
| CYP | Cytochrome P450 |
| $^{o}$C | Degree of Celsius |
| DDFS | Distant disease free survival |
| DFS | Disease free survival |
| DNA | Deoxyribonucleic acid |
| dbSNP | Database SNP |
| del | Deletion |
| dNTP | Deoxynucleotide triphosphate |
| EDTA | Ethylene diamine tetraacetic acid |
| EMs | Extensive metabolizers |
| ER | Estrogen hormone receptor |
| E2 | 17β- estradiol |
| FNA | Fine needle aspiration |
| G | Guanine |
| GC | Gene conversion |
| g | Gram |
| HER2/neu | Human epidermal growth factor type 2 receptor |
| HR | Hazard ratio |
| HWpval | Hardy-Weinberg equilibrium p value |
| IMs | Intermediated metabolizers |
| ins | Insertion |

# LIST OF ABBREVIATIONS (cont.)

| | |
|---|---|
| LD | Linkage disequilibrium |
| M | Molar |
| MAF | Minor allele frequency |
| Mg | Magnesium |
| MRI | Magnetic resonance imaging |
| mg | Milligram |
| mL | Milliliter |
| mM | Milli molar |
| ng | Nanogram |
| nMRI | Nuclear magnetic resonance imaging |
| ObsHET | Observed heterozygosity |
| OS | Overall survival |
| OR | Odd ratio |
| PCR | Polymerase chain reaction |
| PD | Pharmacodynamic |
| PK | Pharmacokinetic |
| PMs | Poor metabolizers |
| PR | Progesterone hormone receptor |
| PredHET | Predicted heterozygosity |
| RFS | Recurrence free survival |
| rs | Reference SNP |
| SERMs | Selective estrogen receptor modulators |
| SNPs | Single nucleotide polymorphisms |
| SULT1A1 | Sulfotransferase 1A1 |
| T | Thymine |
| TAM | Tamoxifen |
| TdT | Terminal deoxynucleotide transferase |
| TTP | Time to disease progression |

# LIST OF ABBREVIATIONS (cont.)

| | |
|---|---|
| U | Unit |
| UGT | Uridinediphosphate - glucuronosyltransferase |
| UMs | Ultra-rapid metabolizers |
| US FDA | United State food and drug administration |
| µL | Microliter ($10^{-6}$) |

# CHAPTER I
# INTRODUCTION

Breast cancer is the most prevalent cancer among women and affects approximately one million women worldwide and Thailand [1]. Tamoxifen is the selective estrogen receptor modulators (SERMs) that can bind to estrogen receptor/ progesterone receptors in breast cancer tissue. After tamoxifen binding with estrogen receptors, the activity of estrogen can be blocked. Therefore, it is the most commonly prescribed drug used therapy for the treatment and prevention of estrogen receptor (ER)-positive or progesterone receptor (PR)-positive breast cancers [2], treatment in women with ductal carcinoma *in situ* (DCIS) [3], and adjuvant treatment in women with pre-menopausal breast cancer [4]. It has been used for more than 30 years to treat breast cancer.

Tamoxifen considered as a prodrug that primarily metabolized by cytochrome P450 (CYP) enzymes (including CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4 and CYP3A5), a group of predominance liver enzymes responsible for the metabolism of tamoxifen into active and inactive form [5]. Tamoxifen is required metabolism into its pharmacologic activity. It is described that 4-hydroxytamoxifen (4-OH-tamoxifen) and 4-hydroxy-*N*-desmethyltamoxifen (endoxifen), are the potent active metabolites. Both metabolites exert a 30- to 100-fold higher affinity to ER compared with tamoxifen in repress estrogen dependent cell proliferation [6-8]. Several studies have reported that endoxifen is a greatest response for activity of tamoxifen, and endoxifen is more potent an antiestrogen than 4-OH-tamoxifen and tamoxifen.

Polymorphisms of *CYP* genes which has an activity importance for metabolism of drugs as certain allele variants determine either altered activity or non-functional of enzyme activity [9]. The CYP isoenzymes which encoded by the *CYP2D6* and *CYP2C19* genes are responsible for the metabolism of a number of prescribed drugs [10]. The *CYP2D6* gene that encode debrisoquine hydroxylase

enzyme and more than 100 allelic variants have been reported [9, 11], which lead to a broad spectrum of enzyme metabolic activity and difference of phenotype in within populations [12]. The *CYP2D6* gene has result in four phenotypic types: individual carry two wild types allele as *CYP2D6*1/*1*, referred to extensive metabolizers (EM) with at least one functional allele. Intermediate metabolizers (IM) as *CYP2D6*10/*10* display two reduced activity alleles or one null activity allele. Poor metabolizers (PM) as *CYP2D6*4/*4* display two alleles gene inactivation and are qualify by deficiency hydroxylation of a number of drug classes and xenobiotic. Ultra-rapid metabolizers (UM) as *CYP2D6*1/*1XN* display excess enzyme activity due to multiple copies of *CYP2D6* alleles from gene duplication, which lead to higher plasma concentration of endoxifen [13, 14]. The distributions of normal functional, partially active functional and inactive functional *CYP2D6* alleles depend on the ethnic/racial [15-17]. Reduced (partially active) functional alleles include *CYP2D6*10* (100C>T) have a reported high frequency in Asian (51%), 6% in African populations and it is rare in Caucasian population (1-2%). Nonfunctional (Inactive) allele includes *CYP2D6*4* (1846G>A) have reported highest frequency in European Caucasians (12-21%) and the lowest frequency in Asian populations (1%) [18, 19]. The UM phenotypes have a reported highest frequency in Ethiopian and Saudi Arabian populations [20].

The *CYP2C19* gene that encode S-mephenytoin hydroxylase enzyme, has two major variant alleles that result in deficiency of enzyme. The most common variation are represented by the *CYP2C19*2* (681G>A) and **3* (636G>A) alleles [21, 22]. It has been reported that patients with PM who receiving tamoxifen have significantly lower level of plasma concentrations of endoxifen [6, 23, 24]. Several studies suggested that the clinical outcome of PM patients with breast cancer on adjuvant tamoxifen lead to have the risk of breast cancer recurrence and a lower prevalence of hot flashes [25].

Consequently, the aims of this study were investigated the impact of *CYP2D6* and *CYP2C19* polymorphisms in predicting tamoxifen efficacy and clinical outcomes in Thai breast cancer patients receiving tamoxifen as adjuvant therapy.

# CHAPTER II
# OBJECTIVE

The aims of this study were:

1. To investigate the association between *CYP2D6* and *CYP2C19* polymorphisms and tamoxifen response in Thai breast cancer patients.
2. To investigate the association between *CYP2D6* and *CYP2C19* polymorphisms and disease free survival in Thai breast cancer patients treated with adjuvant tamoxifen.
3. To determine alleles and genotypes frequency of *CYP2D6* and *CYP2C19* in Thai breast cancer patients.

# CHAPTER III
# LITERATURE REVIEW

## 3.1 Breast cancer

Breast cancer is a malignant tumor that growth of breast cancer cell, and is the most common malignancy in women [26]. A malignant tumor is a group of cancer cells that may grow invade surround breast tissue or metastasis to distant the part of the human body. The malignant tumor can occur nearly in women but men can get it [27]. Because of damage to DNA effect on cells become cancer cells. In a normal cell, when DNA gets damaged the cell either repairs the damage or the cell dies but in cancer cells, cell doesn't die by program cell death when DNA get damaged the cell is not repaired by itself. The environment factors influence on DNA damaged i.e. smoking cigarettes and drinking alcoholic. Breast cancer cells can spread from the original tumor by cell breaking away. Tumor cells go into the blood vessels or lymph vessels and grow into the other parts of body. The cancer cells may be found in lymph nodes near the breast [28]. Anything that increase chance of getting a disease is called risk factors includes older age, early age at menarche, older age at first birth or never having given birth, person history of breast cancer or benign breast disease, mother or sister with breast cancer, treatment with radiation therapy to the breast/chest, breast tissue that is dense on a mammogram, taking hormones such as estrogen and progesterone, obesity status, drinking alcoholic beverages and being white [29].

It has been reported by the National Cancer Institute in 2009 that the 10 leading cancers in Thai women are breast cancer (37%) followed by cervix cancer (14.4%), colon and rectum cancer (8.1%), bronchus and lung cancer (6.8%), liver and bile duct cancer (4%), ovary cancer (3.7%), corpus uteri cancer (3%), oral cavity cancer (2.9%), non-Hodgkin lymphoma (2.4%) and thyroid gland cancer (2.3%)[1].

## 3.2 Diagnosis of breast cancer

The goals of breast cancer testing are to identify genetic risk in high risk patients, detect and diagnose breast cancer in its earliest stages, evaluate the cancer's characteristics in order to guide treatment, determine how far it has spread, monitor the woman over time to detect, monitor the effectiveness of treatment**.** Currently, it has many methods to detect and diagnosis breast cancer includes [28, 29]:

- Mammogram is an x-ray picture of tissues inside of the breast. Women should get screening mammograms to detect breast cancer early and the older age women more than 40 years should have mammograms every 1 or 2 years.
- Biopsy by removal of cells or tissues using a microscope to check for signs of cancer by a pathologist. Four types of biopsies are as follows: Excisional biopsy: The removal of an entire lump of tissue**,** Incisional biopsy: The removal of part of a lump or a sample of tissue**,** Core biopsy: The removal of tissue using a wide needle**,** Fine-needle aspiration (FNA) biopsy: The removal of tissue or fluid, using a thin needle**.**
- Estrogen and Progesterone receptor is test to measure the amount of ER/PR in cancer tissue. The test results show whether treatment to block estrogen and progesterone may inhibit the cancer proliferation.
- MRI (Magnetic resonance imaging) is test procedure relies on principle of nuclear magnetic resonance imaging (NMRI) in order to create detailed pictures of areas inside the body.

## 3.3 Factors associated with prognosis and treatment options

If cancer is found, tests are done to study the cancer cells. Decisions about the best treatment are based on the results of these factors. The major factors are influenced prognosis and treatment options include that the stage of the cancer, the type of breast cancer, estrogen receptor and progesterone receptor levels in the tumor tissue, human epidermal growth factor type 2 receptor (HER2/neu) levels in the tumor

tissue, proliferation of tumor, a woman's age, general health, menopausal status and whether the cancer has just been diagnosed or has recurrence [29].

## 3.4 Breast cancer treatment

### 3.4.1. Surgery

Surgery is medical specialty that uses operative manual on a patient to remove the cancer cell from the breast tissue. Some of the lymph nodes under the arm are usually taken out and looked at under a microscope to see if they contain cancer cells. Breast cancer surgery is mainly consisted of 4 procedures consist of breast conserving surgery is an operation to remove the cancer but not the breast itself, includes the lumpectomy to remove a tumor (lump) and a small amount of normal tissue around and partial mastectomy is remove the part of the breast that has cancer and some normal tissue around it and procedure is also called a segmental mastectomy. Total mastectomy is surgical operation to remove the whole breast that has cancer and procedure is also called a simple mastectomy, some of the lymph nodes under the arm may be removed for biopsy at the same time as the breast surgery or after and it is done through a separate incision. Modified radical mastectomy is surgical operation to remove the whole breast that has cancer, many of the lymph nodes under the arm, the lining over the chest muscles, and sometimes, part of the chest wall muscles and the last of surgery is radical mastectomy use to remove the breast that has cancer, chest wall muscles under the breast, and all of the lymph nodes under the arm and procedure is sometimes called a Halsted radical mastectomy.

### 3.4.2 Radiation therapy

Radiation is a cancer treatment that uses high energy x-rays or other types of radiation to kill cancer cells or keep them from growing. There are two types of radiation therapy. External radiation therapy uses a machine outside the body to send radiation toward the cancer. Internal radiation therapy uses a radioactive substance sealed in needles, seeds, wires, or catheters that are placed directly into or near the

cancer. The way the radiation therapy is given depends on the type and stage of the cancer being treated.

### 3.4.3 Chemotherapy

Chemotherapy is a cancer treatment that uses drugs to stop the growth of cancer cells, either by killing the cells or by stopping them from dividing. When chemotherapy is taken by mouth or injected into a vein or muscle, the drugs enter the bloodstream and can reach cancer cells throughout the body (systemic chemotherapy). When chemotherapy is placed directly into the cerebrospinal fluid, an organ, or a body cavity such as the abdomen, the drugs mainly affect cancer cells in those areas (regional chemotherapy). The way the chemotherapy is given depends on the type and stage of the cancer being treated.

### 3.4.4 Sentinel lymph node biopsy

It is followed by surgery is the removal of the sentinel lymph node during surgery. The sentinel lymph node is the first lymph node to receive lymphatic drainage from a tumor. A radioactive substance and/or blue dye are injected near the tumor. The first lymph node to receive the substance or dye is removed. A pathologist views the tissue under a microscope to look for cancer cells. If cancer cells are not found, it may not be necessary to remove more lymph nodes. After the sentinel lymph node biopsy, the surgeon removes the tumor (breast conserving surgery or mastectomy).

### 3.4.5 Targeted therapy

Targeted therapy is a type of treatment that uses monoclonal antibodies and tyrosine kinase inhibitors for the treatment of breast cancer. The antibodies attach to the substances and kill the cancer cells, block their growth, or keep them from spreading. They may be used in combination with chemotherapy as adjuvant therapy. Trastuzumab (Herceptin) is a monoclonal antibody that blocks the effects of the growth factor protein HER2, which sends growth signals to breast cancer cells. About one-fourth of patients with breast cancer have tumors that may be treated with trastuzumab combined with chemotherapy. Lapatinib is a tyrosine kinase inhibitor that blocks the effects of the HER2 protein and other proteins inside tumor cells. It may be

used to treat patients with HER2-positive breast cancer that has progressed following treatment with trastuzumab.

#### 3.4.6 Hormone therapy

Hormone therapy is a cancer treatment that removes hormones or blocks their action and stops cancer cells from growing. Hormones are substances produced by glands in the body and circulated in the bloodstream. The female hormone estrogen, which makes some breast cancers grow, is mainly produced by the ovaries. Treatment to stop the ovaries from making estrogen is called ovarian ablation. Hormone therapy with tamoxifen or estrogens can act on cells all over the body and may increase the chance of developing endometrial cancer. Aromatase inhibitor is given to some postmenopausal women who have hormone-dependent breast cancer. Aromatase inhibitors decrease the body's estrogen by inhibiting aromatase activity converting androgen to estrogen.

## 3.5 Indication guideline of adjuvant hormonal therapy for early breast cancer [1]

#### 3.5.1 Tamoxifen

Tamoxifen is an antiestrogen drug for used to treat all stage of breast cancer patients in worldwide and treated with ER and PR positive and uncertain hormone receptor status is considered by more than 50 year old in breast cancer patients and post-menopausal status. Tamoxifen given for approximately five years after surgery to patients with early breast cancer and is the current standard of treatment breast cancer. It is reduced the risk of death about 25 percent and improved 10 years survival of more than 10 percent for patients with involve node [30]. Several studies suggested that used tamoxifen monotherapy or tamoxifen alone follow by aromatase inhibitors (letrozole) as initial adjuvant endocrine therapy for hormone receptor-positive postmenopausal breast cancer patients. Breast cancer patients were assigned to receive 5 years of tamoxifen alone or 2 years of treatment with tamoxifen followed by 3 years of treatment with the aromatase inhibitors. The important factor

influence the treatment initiation with tamoxifen that approval for use include that hormonal status, loss of bone density, arthralgia, patient at high risk of late recurrence, and treatment cost [31-33].

### 3.5.2 Aromatase Inhibitors (AIs)

Aromatase inhibitors is the considered for endocrine treatment of post-menopause breast cancer patients with ER positive and use in treating early stage, hormone receptor positive breast cancer and post menopause [34]. Aromatase inhibitors are different from tamoxifen in long term adverse effects. Breast cancer patients who receiving aromatase inhibitors (anastrozole) had significantly lower incidence of hot flashes, vaginal discharge, vaginal bleeding venous thromboembolism and had significantly higher incidence of fractures (hip, spine and wrist or radius) and musculoskeletal disorder [31, 33, 34]. The aromatase inhibitors in the adjuvant treatment of postmenopausal women have led to the following considerations in premenopausal patients:

- If AI is need; switching to an AIs after taking tamoxifen for 2 to 3 years.
- Tamoxifen contraindication and/or tamoxifen resistance and time course over 5 years.
- High risk of recurrence as metastasis to lymph node following 5 years tamoxifen (never received AIs).

The factors that approval the use of AI include: stronger activity, less incidence of gynecological and thromboembolic events, and patient at high risk of early recurrence. *CYP2D6* polymorphisms are an important factor influences the selection of adjuvant hormone treatment at the present [33].

## 3.6 Pharmacogenetics

Pharmacogenetics is a study of association between genetics of the individual person and drug response for individualized treatment. Pharmacogenetics focuses on the influence of single genes on drug response but pharmacogenomics take a broader view of the influence of an individual's entire genome on their response to drug therapy. Pharmacology is the study of drugs and their interactions with the body.

Clinical responses via drug action could be beneficial or adverse effect depending on two major principles, pharmacodynamics and pharmacokinetics. PD is the study of what the drug act to our body does. PK is the study of what does our body act to the drug which is consisted of 4 major processes; absorption, distribution, metabolism and elimination. Machinery units of all processes in PD and PK are indeed functional proteins. Theirs property are depended on various factors including genetics. The knowledge of individual genetic background affects PD and PK resulting in different drug response is called pharmacogenomics [35, 36].

Pharmacogenetics variations are associated with genetics of the individual patient and depend on PK and PD and include that: drug metabolizing enzymes, drug transporters, biomarker genes and drug target genes [35].

Personalized medicine is applied from the understanding of pharmacogenetics in order to reach optimum drug therapy and select appropriate drug dose for treatment. Specific advantages that personalized medicine may offer patients and clinicians include that ability to make more informed medical decisions, higher probability of desired outcomes due to better-targeted therapies, reduced probability of negative side effects, focus on prevention and prediction of disease rather than reaction to it and reduced healthcare costs [37].

## 3.7 Pharmacokinetics and genetic variation

The most common basis for genetic variation and thus the basis or a pharmacogenetics approach to drug therapy is the single nucleotide polymorphism (SNP). An SNP occurs when a single nucleotide is exchanged for another at a point in an individual's genome. It is estimated that the human genome consist of approximately 3 billion nucleotides which in specific combinations form 25,000 to 40,000 genes and encode approximately 100,000 proteins. SNPs that occur in coding regions of the genome have the potential to influence protein expression by altering an amino acid and causing premature stop codon within the protein called non synonymous and missense SNPs respectively. Substitution of this amino acid may change the structure or function of the protein. Certain alleles occur more commonly in some ethnic groups than in others. The impact of these SNPs on phenotypes and

their subsequent clinical consequence can again be divided into two fundamental branches of pharmacology, those that influence pharmacokinetics and pharmacodynamics [37-39]. The polymorphisms in genes that encode cytochrome P450 enzymes influence patient's treatment outcome because of genes encoding drug metabolizing enzymes could be effect on drug elimination, which process has greatest role in the treatment of many different diseases. Polymorphisms of genes will have an effect on treatment outcome about 20-25% of all drug therapies [35].

## 3.8 CYP polymorphisms

CYP superfamily is the most important human drug-metabolizing enzymes, which oxidation many of drug substrates. The cytochrome P450 superfamily has 57 functional *CYP* genes and 58 pseudogenes comprised of 18 families (i.e. *CYP1A2, CYP1B1, CYP2A6, CYP2A13, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP2F1, CYP2J2, CYP2R1 CYP2S1, CYP2W1, CYP3A4, CYP3A5, CYP3A7, CYP3A43, CYP4A11, CYP4A22, CYP4B1, CYP4F2, CYP5A1, CYP8A1, CYP19A1, CYP21A2 and CYP26A1* genes) [40]. More than 90% of human drug metabolism can be attributed to the following CYP3A4 (50%), 2D6 (30%), 2E1 (2%), 2C19 (2%), 2C9 (10%), 2A6 (2%) and 1A2 (4%) [41]. CYP2D6, 2C19, 2C9 and 2A6 enzymes are subject to stimulation and inhibition, and genetic variation has significant for enzyme activity distinction [35]. The cytochrome P450 polymorphisms compose of single nucleotide polymorphisms (base substitution), insertion, gene deletion, and gene duplications. The variation of CYP polymorphisms include copy number variation, which multiple gene copies influence on increase enzyme activity and reduce drug efficacy.

### 3.8.1 CYP2D6

CYP2D6 is the major enzyme active in metabolism of many drugs. The CYP2D6 enzyme is a metabolic liver enzyme explanation for 2-4 % of hepatic cytochrome P450 enzyme [36] and CYP2D6 enzyme is an important phase I drug enzyme related with metabolism approximately 25 % of prescribe drugs [18]. Many of drug substrates for CYP2D6 enzyme have been metabolized, listed in **Table 3.1**. The

*CYP2D6* gene is located on chromosome 22 (22q13.1) includes *CYP2D6* gene and *CYP2D7P1* (cytochrome P450, family 2, subfamily D, polypeptide 7 pseudogene 1), *CYP2D7P2* (cytochrome P450, family 2, subfamily D, polypeptide 7 pseudogene 2), *CYP2D8P1* (cytochrome P450, family 2, subfamily D, polypeptide 8 pseudogene 1), *CYP2D8P2* (cytochrome P450, family 2, subfamily D, polypeptide 8 pseudogene 2). The *CYP2D6* gene composed of nine exons with an open reading frame of 1491 base pairs coding for 497 amino acids and eight introns [42-44]. The *CYP2D6* gene is highly polymorphism, presently with over 90 known allelic variant (http://www.imm.ki.se/cypalleles/cyp2d6.htm). Gene alteration originated in single base substitution causing missense, nonsense or splice site mutation, frameshift and gene conversions with related pseudogenes. Genetic variation of *CYP2D6* gene is association with CYP2D6 enzyme activity include decreased, increased and non-functional enzyme. The *CYP2D6* phenotypes include four phenotypic as show below [45]:

- Poor metabolizers (PM) lead to lacking of functional enzyme activity because of gene deletion or change of amino acid. Defective of gene caused altered drug metabolism or eliminated in pharmacokinetic phase II (sulphation or glucuronidation).

- Intermediate metabolizers (IM), carrying two reduces functional alleles or one reduce functional allele and nonfunctional allele

- Extensive metabolizers (EM), which carrying two functional alleles or one functional allele and this result in normal enzyme activity and drug concentration.

- Ultra-rapid metabolizers (UMs), carrying more than two gene copies, duplicated, multiduplicated or amplified *CYP2D6* genes. This result in enzyme activity that exhibit increased CYP2D6 enzyme activity and lead to higher plasma drug concentration in prodrug or lower plasma drug concentration in active drugs.

**Table 3.1 Clinically relevant drug substrates for metabolism by CYP2D6 enzymes [46].**

| CYP2D6 substrates | | | |
|---|---|---|---|
| **Antidepressants** | **Beta blockers** | **Antipsychotics** | **Others** |
| Amitriptyline | Alprenolol | Haloperidol | Atomoxetine |
| Clomipramine | Carvedilol | Risperidone | Codeine |
| Desipramine | Propafenone | Thioridazine | Dextromethorphan |
| Imipramine | Brupranolol | Clozapine | Flecainide |
| Fluoxetine | Clonidine | Olanzapine | Mexiletine |
| Paroxetine | Debrisoquine | Pimozide | Ondansetron |
| Tamoxetine | Metoprolol | Sertindole | Tamoxifen |
| Trimipramine | Propranolol | Thioridazine | Tramad ol |
| Venlafaxine | Timolol | Zuclopenthixol | Tacrine |

Recently, it has been reported that analysis of tamoxifen pharmacology by evaluation the combined effect of 33 variant alleles of *CYP2D6* on the plasma concentrations of tamoxifen and its metabolites. The result interpretation from *CYP2D6* alleles nomenclature base on heterozygous or homozygous alleles for reduced functional (i.e., *CYP2D6*9, *10, *17, *29,* *36 and **41*) and null alleles (i.e., *CYP2D6*3-*8, *11, *14, *15, *19-*20,* and **40*) had related endoxifen concentrations. There is an important of allele variant in the activity of *CYP2D6* in different ethnic/racial groups. The frequencies of gene duplication (PM phenotype, *CYP2D6*3, *4* and **5*) are higher in Caucasian, African population and Saudi Arabia. CYP2D6*4 is the most frequent variant allele in European population (12-23%) and present in 70-90% of all PM phenotype [47]. It is rare frequency in Asian population, however, IM phenotype (*CYP2D6*10*) is found higher frequencies in Asian population, especially Chinese and southeast Asian population but it is rare in Caucasian [4, 15, 40, 41], listed in **Table 3.2**. The concentrations of tamoxifen plasma are higher in UM compared with EM phenotype, which indicated the significant of

comprehensive *CYP2D6* genotype to explanation of the variation in endoxifen plasma concentrations [48].

**Table 3.2 Most *CYP2D6* alleles, enzyme effect and allele frequencies in ethnic groups [18, 49-51]**

| ***CYP2D6* alleles** | **Nucleotide change** | **Enzyme activity** | **Allele frequencies (%)** | | |
|---|---|---|---|---|---|
| | | | Asian | Caucasian | African-American |
| **1* | None | Normal | 20-40 | 30-40 | 28-50 |
| **2* | -1584C>G | Normal | 9-20 | 20-35 | 10-80 |
| **3* | 2549Adel | No enzyme | 0.8-1 | 1-4 | 0-0.5 |
| **4* | 1846G>A | No enzyme | 0.5-3 | 12-23 | 2-7 |
| **5* | *CYP2D6* deleted | No enzyme | 4-6 | 1.5-7 | 0.5-6 |
| **10* | 100C>T | Decreased | 40-70 | 2-8 | 3-8 |
| **17* | 1023C>T,2850C>T | Decreased | 0.5 | 0.1-0.3 | 10-30 |
| **41* | -1584C>G, 2850C>T 4180G>C | Decreased | 0-2 | 8 | - |
| **1XN* | duplicate active gene | Increased | 0.5 | 0.2-1 | 2-5 |
| **2XN* | duplicate active gene | Increased | 0-2 | 1-5 | - |

### 3.8.2 CYP2D6 inhibitors

There are CYP2D6 inhibitors such as drugs that make extensive meatabolizers perform to poor matebolizers. Some inhibitors are CYP2D6 substrates or some inhibitors are not CYP2D6 substrates. Quinidine is not substrate of CYP2D6 but it is the most potent inhibitor of CYP2D6 because of quinidine sulphate is converted extensive metabolizers to poor metabolizers [52]. Many drugs are CYP2D6 inhibitors can be found in **Table 3.3**. Antidepressants are the most important CYP2D6 inhibitors in patient receiving adjuvant tamoxifen such as fluoxetine (Prozac), paroxetine (Paxil), bupropion (Wellbutrin), and duloxetine (Cymbalta). They can inhibit CYP2D6 enzyme activity and may reduce tamoxifen plasma level. Patients

receiving tamoxifen drug should be advised not to start new prescription drug without checking the CYP2D6 inhibitors [53].

**Table 3.3 Inhibitors of CYP2D6**

| CYP2D6 inhibitors | | |
|---|---|---|
| Amitriptyline | Propoxyphene | Perphenazine |
| Bupropion | Imatinib | Haloperidol |
| Chlorpheniramine | Fluoxetine | Duloxetine |
| Chloroquine | Cimetidine | Terbinafine |
| Chlorpromazine | Paroxetine | Diphenhydramine |
| Quinacrine | Propafenone | Venlafaxine |
| Quinidine | Quinine | Yohimbine |

### 3.8.3 CYP2C19

CYP2C19 enzyme plays the minor role in tamoxifen metabolism. This enzyme is a member of CYP450 family 2C and it is a metabolic liver enzyme explanation for 25% of hepatic cytochrome P450 enzyme [54]. Many of drug substrates for CYP2C19 enzyme have been metabolized and eliminated common prescription drugs, including anti-depressants, anti-convulsants, anti-cancer, proton pump inhibitors, anti-malaria, anti-ulcer, and antithrombotics, as shown in **Table 3.4** and other drugs has been metabolized by CYP2C19 enzyme and approved by FDA (www.fda.gov) for genetic testing before treatment. CYP2C19 enzyme produced from the *CYP2C19* (cytochrome P450, family 2, subfamily C, polypeptide 19) gene that it has nine exons and is located on chromosome 10q24 [55]. The CYP2C19 isoenzyme has considerable genetic variation. The most common variant polymorphisms result in many null alleles (i.e. *CYP2C19*2, *3, *4, *5, *6, *7,* and **8*), resulting in a phenotype of absent CYP2C19 enzyme activity. This phenotype is the most common phenotype in Asians and Oceania population (approximately 20%) and occurs in Caucasians and African (approximately 3-5%) [56]. In recently, it has been reported

that clinical impact of *CYP2C19*17* has been evaluated and demonstrated the association with increased CYP2C19 enzyme activity in vivo [57]. The distribution of *CYP2C19*17* allele frequencies is 25% in German population [58], 18% in Swedish and Ethiopian population, and it is rare in Chinese population (4%) [57]. The poor metabolizer (PM) is association with decreased enzyme activity in patients who have drug therapy such as patients who received clopidogrel treatment of cardiovascular disease that have been shown to response poorly to standard doses due to reduced metabolic activation of the drug [4, 59-61].

**Table 3.4 Clinically relevant drug substrates for metabolism by CYP2C19 enzymes [46].**

| CYP2C19 substrates | | |
|---|---|---|
| **Anti-epileptics** | **Proton pump inhibitors** | **Others** |
| Diazepam | Omeprazole | Prasugrel |
| Phenytoin | Dexlansoprazole | Ticagrelor |
| Phenobarbitone | Pantoprazole | Cyclophosphamide |
| Citalopram | Esomeprazole | Progesterone |
| | Rabeprazole | Voriconazole |
| | | Clopidogrel |

The clinical impact of the *CYP2C19* genotype is influenced by whether a drug is activated, listed in **Table 3.5**. The two most common variant alleles are *CYP2C19*2* (681G>A, rs4244285) and *CYP2C19*3* (636G>A, rs4986893), which has nonfunctional enzyme activity.

**Table 3.5 Most *CYP2C19* variant alleles, enzyme effect and allele frequency in ethnic groups [54, 61, 62]**

| ***CYP2C19* alleles** | **Nucleotide change** | **Enzyme activity** | **Allele frequencies (%)** | | |
|---|---|---|---|---|---|
| | | | Asian | Caucasian | African-American |
| **1* | none | Normal | 63.1 | 86.4 | 81 |
| **2* | 681G>A | No enzyme | 31.2 | 12.7 | 18.2 |
| **3* | 636G>A | No enzyme | 5.7 | 0.9 | 0.8 |

Several studies had compared to *CYP3A4/5*, *CYP2D6* and *CYP2C19* variation effect to tamoxifen and its metabolites level. Then it could be concluded that CYP2D6 is the rate-limiting enzyme suggesting as a predictor of drug response [19]. Many studies have been examined the reliable of *CYP2D6* polymorphisms in order to predict the clinical outcome of adjuvant tamoxifen therapy. It is conflicting results have been appeared up to date [19, 63, 64]. The latest ongoing prospective research is doing by NCI [26].

## 3.9 Tamoxifen metabolisms

In primary breast cancer, adjuvant tamoxifen significantly decreases relapse rates and mortality in pre- and postmenopausal patients and the therapy benefit from 5 years of adjuvant tamoxifen is maintained, even 10 years after primary diagnosis [65]. However, up to 50% of patients who receive adjuvant tamoxifen had recurrence or die from tumor-specific resistance or host genetic factors. Since tamoxifen is a prodrug requiring metabolic activation to exert pharmacological activity. The major and minor enzymes for tamoxifen metabolism are CYP2D6 and CYP2C19, which these enzyme convert tamoxifen (inactive form) to active metabolites. It has been reported that 4-hydroxytamoxifen, a minor metabolite of tamoxifen, display 100-fold more affinity for the estrogen receptor than tamoxifen and *N*-desmethyl tamoxifen. Recent several research have demonstrated that 4-hydroxy-*N*-desmethyl tamoxifen (endoxifen), major secondary metabolite of tamoxifen, is more

potent than 4-hydroxytamoxifen in terms of binding affinity to estrogen receptor [6, 7]. Thus drug metabolism enzyme polymorphisms possible indicate group of patients that being benefit or non-benefit from receiving adjuvant tamoxifen.

### 3.9.1 Primary tamoxifen metabolism pathway

Formation of the major metabolite N-desmethyltamoxifen (NDM-tam) is primarily catalyzed by CYP3A4 and 3A5, with minor contributions by CYP2D6 and other CYP isoforms (Figure 1). 4-hydroxy tamoxifen is an active metabolite in minor pathway and it is catalyzed by CYP2D6 (minor metabolized by CYP2B6, CYP2C9, CYP2C19 and CYP3A). The steady state plasma concentration of N-desmethyltamoxifen after 20 mg tamoxifen is administered daily are 654.9 nM about 2 times of [tamoxifen][23]. Although CYP3A4/5 is identified as major enzymes involved in the principal TAM sequential metabolic routes. Their genetic variations has been reported that no statistical significant association with plasma concentration of tamoxifen and its metabolites. Therefore *CYP3A4/5* polymorphisms might not effect to tamoxifen treatment outcome.

### 3.9.2 Secondary tamoxifen metabolism pathway

Another clinically active metabolite, endoxifen, is primarily catalyzed by CYP2D6, with minor contributions by CYP3A4/5 (**Figure 3.1**). CYP2D6 is the most significant enzymes that modulate plasma endoxifen concentration [6]. Recent clinical studies have demonstrated that common *CYP2D6* genetic variation (leading to low or absent CYP2D6 enzyme activity), or the inhibition of CYP2D6 enzyme activity significantly lower the plasma concentrations of endoxifen. Patients homozygous for a *CYP2D6* null allele had significantly lower endoxifen concentrations (mean, 21.9 nm) than patients with one (mean, 62.4 nm) or two (mean, 88.6 nm) *CYP2D6* functional alleles [19].

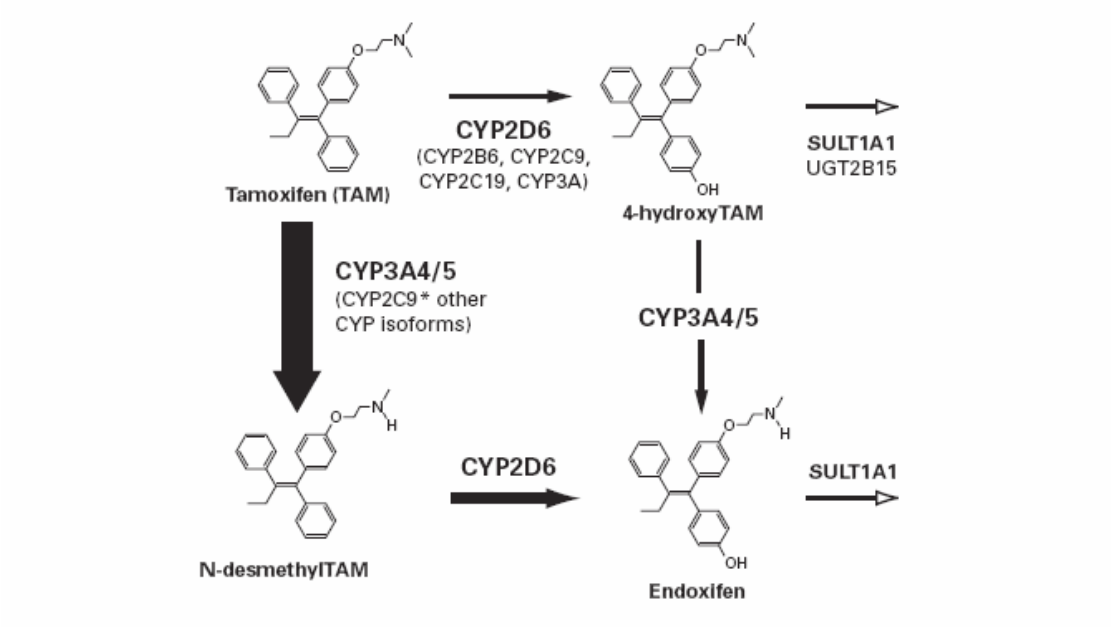



**Figure 3.1** Biotransformation pathways of tamoxifen [19, 23]. The relative contribution of each pathway to the overall oxidation of tamoxifen is shown by the thickness of the arrow, and the principal *P*450 isoforms responsible are highlighted in larger fonts. Modified slightly from Jin,Y. *et al. J. Natl Cancer Inst*. 97(1):30-39 (2005).

CYP2C19 is the minor enzyme that effect to tamoxifen metabolism. Furthermore, previous studies reported that the clearance tamoxifen metabolites mediated by many enzyme others including sulfotransferase (SULT1A1), UDP-glucurunosyltransferase (UGT) 2B15 and UGT1A4 [66]. Tamoxifen has limitations; only about 20–30% of patients with advanced disease respond to tamoxifen, and many patients who initially respond to treatment ultimately become resistant [67-69].

## 3.10 *CYP2D6* predicted phenotype and tamoxifen efficacy

It has been known for long time that tamoxifen metabolized by major CYP2D6 and it is requiring metabolic activation to 4-OHtam to exert pharmacological activity [8] but endoxifen that it is an important secondary metabolite, only recently it had been reported that endoxifen play the major role in suppress breast cancer cell

proliferation similar to 4-OHtam but it has high-ER affinity and more potent than 4-OHtam by studied of Sterns V and colleagues [6]. From their research found that both of two metabolites (4-OHtam and endoxifen) had induced similar change on the pattern of gene expression in human breast cancer cell (MCF7) and plasma concentration of endoxifen had 10 fold higher than 4-OHtam. Buck MB and colleagues [70] had investigated the growth inhibitory effect of tamoxifen and its major metabolites in MCF-7 and T47D treated with tamoxifen and tamoxifen metabolites and mRNA expression of TGFb2. They had found that only two metabolites 4-OHtam and endoxifen had significant associated with antiproliferative activity and were able to induce TGF$\beta$2 and T$\beta$RII. Johnson MD and colleagues [7] had been reported that endoxifen play the major role in inhibiting estrogen stimulated MCF-7 and the regulation of estrogen responsive genes. Jin Y and colleagues [23] investigated the association between *CYP2D6* genotype and plasma concentrations of tamoxifen and its metabolites. The result shown that had significantly lower plasma concentration of endoxifen in patients with *CYP2D6* homozygous variant genotype (20.0 nM, 95% CI = 11.1 to 28.9 nM) or a heterozygous genotype (43.1 nM, 95% CI = 33.3 to 52.9 nM) compared with a homozygous wild type genotype (78.0 nM, 95%CI = 65.9 to 90.1 nM), both $P = 0.003$.

## 3.11 *CYP2D6* polymorphisms and conflicting of tamoxifen treatment outcome

Several studies investigated the clinical impact of tamoxifen pharmacokinetics that influence on the clinical outcome of breast cancer patients who received adjuvant tamoxifen. The evidence from published studies investigating drug metabolizing enzymes and transporter genes as predictive factors of tamoxifen efficacy and clinical outcome of breast cancer patients are conflicting study results. Most of researcher recommended identify of polymorphisms of drug metabolizing enzymes may be useful in optimizing therapy with adjuvant tamoxifen but any researcher found that no association between polymorphisms of drug metabolizing enzymes and transporter genes and clinical outcome of breast cancer patients receiving adjuvant tamoxifen. It divided into two groups include positive and negative results.

**3.11.1 Positive studies on breast cancer patients using adjuvant tamoxifen**

Goetz MP and colleagues [25] reported that breast cancer patients with the *CYP2D6*4/*4* genotype had significantly worse relapse free time (RF-time, *P* = 0.023) and disease free survival (DFS, *P* = 0.012), but not overall survival (*P* = 0.169) compared with heterozygous or homozygous for the wild type allele and patients with the *CYP2D6*4/*4* genotype had significantly worse RFS (HR= 1.85, *P* = 0.176) and DFS (HR = 1.86, *P* = 0.089). The *CYP3A5*3* variant was not associated with any of these clinical outcomes.

Schroth W and colleagues [71] evaluated the polymorphisms in *CYP2D6* relationship with clinical outcomes in breast cancer patients receiving adjuvant tamoxifen. The result shown that had significantly increased risk of recurrence for heterozygous extensive /intermediate metabolizers (HR = 1.40) and homozygous poor metabolizers (HR = 1.90) compared with homozygous extensive metabolizers. There were significantly worse disease free survival (HR = 1.29) and event free survival (HR = 1.33) but no significant difference in overall survival (HR = 1.15).

Serrano D and colleagues [72] investigated the polymorphisms in *CYP2D6*, *CYP2C19* and *SULT1A1* correlation with tamoxifen efficacy in Italian breast cancer treated with tamoxifen. The results shown that breast cancer patients with a *CYP2D6*2A* allele had significantly associated with increased tamoxifen efficacy (*P* = 0.00001) and *CYP2D6* poor metabolizer alleles shown the increased risk for breast cancer recurrence compared to the others phenotype (*P* = 0.035) but in contrast, *CYP2C19* and *SULT1A1* polymorphisms had not significantly correlation with the tamoxifen efficacy.

Abraham JE and colleagues [73] evaluated *CYP2D6* variants as predictive factors of tamoxifen efficacy and clinical outcome in breast cancer patients from United Kingdom treated with adjuvant tamoxifen. They found that only *CYP2D6*6* was associated with decreased breast cancer specific survival (BCSS) (*P* value = 0.02, HR = 1.95) and it had not been reported the association with other variants that different from the previous reported to be associated with poor metabolizer (*CYP2D6*4*) clinical outcomes, had significantly difference in BCSS.

Madlensky L and colleagues [74] explored breast cancer outcomes are associated with endoxifen and other metabolites of tamoxifen and long term breast cancer clinical outcomes. It is shown that had significantly the relationship between endoxifen concentrations and reduced the recurrence rate. It is the first report on the association between endoxifen concentrations and clinical outcomes in breast cancer patients treated with tamoxifen.

Kiyotani K and colleagues [75] investigated the relationships of polymorphisms in *CYP2D6* and transporter genes on clinical outcome of Japanese breast cancer patients receiving tamoxifen. *CYP2D6* variants were significantly associated with shorter recurrence free survival (HR= 9.52) in patients with two variant alleles compared with wild type alleles and result shown that *CYP2D6* variant alleles were associated with lower plasma level of endoxifen and 4-hydroxytamoxifen (*P* value = 0.0000043 and 0.00052) but no difference was found in transporter genes polymorphisms.

Xu Y and colleagues [76], established the relationship between *CYP2D6*10* genotype and tamoxifen efficacy and survival time of breast cancer patients receiving tamoxifen. *CYP2D6*10* (100C>T) homozygous variants had significantly lower plasma level of 4-OHtam compared with homozygous wild type (*P* value = 0.04) and found that patients with homozygous variants had significantly reduced DFS compared with homozygous or heterozygous wild type of *CYP2D6*10* (*P* value = 0.04, HR = 4.7).

Lim JSL and colleagues [77], investigated the influence of *CYP2D6*, *CYP3A5*, *CYP2C9* and *CYP2C19* polymorphisms on tamoxifen efficacy in Asian breast cancer patients. This study revealed that polymorphisms in *CYP2D6*5* and **10* were significantly associated with higher N-desmethyltamoxifen (NDM) and lower plasma of endoxifen concentration. No significant relationship between polymorphisms in *CYP3A5, CYP2C9, CYP2C19* and tamoxifen metabolites.

### 3.11.2 Negative studies on breast cancer patients using adjuvant Tamoxifen

Okishiro M and colleagues [78], their result shown that no significant difference recurrence free survival (RFS) between *CYP2D6*10/*10* and patients with homozygous *CYP2D6* wild type or heterozygous *CYP2D6* genotype. RFS did not significant difference between *CYP2C19*2/2, *2/*3, *3/*3* and *CYP2C19 wt/wt, wt/*2, wt/*3*. So, they concluded that the *CYP2D6*10/*10* and *CYP2C19* genotypes were not associated with recurrence free survival of primary breast cancer patients treated with adjuvant tamoxifen.

Wegman P and colleagues [79] reported the conflicting result that had significantly better disease free survival in post-menopause patients with homozygous *CYP2D6*4* and had significantly improved RFS in patients with homozygous *CYP3A5*3* alleles (HR = 0.20, *P* = 0.002) but there were no difference between genotypes of *CYP2D6, SULT1A1* or *UGT2B15* and recurrence free survival time.

Toyama T and colleagues [80], found that the result had no significantly correlations between *CYP2D6*10* genotype and clinical pathologic parameters and there was no significantly association between *CYP2D6*10* and DFS, DDFS, BCSS or OS in breast cancer patients who had received adjuvant tamoxifen.

## 3.12 Pharmacogenetics of Aromatase Inhibitor therapy

Aromatase inhibitors used for treatment in post-menopausal women because of aromatase enzyme are mediated conversion of androstenedione to estrone in peripheral tissue. So, it is drug of choice for therapy in breast cancer patients with post-menopause status. It divided into two mechanisms included that first, Anastrazole and letrozole inhibit aromatase that the enzyme reversibly bind to heme component of aromatase enzyme and they are catalyzed the conversion of androstenedione to estrone. Both drugs are used in advance breast cancer. The secondary enzyme is exemester that irreversibly bind to substrate complex, permanently inactivating aromatase and had led to prolong estrogen deficiency [81]. Aromatase, an enzyme of the cytochrome P450 subfamily and the product of the *CYP19A1* gene, is highly expressed in the placenta and in the granulose cells of ovarian follicles in

premenopausal women. Its expression depends on cyclical gonadotropin stimulation [3]. In addition, aromatase is also present at lower levels in several non-glandular tissues that include subcutaneous fat, liver, muscle, brain, normal breast and breast cancer tissue [4]. Estrogen production after menopause is solely from non-glandular sources, particularly subcutaneous fat. In menopause, androstenedione produced in the adrenals and, to a small extent, testosterone produced in the ovaries are released to the circulation and then sequestered to non-glandular tissues (e.g. liver and breast cells), where they are converted to estrone and estradiol, respectively, by aromatase located in these tissues [5]. In the liver and in breast tissue, estrone and estradiol undergo oxidation by cytochrome P450s to a number of hydroxylated metabolites [6]. Estrone and estradiol in these tissues also undergo conjugation by sulfotransferases or deconjugation by steroid sulfates [7]. In all tissues, hydroxysteroid (17-beta) dehydrogenase (HSD17B) converts androstenedione to testosterone and estrone to estradiol [5]. Furthermore, from previous studied [81], Yu KD and colleagues had been suggested that the efficacy of tamoxifen treatment in post-menopausal women with breast cancer who present with *CYP2D6* homozygous (EM) wild type is similar to patients receiving aromatase inhibitors (AI).

## 3.13 Estrogen receptor

An estrogen receptor is a protein molecule found inside those cells that are targets for estrogen action. Estrogen receptor plays the major role for pharmacodynamics and plays an important role in the development and growth of the mammary gland during puberty, pregnancy, and lactation. Increased exposure of E2 (natural estrogen receptor agonist) including early menarche and later menopause, is associated with increased risk of breast cancer, and may contribute to tumor growth [83-85]. There are two different forms of the estrogen receptor consist of ER*α* and ER*β*. Two form of estrogen receptors are encoded of dissimilar genes, *ESR1* and *ESR2* on the sixth and fourteenth chromosome (6q25.1 and 14q23.2), respectively. Estrogen hormone activated estrogen receptors predominantly to form ERα (αα) or ERβ (ββ) homodimers and sometime ERαβ (αβ) heterodimers which depend on the different type of estrogenic ligands, cell types or cell status whether is normal or disease state

[83, 86]. E2 treatment stimulates breast cancer cell proliferation and the growth of human tumor. Tamoxifen binds to the estrogen receptor by competition with natural estrogen hormone. After tamoxifen binding, breast cancer cell proliferation undergoes down regulation due to the lack of estrogen stimulate signaling [87]. Therefore estrogen receptor plays the major role in predictive factors to hormonal treatment response. Immunohistochemistry is the standard technique to determine the individuals' estrogen receptors status to being the criteria for tamoxifen or aromatase inhibitors adjuvant therapy. Patients who are hormonal sensitive, ERα positive, or with expression of the E2-stimulated PR are received the anti-hormone or aromatase drugs and have longer time to recurrence (disease-free or relapse-free survival time), than the patients who are not hormonal sensitive [84, 85, 88-91]. However, not all of the ERα positive patients' response to the tamoxifen so the additional markers to predict clinical responses are still needed and sought. The absence of PR has been one predictor for poor responses to tamoxifen [90, 91]. Because the PR gene is ER-regulated, its expression has been interpreted to indicate a functioning ER and, therefore, an E2-responsive tumor. Alternative growth factor-sensitive pathways, identified by the presence of the EGF receptor family member HER2, are also associated with poor response to endocrine therapy and may cause decreased PR expression [91-93]. As mention before, there are two subtypes of ER, ERα and the more recently isolated receptor named ERβ with different expression profiles in normal and malignant tissues, let us to the new knowledge that ER+ breast tumors might be even more heterogeneous than originally supposed [87]. The biological information of ERβ has become available to date, its potential role in breast tumor formation and response to endocrine therapy is of considerable interest and investigation [94]. The study shows that expression of $ER_{\beta}$ is an independent marker for favorable prognosis after adjuvant tamoxifen treatment in ERα -negative breast cancer patients [95].

## 3.14 Current status of microarray based testing

### 3.14.1 Clinical microarray testing

In recent years, a new technology, DNA microarray is measure of the expression of thousands of genes simultaneously. DNA microarrays rely on the hybridization properties of nucleic acids to monitor DNA or RNA abundance on a genomic scale in different types of cells. It seems to be an important tool for diagnosis of diseases at a molecular level. Applications are for example the improvement of diagnosis and treatment of cancer and the improvement of the effectiveness of drug. Clinical microarray based test has led to study in the treatment and diagnosis of diseases. Recently, it used to detect disease marker and various genotypes, have significant part of clinical diagnostic, and hold promise in improve disease diagnosis, patient stratification in clinical trial, and selection of the appropriate drug and to guide dosage adjustment. The AmpliChip P450 Test contained for two cytochrome P450 genes (included *CYP2D6* and *CYP2C19*). This information genotype may be used for help the clinician in determining the appropriate drug and dose for therapeutics, and determining the therapeutic strategy for improvement patient outcome by improve drug efficacy and increase patient outcome [96-98].

Microarray based technology has recently introduced into various clinical tests as the advantage over to the traditional DNA based tests and histopathological assays. Their unique ability are including to simultaneously measure the relative expression level of a large number of clinically relevance genes, or to genotype a large number of allelic variants at one or more loci at once. These features are essential for the accurate diagnosis and prognosis of disease with genetic complexity contribution. The increasing number applications of microarray technology to drugs development, drugs safety and efficacy are exponentially growing in pharmacogenomics field. Gene technology pave a way toward personalize medicine. This microarray technology has led to improve patient outcome by decrease adverse drug reactions and improve drug efficacy [97, 99]. The AmpliChip CYP450 Test, for example is the first US FDA approved based tests for diagnostic applications.

The test is intended to identify *CYP2D6* and *CYP2C19* genotypes and predicted phenotypes from genomic DNA extracted from a whole blood sample. These

genes regulate the metabolism of prescribed drugs of approximately 25 % [100]. There are four predicted phenotypes: poor, intermediate, extensive and ultra-rapid metabolizers. The interpreted results may help the clinician for determining the appropriate drug and doses. This test has the potential to replace lengthy trial approaches and provide a quick and more efficient mean for individual optimizing treatment (**Figure 3.2**). Several microarray based clinical testing are currently available (**Table 3.**7).

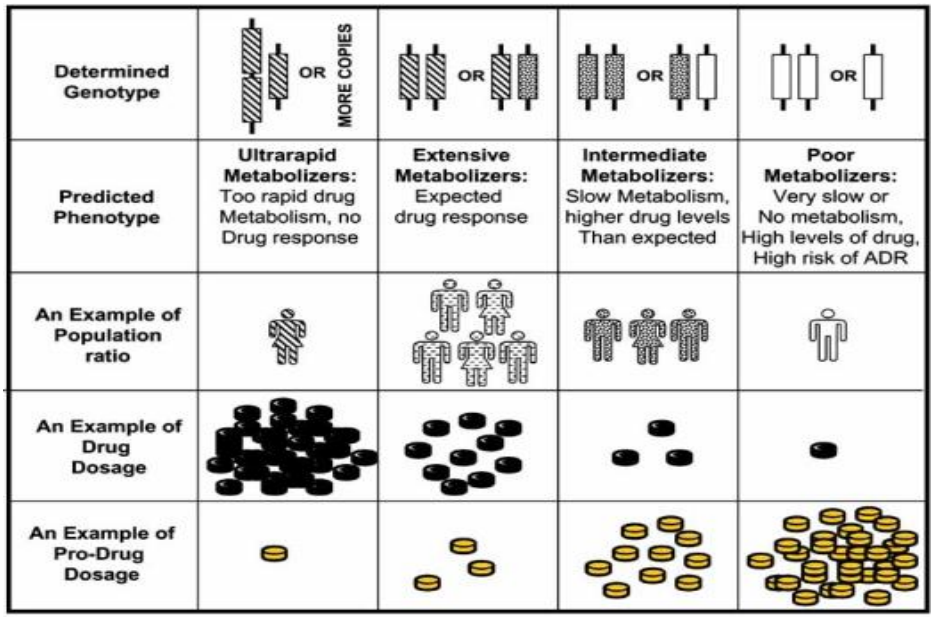



**Figure 3.2 Patterns of AmpliChip CYP450 test: from establish genotype, predicted phenotype to optimized drug dose [97].**

The AmpliChip CYP450 Test has been designed to be comprehensive in reporting the most common allelic variants of both *CYP2D6* and *CYP2C19*. Nonfunctional *CYP2C19* composed of two allelic variants (*CYP2C19*2* and **3*), each leading to enzyme activity, account for the virtually 100% of Asian poor metabolizers, and between 85%–90% of Caucasian and African poor metabolizers [100]. In contrast, the *CYP2D6* locus is far more polymorphic and has unique challenges in terms of the breath of genetic variation that underlies inherited enzyme activity aberrancies, including gene deletion (*CYP2D6*5*), various types of point mutations, in-frame deletions and gene conversion events. *CYP2D6* gene duplication of functional alleles

can lead to excesses in enzyme activity [20]. The AmpliChip CYP450 test microarray contains more over 15,000 oligonucleotide probe querying for over 30 polymorphisms of the *CYP2D6* gene and 2 single nucleotide polymorphisms within the *CYP2C19* gene, reporting 33 variant alleles, including 7 duplicated alleles of *CYP2D6* and 3 alleles of *CYP2C19*. The genotyping accuracy of the AmpliChip CYP450 Test was excellent when tested against direct sequencing analysis of over 400 clinical samples with a sensitivity of 99.2% and a specificity of 100% for *CYP2D6* and a sensitivity of and specificity of 100% for *CYP2C19* (**Table 3.6**). This accuracy is due primarily from the use of redundant probe tiling on the microarray, which in turn allows one to set very robust cutoffs to discriminate between homozygous wild-type or mutants or heterozygotes. The single block contains with 40 oligonucleotide sequences and up to six blocks contain with a total of 240 oligonucleotide probes are used for the determination of each genetic alteration, and over 15,000 probes are synthesized on the microarrays' chip. From an assay development point of view, the human *CYP2D6* locus provides many of the challenges seen in other parts of the genome, including the presence of highly conserved nonfunctional pseudogenes [44]. Several studies reported that the associations between *CYP2D6* or *CYP2C19* genotype and drug responses, and the genes are considered valid biomarkers by the FDA. The medical community is not well versed in the use of such genetic information in making treatment decisions. Thus, even when robust FDA-approved in vitro diagnostics such as the AmpliChip CYP450 Test are available, further clinical research will be needed to establish where such genotype information will provide the greatest benefit to maximize drug efficacy and enhance drug safety [102].

**Table 3.6 Performance of AmpliChip CYP450 Test[a]**

| | ***CYP2D6*** | | ***CYP2C19*** | |
|---|---|---|---|---|
| | **Samples** | **Accuracy (%)** | **Samples** | **Accuracy (%)** |
| Specificity (normal alleles) | 100 | 100 | 270 | 100 |
| Sensitivity (mutant alleles) | 492 | 92.2 | 246 | 99.5 |

[a]Data were quoted from the package insert of AmpliChip CYP450 Test

### 3.14.2 Types of Microarrays [97, 103]

The Microarray experiments can be categorized in three methods depends on the kind of immobilized sample used construct arrays and the information fetched. Microarray expression analysis**:** the cDNA derived from the mRNA of known genes is immobilized. The sample has genes from both the normal as well as the diseased tissues. Spots with more intensity are obtained for diseased tissue gene if the gene is over expressed in the diseased condition. This expression pattern is then compared to the expression pattern of a gene responsible for a disease. Microarray for mutation analysis**:** the researchers use gDNA. The genes might differ from each other by as less as a single nucleotide base. A single base difference between two sequences is known as Single Nucleotide Polymorphism (SNP) and detecting them is known as SNP detection. Comparative Genomic Hybridization**:** It is used for the identification in the increase or decrease of the important chromosomal fragments harboring genes involved in a disease.

### 3.14.3 Applications of Microarrays [99, 103, 104]

Gene discovery**,** DNA microarray technology helps in the identification of new genes, know about their functioning and expression levels under different conditions. Disease diagnosis**,** DNA microarray technology helps researchers learn more about different diseases such as heart diseases, mental illness, infectious disease and especially the study of cancer. Until recently, different types of cancer have been classified on the basis of the organs in which the tumors develop. Now, with the evolution of microarray technology, it will be possible for the researchers to further classify the types of cancer on the basis of the patterns of gene activity in the tumor cells. This will tremendously help the pharmaceutical community to develop more effective drugs as the treatment strategies will be targeted directly to the specific type of cancer. Drug discovery**;** microarray technology has extensive application in pharmacogenomics. Pharmacogenomics is the study of correlations between therapeutic responses to drugs and the genetic profiles of the patients. Comparative analysis of the genes from a diseased and a normal cell will help the identification of the biochemical constitution of the proteins synthesized by the diseased genes. The researchers can use this information to synthesize drugs which combat with these

proteins and reduce their effect. Toxicological research**;** microarray technology provides a robust platform for the research of the impact of toxins on the cells and their passing on to the progeny. Toxicogenomics establishes correlation between responses to toxicants and the changes in the genetic profiles of the cells exposed to such toxicants.

**Table 3.7 A summary of currently available or upcoming microarray based tests [97]**

| Testing | Specimens | RNA/DNA | Measured | Number of Gene/SNPs | Availability | FDA-Approval |
|---|---|---|---|---|---|---|
| **Clinical Testing** | | | | | | |
| AmpliChip CYP450 | Blood | DNA | CYP450 SNPs | 31+controls | Yes | Yes |
| AmpliChip p53 | Tumor | DNA | P53 mutations | Coding region + splice sites | No | No |
| MammaPrint | Tumor | RNA | Relevant genes | 70 | Yes | Yes |
| Tissue of origin | Tumor | RNA | Relevant genes | 1550+110 controls | No | Under review |
| aCGH | Blood/Tissues | DNA | Chromosomal abnormalities | Up to 1.8 million markers | Yes | No |
| **Consumer-Based Testing** | | | | | | |
| Health Compass | Saliva | DNA | SNPs | 1 million | Yes | No |
| 23andme | Saliva | DNA | SNPs | 0.58 million + proprietary | Yes | No |
| deCODEme | Buccal swab | DNA | SNPs | 1million | Yes | No |

# CHAPTER IV
# MATERIALS AND METHODS

## 4.1 Subjects

Between February 1997 and January 2008, forty eight patients were recruited from department of medicine, Ramathibodi hospital. The inclusion criteria included Thai women age over 18 years old, breast cancer patients who received adjuvant tamoxifen and histological diagnosis of breast cancer with estrogen and/or progesterone receptor positive. Patients had received coincident or previous other malignancy and had received co-medication of SSRIs (selective serotonin reuptake inhibitors) was excluded from this study because of the information of SSRIs was interact with tamoxifen. These breast cancer patients were classified in two groups (**Figure 4.1**). To increase the statistical power of this study, we ruined the individual matching and used all available control after verifying by the clinician. However, that the breast cancer patients cases and controls had similar characteristics. The patients in group 1 consisted of breast cancer patients who had recurrence of breast cancer while receiving adjuvant tamoxifen was defined as a case group. The group 2 consisted of breast cancer patients who had already completed 5 years of adjuvant tamoxifen treatment been defined as a control group. The information of patients clinical data which collected from medical records composed of age at diagnosis of breast cancer, date of surgery, type of surgery, menstruation status, ER/PR status, her-2 status, histologic grading tumor, lymphovascular involvement status, surgery margin status, T stage of tumor, nodal involvement, number of nodes dissection, start and stop date of chemotherapy either neoadjuvant or adjuvant setting, start and stop date of adjuvant radiotherapy, start and stop date of tamoxifen and date and site of the first disease recurrence were recorded. The study was reviewed by the ethics committee of Ramathibodi Hospital (MURA2010/541/$S_3$) and all patients written informed consent before enrolled. In this study, the definition of disease free survival as the survival endpoint was defined as the time from surgery to the occurrence of breast event.

(local, regional, or distant occurrence or contralateral breast cancer) or death from any cause. Patients who were alive without a breast recurrence were censored at the date of their last disease evaluation.

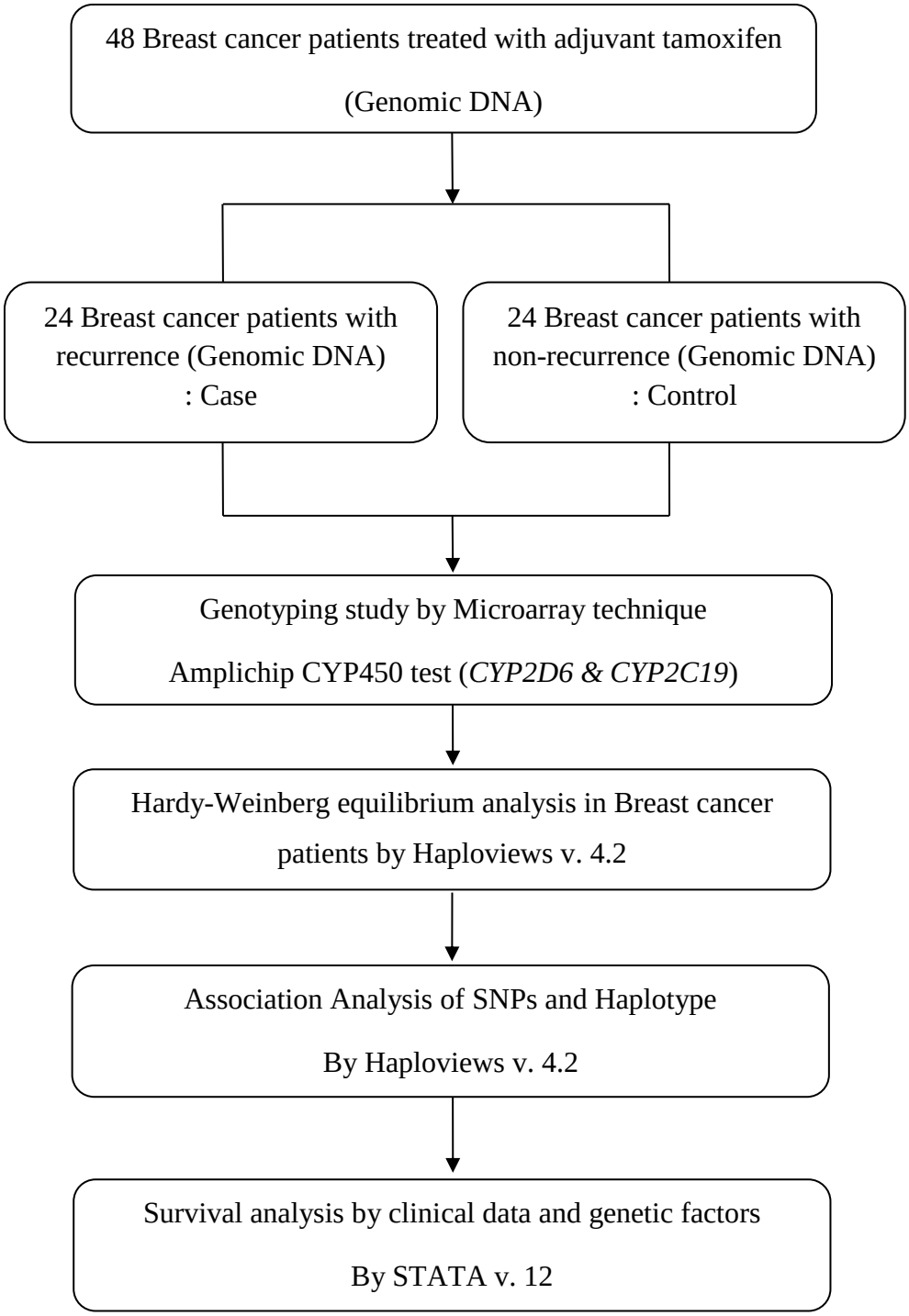



**Figure 4.1 Research design procedure**

## 4.2 Materials

### 4.2.1 Instruments

- Applied Biosystems Gold-plated 96-Well GeneAmp System 9700 thermal cycler (Applied Biosystems, Foster City, CA)
- Affymetrix GeneChip Fluids Station 450Dx
- Affymetrix GeneChip Scanner 3000Dx

### 4.2.2 Reagents and GeneChip

- DNase I, RNase-free recombinant (Roche Diagnostics)
- Alkaline Phosphatase, from calf intestine (Roche Applied Science)
-Terminal transferase, recombinant (includes 5X terminal Transferase reaction buffer and $CoCl_2$ solution) (Roche Applied Science)
- Streptavidin, R-phycoerythrin conjugate, 1 mg/mL (Roche Diagnostics)
- CYP450 master mix
- CYP450 primer A
- CYP450 primer B
- $Mg^{2+}$
- TdT labeling
- B1 oligonucleotide solution
- CYP450 microarray Gene Chip
- CYP450 positive control
- CYP450 negative control
- 0.5 M EDTA, pH 8.0 (Invitrogen Corp., Carlsbad, CA)
- Water, distilled or deionized
- Water, molecular biology grade, nuclease-free
- AccuGENE 20X SSPE buffer (Cambrex, Rockland, ME)
- Triton® X-100 surfactant (Sigma-Aldrich, St. Louis, MO)
- Denhardt's solution, 50X concentrate (Sigma-Aldrich)
- Sodium azide solution, 5% (w/v) (VWR international, Mississauga, Ontario)
- Acetylated bovine serum albumin, 20 mg/mL (Sigma-Aldrich)

## 4.3 Methods

The AmpliChip CYP 450 (*CYP2D6* and *CYP2C19*) Test is composed of five main steps; PCR amplification of DNA extraction; fragmentation and labeling of the amplified products; hybridization and staining; scanning of microarray; and analysis of the *CYP450* genotypes and predicted phenotypes.

### 4.3.1 Specimen and control preparation

Genomic DNA was extracted from each whole blood (EDTA tube). The DNA must be at a concentration of approximately 2-20 ng/µL (50-500 ng/PCR) and have an $A_{260}/A_{280}$ ratio of 1.50-1.85. If the DNA must be diluted, use 10 mM Tris-HCl, 0.1 mM EDTA, 0.09% sodium azide, pH 8.0 as diluent. Store undiluted and diluted specimen DNA at 2-8 $^{o}$C for up to one week or frozen at -20 $^{o}$C for up to one month with no more than three freeze-thaw cycles. Prepare the working CYP450 positive control as follow: To a labeled 2.0 mL screw-cap tube, add 54 µL A-CHIP (-) C and CYP450 (+) C. Cap the tube and vortex for 5 seconds.

### 4.3.2 Reagent preparation

Place 96 well plate into the MicroAmp tray and lock in place with retainer. Equilibrate working master mix components (**Table 4.1**) for 15 minutes at room temperature. Mix working master mix components by inverting 10-15 times and label two 2.0 mL tubes as A and B and then prepare working master mix A and working master mix B by pipetting the reagent volumes. Cap tubes and invert 10-15 times to mix.

**Table 4.1 Preparation of working master mixes A and B**

| **Working master mixes A and B** | |
|---|---|
| **Reagents** | **1 reaction** |
| AmpliChip CYP450 Test master mix | 25µl |
| AmpliChip CYP450 Test primer mix A or primer mix B | 25µl |
| AmpliChip CYP450 Test magnesium chloride solution | 25µl |
| **Total volume** | **75µl** |

Working master mix A and B must be used within 1 hour of preparation. Pipette 75 µL working master mix A and B into two amplification tube for each specimen and control. Do not cap the reaction tubes at this time. Fill remaining amplification tubes that will not be used for specimen or control with 100 µL water. Place the tray containing working master mix A and B in resealable plastic bag and seal the plastic bag securely until ready to use. Working master mix A and B are stable for 1 hour at room temperature. Add 25 µL of each prepared specimen, working CYP450 positive control and negative control to the appropriate amplification tube containing working master mix A and B. Cap the amplification tubes. Once prepared specimens and controls are added to working master mix A and B, amplification must be started within 15 minutes. Transfer the prepared specimens and controls in the amplification tray. The remainder of the prepared specimens may be stored at 2-8 $^{o}$C for 1 week or at -20 $^{o}$C for up to 1 month, with up to three freeze-thaw cycles.

**4.3.3 Amplification**

Program the thermal cycler 9700 for the AmpliChip CYP450 Test as follows:

| | |
|---|---|
| HOLD Program: | 2 min 50 $^{o}$C |
| HOLD Program: | 10 min 95 $^{o}$C |
| CYCLE Program (35 cycles): | 20 sec 95 $^{o}$C, 4 min 67 $^{o}$C |
| HOLD Program: | 7 min 72 $^{o}$C |
| HOLD Program: | 4 $^{o}$C Indefinitely |

Place the tray or retainer assembly into the thermal cycler block and ensure that all of the tubes are tightly capped. Before start the method program. Set the ramp speed to max and reaction volume to 100 µL in the method options screen. Press start again. The program runs approximately 3 hours and 30 minutes. Upon completion of amplification including the 72 $^{o}$C HOLD step, removed the tray from the thermal cycler and place in the MicroAmp base. If necessary, reaction tubes can be left in the thermal cycler at 4 $^{o}$C for up to 18 hours after amplification has completed. If amplicon fragmentation will not be performed within 30 minutes of removing reaction tubes from the thermal cycler, store the amplification tray at -20 $^{o}$C. The amplified products can be stored at -20 $^{o}$C up to 1 week.

### 4.3.4 Fragmentation and labeling of the amplified products

Prepare a solution of 20 mM EDTA by adding 1 mL 0.5 M EDTA to 24 mL of deionized water and mix thoroughly. 20 mM EDTA is stable for 6 months from the date of preparation, stored at 2-8 $^{o}$C in a clean and closed plastic container. Prepare working fragmentation mix for 24 fragmentation reactions as followed by pipetting the volumes listed in the (**Table 4.2**). Below in the specified order into a 2.0 mL tube that is kept on ice and briefly vortex working fragmentation mix.

**Table 4.2 Preparation of working fragmentation mix**

| **Fragmentation mix** | |
|---|---|
| **Reagents** | **24 reactions (µL)** |
| Water, nuclease-free | 191.4 |
| 20 mM EDTA | 3.3 |
| Alkaline Phosphatase(1 U/ µL ) | 22 |
| DNase I, RNase-free recombinant (10 U/µL) | 3.3 |
| **Total volume** | **220 µL** |

Label a new MicroAmp tray/retainer assembly as fragmentation. One reaction tube is needed for each specimen and control. Place the tubes in the MicroAmp tray and lock in place with retainer, and place on ice. Remove the caps from the working master mix A and B amplification tubes. Gently mix amplicon by pipetting up and down three times before removing and transferring 8 µL of the amplicon from each working master mix A and B reaction into the appropriate tube of the fragmentation tray on ice. Add 8 µL working fragmentation mix to each of the tubes containing amplicon in the fragmentation tray on ice. Gently mix each specimen and control by pipetting up and down three times with the pipette. Cap the reaction tubes. Immediately transfer the Fragmentation tray/retainer assembly into the 9700 thermal cycler programming as follow with the DNase fragmentation thermal cycling Protocol:

HOLD Program: 20 min 25 $^{o}$C

HOLD Program: 10 min 95 $^{o}$C

HOLD Program: 4 $^{o}$C Indefinitely

Before start the method program. Set the ramps to max and reaction volume to 24µL in the method options screen. Press start again. The program runs approximately 40 minutes. Upon completion of fragmentation, prepare the working labeling for 24 labeling reaction as follows by pipetting the volumes listed in the (**Table 4.3**) below in the specified order into a 2.0 mL tube that is kept on ice:

**Table 4.3 Preparation of working labeling mix**

| **Working labeling mix** | |
|---|---|
| **Reagents** | **1 reaction (µL)** |
| 5X Terminal Transferase Reaction Buffer | 6.8 |
| 25 mM $CoCl_2$ | 0.8 |
| AmpliChip TdT Labeling Reagent (TdT) | 0.8 |
| Terminal transferase, recombinant (400 U/ µL) | 1.6 |
| **Total volume** | **10 µL** |

Briefly vortex the working labeling mix. Remove the fragmentation tray from the thermal cycler and place into an amplification base. Remove the caps from the fragmentation tubes carefully to avoid creating aerosols of the fragmentation amplicon. Add 10 µL Working Labeling Mix into each of the tubes containing amplicon in the Fragmentation Tray. Gently mix each specimen or control by pipetting up and down three times with the pipettor. Cap the reaction tubes with new caps. Immediately transfer the fragmentation tray or retainer assembly into the 9700 thermal cycler programmed as follows with the labeling thermal cycling protocol:

HOLD Program: 35 min 37 $^{o}$C

HOLD Program: 5 min 95 $^{o}$C

HOLD Program: 4 $^{o}$C Indefinitely

Start the method program. Set the ramp speed to max and reaction volume to 34 µL in the method options screen. Press start again. The program runs approximately 45 minutes. Upon completion of labeling of the fragmented amplicon, remove the fragmentation tray from the thermal cycler and place into an amplification base. Store the fragmentation tray at 2-8 $^{o}$C for up to 18 hours until ready to perform

the microarray hybridization. If not proceeding to the hybridization step within 18 hours, store the labeled fragmentation tray at -20 $^{o}$C. The labeled fragmented amplified products can be stored at -20 $^{o}$C for up to 1 week.

### 4.3.5 Hybridization

Prepare a solution of 10% Triton X-100 by slowly pipetting 10 mL Triton X-100 into a clean empty container, then adding 90 mL water. Mix well. 10% Triton X-100 is stable for 6 months from the date of preparation, stored at 15-30 $^{o}$C in a clean, closed plastic container that protects the Triton X-100 from exposure to light. Prepare hybridization buffer by adding the component volumes listed in the following (**Table 4.4**) to a 0.5 mL tube and mixing by inversion:

**Table 4.4 Preparation of hybridization buffer**

| **Hybridization buffer** | |
|---|---|
| **Reagents** | **1 reaction (µL)** |
| 20X SSPE | 125 |
| 10% Triton X-100 | 2.5 |
| B1 Oligo | 50 |
| 50X Denhardt ’s Solution | 10 |
| 5% Sodium azide | 9 |
| Water, deionized or distilled | 303.5 |
| **Total volume** | **0.5 mL** |

Hybridization buffer is stable for 6 months from the date of preparation, stored at 2-8 $^{o}$C in a clean, closed plastic container. Prepare stain solution by adding the component volumes listed in the following (**Table 4.5**) to a 0.5 mL tube wrapped in foil and vortex for 30 seconds:

**Table 4.5 Preparation of stain solution**

| Stain solution | |
|---|---|
| **Reagents** | **1 reaction (µL)** |
| 20X SSPE | 140 |
| Acetylated Bovine Serum Albumin (20 mg/mL) | 25 |
| Streptavidin, R-phycoerythrin conjugate (1 mg/mL) | 5 |
| 5% Sodium azide | 9 |
| Water, deionized or distilled | 321 |
| **Total volume** | **0.5 mL** |

Stain Solution is stable for 6 months from the date of preparation, stored at 2-8 $^{o}$C in a clean, closed plastic container that protects the Stain Solution from exposure to light.
4.3.5.4 Prepare Wash Buffer by combining the component volumes listed in the following (**Table 4.6**) in a suitable container and mixing thoroughly:

**Table 4.6 Preparation of wash buffer**

| Wash buffer | |
|---|---|
| **Reagents** | **1 liter** |
| 20X SSPE | 150 mL |
| 10% Triton X-100 | 0.5 mL |
| 5% Sodium azide | 18 mL |
| Water, distilled or deionized | 831.5 mL |
| **Total volume** | **1.0 L** |

Label one 1.5 mL tube for each specimen and control. Add 500 µL hybridization buffers to each tube. Mix each fragmented/labeled specimen and control amplicon with the pipette and then add 20 µL to the appropriately labeled tube. Cap the tubes and vortex each tube for 10 seconds. Incubate the tubes at 95 $^{o}$C for 10 minutes in a dry heat block. Remove the tubes from the heat block and immediately place each tube in the ice water bath. Add 500 µL stain solution into the appropriate number of 1.5 mL tubes, one for each specimen and control. Label one array for each specimen and control.

#### 4.3.6 Hybridization and staining

Load the appropriated tube that contain hybridization buffer and denatured fragmented/labeled amplicon to position 1 and a tube containing stain solution to position 2 of each module to be used on the GeneChip fluidics station. After hybridization and staining are completed, remove the array from the GeneChip fluidics station before closing the washblock door. Visually inspect the array window for air bubbles. If air bubbles are present, reinsert the array, close the washblock door and the GeneChip fluidics station will automatically refill the array with wash buffer. Follow the module instructions to complete the protocol.

#### 4.3.7 Microarray scan and report

To prevent leaked of liquid from the array, apply one tough-spot over each of the two septa located on the back of each Array and press to ensure that the spots remain flat. Load the array into the scanner autoloader; they may be loaded in any order and scan arrays with AMDS and AmpliChip CYP450 data analysis software. Before start scanner, select *CYP2D6*, *CYP2C19* or both to define the desired report on the additional information window or batch information window within the AmpliChip CYP450 test data analysis software. Start the scanning function. The results will be automatically generated after scanning and can be reviewed by following the links in AMDS.

## 4.4 Results interpretation

#### 4.4.1 *CYP2D6* genotypes and phenotypes

DNA samples were isolated by the salting out procedure and were adjusted to a concentration of 20 ng/µl, and purified DNA was amplified by PCR. AmpliChip CYP 450 test used for detection of *CYP2D6* and *CYP2C19* polymorphisms according to the manufacturer's instructions. The AmpliChip CYP 450 microarray contained with over 15,000 different oligonucleotide probes that was synthesized on a glass surface to analyze both sense and antisense strands of an amplified target DNA samples. The AmpliChip CYP450 microarray assay distinguished 29 known

polymorphisms in the *CYP2D6* gene including gene duplication and gene deletion. The polymorphisms of *CYP2D6* were detected results in the identification of 33 alleles (**Table 4.7**). i.e.

Normal functional alleles : **1,*2,*35*

Reduced functional alleles : **9,*10, *14B, *17,*29,*36,*41,*10XN, *17XN* and **41XN*

Nonfunctional alleles : **3, *4, *5, *6, *7, *8, *11, *14A, *15, *19,*20*, **31, *40* and **4XN*

Duplicated alleles (increased functional): **1XN, *2XN* and **35XN*

Unknown functional alleles : **25, *26, *30*

The combination of polymorphisms allows for the prediction of the likely enzymatic activity of the *CYP2D6* allelic gene product [11]. The nucleotide changes listed in bold font define the allele.

**Table 4.7 Cytochrome P450 *2D6* mutation detected 33 alleles**

| ***CYP2D6* alleles** | **Nucleotides change** | **Effect** | **Enzyme Activity** |
|---|---|---|---|
| **1* | None | | Normal |
| **2ABD* | -1584G, 1039C>T, 1661G>C, **2850C>T,4180G>C** | R296C, S486T | Normal |
| **3* | **2549Adel** | 259Frameshift | None |
| **4ABDJK* | 100C>T, 1039C>T, 1661G>C**,** **1846G>A**, 2850C>T, 4180G>C | splicing defect | None |
| **5* | **Gene deletion** | *CYP2D6* deleted | None |
| **6ABC* | **1707Tdel**, 1976G>A, 4180G>C | 118Frameshift | None |
| **7* | **2935A>C** | H324P | None |
| **8* | 1661G>C, **1758G>T**, 2850C>T,4180G>C | G169X | None |
| **9* | **2613-2615delAGA** | K281del | Reduced |
| **10AB* | **100C>T**, 1039C>T, 1661G>C, 4180G>C | P34S | Reduced |
| **11* | **883G>C**, 1661G>C, 2850C>T,4180G>C | Splicing defect | None |
| **14A* | 100C>T, **1758G>A**, 2850C>T,4180G>C | G169R | None |
| **14B* | 1661G>C,**1758G>A**, 2850C>T,4180G>C | G169R | Reduced |
| **15* | **138 ins T** | 46Frameshift | None |
| **17* | **1023C>T**, 1661G>C, **2850C>T**,4180G>C | T107I, R296C | Reduced |
| **19* | 1661G>C, **2539-2542delAACT**, 2850C>T,4180G>C | 255Frameshift | None |
| **20* | 1661G>C, **1973insG**,1978C>T, 2850C>T, 4180G>C | 211Frameshift | None |
| **25* | **3198C>G** | R343G | Unknown |
| **26* | **3277T>C** | I369T | Unknown |
| **29* | **1659G>A**, 1661G>C, 2850C>T, **3183G>A**, 4180G>C | V136I, V338M | Reduced |

| *CYP2D6* alleles | Nucleotides change | Effect | Enzyme Activity |
|---|---|---|---|
| **30* | 1661G>C, **1855-1863ins (TTTCGCCCC)repeat**, 2850C>T,4180G>C | 174_175insFRP | Unknown |
| **31* | 1661G>C,2850C>T,**4042G>A**,4180G>C | R440H | None |
| **35* | -1584G, **31G>A**,1661G>C, 2850C>T,4180G>C | V11M | Normal |
| **36* | **100C>T**,1039C>T, 1661G>C,4180G>C, **gene conversion to CYP2D7 in exon 9** | P34S | Reduced |
| **40* | **1023C>T**, 1661G>C,**1863ins(TTT CGC CCC)2**; 2850C>T,4180G>C | T107I, 174_175insFRP x2 | None |
| **41* | -1584C, 1661G>C, **2850C>T**, **2988G>A, 4180G>C** | R296C, splicing defect, S486T | Reduced |
| **1XN* | Duplicate active *1 genes (n is not determined-range 2-13) | *N* active genes | Increased |
| **2XN* | Duplicate active *2 genes (n is not determined-range 2-13) | *N* active genes | Increased |
| **4XN* | Duplicate inactive *4 genes (n is not determined) | | None |
| **10XN* | Duplicate partially active *10 genes (n is not determined) | | Reduced |
| **17XN* | Duplicate partially active *17 genes (n is not determined) | | Reduced |
| **35XN* | Duplicate partially active *35 genes (n is not determined) | | Increased |
| **41XN* | Duplicate partially active *41 genes (n is not determined) | | Reduced |

### 4.4.2 Predicted *CYP2D6* phenotypes

The combination of the activity of the enzymes encoded by the two *CYP2D6* alleles determines the overall metabolic activity for individual. There are four phenotypic types (**Table 4.8**):

Poor metabolizers (PM): No enzyme activity, increased risk for drug toxicity/side effects and little to no therapeutic effect with pro-drugs. Genotypes consistent with the poor metabolizer phenotype are those with no active *CYP2D6* alleles.

Intermediate metabolizers (IM): Reduced enzyme activity, some therapeutic effect with increased potential for toxicity/side effects. Genotypes consistent with the intermediate metabolizer phenotype are those with one active and one inactive *CYP2D6* allele, one inactive and one partially active *CYP2D*6 allele, or two partially active *CYP2D6* alleles

Extensive metabolizers (EM): Normal enzyme activity, drug is metabolized efficiently, resulting in therapeutic effect with minimal toxicity. Genotypes consistent with the normal metabolizer phenotype include two active *CYP2D6* alleles or one active and one partially active *CYP2D6* allele. Increased caution may be appropriate for individuals having one partially active allele.

Ultra-rapid metabolizers (UM): Excess enzyme activity, drug is extensively metabolized, resulting in increased or lack of therapeutic effect depending on pro-drug status. Genotypes consistent with ultra-metabolizer phenotype include three or more active *CYP2D6* alleles due to duplication of an active allele.

### 4.4.3 *CYP2C19* genotypes and phenotypes

*CYP2C19* genotypes distinguished 2 polymorphisms and determination of 3 alleles (**Table 4.9**). The two of most common allele variant of *CYP2C19* (i.e. *CYP2C19*2* and *CYP2C19*3)* result in a nonfunctional enzymes (**Table 17**).

**Table 4.8 Cytochrome P450 *2C19* mutations detected 3 alleles**

| ***CYP2C19* allele** | **Nucleotide change** | **Predicted Enzyme Activity** |
|---|---|---|
| **1* | None (wild type) | Normal |
| **2* | 681G>A | None |
| **3* | 636G>A | None |

**Table 4.9 Drug metabolism phenotypes associated with *CYP2C19* allelic variants**

| Allele | 1 | 2 | 3 |
|---|---|---|---|
| 1 | E | E | E |
| 2 | | P | P |
| 3 | | | P |

The combination of the activity of the enzymes encoded by the two *CYP2D6* alleles determines the overall metabolic activity for individual. There are two phenotypic types:

Poor metabolizers (PM): are at increased risk of drug-induced side effects due to diminished drug elimination or for pro-drugs lack of therapeutic effect resulting from failure to generate the active form of the drug. Genotypes consistent with the poor metabolizer phenotype are those with no active *CYP2C19* alleles.

Extensive metabolizers (EM): Normal enzyme activity; drug is metabolized efficiently, resulting in therapeutic effect with minimal toxicity. Genotypes consistent with the normal metabolizer phenotype include two active *CYP2C19* alleles.

## 4.5 Statistical analysis

Descriptive statistics were used to describe the clinical characteristics of the subjects. Hardy-Weinberg equilibrium was conducted with Haploview 4.2. The Fisher's exact test or Chi-square test was used to compare the different alleles and patients characteristics between cases and controls. Disease free survival (DFS) was defined as the time from surgery to the occurrence of breast event (local, regional, or distant occurrence or contralateral breast cancer) or death from any cause. Patients who were alive without a breast cancer relapse were censored at the last follow up date. Survival curves were estimated with the Kaplan-Meier method. Statistical significance of a relationship between breast cancer outcomes and each of the genetic polymorphisms was compared by the log-rank test. The Univariate Cox proportion

hazard model was used to estimation the hazard ratio (HR) for comparing the genotype of each group. All tests were two sided and *P* values of less than 0.05 were considered statistically significant. Statistical analyses were conducted using STATA version 12.

# CHAPTER V
# RESULTS

## 5.1 The clinical data of breast cancer patients

This study used microarray technique for genotyping and identifies polymorphisms in *CYP2D6* and *CYP2C19*. The characteristics of these patients can be found in **Table 5.1**. All breast cancer patients who received adjuvant tamoxifen treatment for 5 years were recruited. Cases group composed of 24 patients defined as patients who had completed 5 years of adjuvant tamoxifen and controls group composed of 24 patients defined as patients who had disease recurrence while receiving adjuvant tamoxifen therapy. There were no statistically significant differences in the clinical characteristics of patients in these two groups. The median ages of all women in this study were 50 ± 11 years. Patients received adjuvant tamoxifen treatment from February 1997 to January 2008 with a median follow up time of control and case group of 100.2 (range, 66.5-173.6) months and 32.5 (range, 9.0-70.7) months, respectively. The numbers of pre- and post-menopausal women were 30 and 18, respectively. All women were estrogen receptor positive by immunohistochemistry except one subject estrogen receptor negative but had progesterone receptor positive. All women baseline characteristics were similar. Twenty four patients had positive axillary lymph nodes. Most patients were treated with a modified radical mastectomy. The regimen of adjuvant chemotherapy composed of CMF, Adriamycin based and Adriamycin-Taxane based regimens. Three patients in this study did not receive adjuvant chemotherapy despite being eligible for treatment because they had positive axillary lymph nodes (N1) (two patients in control arm and one patient in case arm). The overall median follow up time was 67.3 (range, 9.1-157.1) months.

**Table 5.1 Characteristics of breast cancer patients according to two groups**

| Characteristics | Non-recurrence | Recurrence | *P* |
|---|---|---|---|
| **Age (years), n (%)** | (n=24) | (n=24) | 0.248[c] |
| ≤ 50 | 10 (41.67) | 14 (58.33) | |
| >50 | 14 (58.33) | 10 (41.67) | |
| **Menstrual status, n (%)** | | | 0.233[c] |
| Pre-menopause | 13 (54.17) | 17 (70.83) | |
| Post-menopause | 11 (45.83) | 7 (29.17) | |
| **Tumor size (cm.), n (%)** | | | 1.000[b] |
| ≤ 2 | 5 (20.83) | 4 (16.67) | |
| 2.1-5 | 16 (66.67) | 17 (70.83) | |
| >5 | 3 (12.50) | 3 (12.50) | |
| **ER[d], n (%)** | | | 1.000[b] |
| Positive | 24 (100.00) | 23 (95.83) | |
| Negative | 0 (0.00) | 1 (4.17) | |
| **PR[e], n (%)** | | | 1.000[b,*] |
| Positive | 5 (20.83) | 15 (62.50) | |
| Negative | 3 (12.50) | 7 (29.17) | |
| Unknown | 16 (66.67) | 2 (8.33) | |
| **Her-2, n (%)** | | | 1.000[b,*] |
| Positive | 0 (0.00) | 1 (4.17) | |
| Negative | 8 (33.33) | 17 (70.83) | |
| Unknown | 16 (66.67) | 6 (25.00) | |
| **Grading, n (%)** | | | 1.000[b,*] |
| 1 | 2 (8.33) | 2 (8.34) | |
| 2 | 9 (37.50) | 12 (50.00) | |
| 3 | 4 (16.67) | 5 (20.83) | |
| Unknown | 9 (37.50) | 5 (20.83) | |
| **Lymph node status, n (%)** | | | 0.920[c] |
| 0 | 12 (50.00) | 12 (50.00) | |
| 1-3 | 5 (20.83) | 6 (25.00) | |
| ≥4 | 7 (29.17) | 6 (25.00) | |
| **LVI[f], n (%)** | | | 0.796[c,*] |
| Positive | 8 (33.33) | 8 (33.33) | |
| Negative | 11 (45.83) | 13 (54.17) | |
| Unknown | 5 (20.84) | 3 (12.50) | |
| **Margin, n (%)** | | | 0.701[b] |
| Positive | 3 (12.50) | 5 (20.83) | |
| Negative | 21 (87.50) | 19 (79.17) | |
| **Chemotherapy, n (%)** | | | 0.179[b] |
| No chemotherapy | 1 (4.17) | 2 (8.33) | |
| CMF[g] | 15 (42.50) | 9 (37.50) | |
| Adrinamycin base | 8 (33.33) | 10 (41.67) | |
| Adrinamycin-Taxane base | 0 (0.00) | 3 (12.50) | |
| **Radiation, n (%)** | | | 0.376[c] |
| Yes | 8 (33.33) | 11 (45.83) | |
| No | 16 (66.67) | 13 (54.17) | |

[b]Fisher 's exact test; [c]Chi-square test; [d]ER, estrogen receptor; [e]PR, progesterone receptor; [f]LVI, lymphovascular invasion; [g]CMF; cyclophosphamide,methotrexate,fluouracil, [*]The data were not included in *P* value analysis

## 5.2 Hardy-Weinberg equilibrium of breast cancer patients

*CYP2D6* and *CYP2C19* genotype frequencies were tested for Hardy-Weinberg equilibrium by using Haploview 4.2. Samples were successfully genotyped 100 percent for each SNP, list in **Table 5.2**. Genotype frequencies for both *CYP2D6* and *CYP2C19* of cases and control were in Hardy-Weinberg equilibrium. Hardy-Weinberg equilibrium probability value (HWpval) of *CYP2D6* included that -1584C>G (0.1911), 100C>T (0.944), 1039C>T (0.7253), 1661G>C (0.1715), 2850C>T (0.9505), and 4180G>C (0.3062), respectively and HWpval of *CYP2C19* included that 681G>A (1.0) and 636G>A (1.0). Minor allele frequency (MAF) of polymorphisms in *CYP2D6* including -1584C>G, 100C>T, 1039C>T, 1661G>C, 2850C>T, and 4180G>C of *CYP2D6* were 0.115, 0.49, 0.479, 0.417, 0.115, and 0.406, respectively and two single nucleotide polymorphisms (SNPs) had MAF less than 0.05 including 1846G>A (0.010) and **36* (0.010). Minor allele frequency of polymorphisms in *CYP2C19* including 681G>A, 636G>A were 0.26 and 0.062, respectively.

**Table 5.2 Minor allele frequency of polymorphisms in *CYP2D6* and *CYP2C19***

| SNPs | Position | SNP ID | ObsHET | PredHET | HWpval | MAF |
|---|---|---|---|---|---|---|
| ***CYP2D6*** | | | | | | |
| -1584C>G | 42528382 | rs1080985 | 0.15 | 0.20 | 0.191 | 0.115 |
| 100C>T | 42526694 | rs1065852 | 0.48 | 0.50 | 0.944 | 0.490 |
| 1039C>T | 42525756 | rs1081003 | 0.46 | 0.50 | 0.725 | 0.479 |
| 1661G>C | 42525132 | rs1058164 | 0.38 | 0.49 | 0.172 | 0.417 |
| 1846G>A | 42524947 | rs1800716 | 0.02 | 0.02 | 1.000 | 0.010 |
| 2850C>T | 42523943 | rs16947 | 0.19 | 0.20 | 0.951 | 0.115 |
| 4180G>C | 42522613 | rs1135840 | 0.40 | 0.47 | 0.392 | 0.377 |

| SNPs | Position | SNP ID | ObsHET | PredHET | HWpval | MAF |
|---|---|---|---|---|---|---|
| ***CYP2C19*** | | | | | | |
| 681G>A | 96541616 | rs4244285 | 0.40 | 0.39 | 1.000 | 0.260 |
| 636G>A | 96540410 | rs4986893 | 0.13 | 0.12 | 1.000 | 0.062 |

ObsHET, Observed Heterozygosity; PredHET, Predicted Heterozygosity; HWpval, Hardy-Weinberg *P* value; MAF, Minor Allele Frequency
The rs numbers in parentheses are the accession numbers in the National Center for Biotechnology information single nucleotide polymorphism (SNP) database, dbSNP

## 5.3 Linkage disequilibrium map of *CYP2D6* and *CYP2C19*

The results of linkage disequilibrium (LD) map of *CYP2D6* and *CYP2C19* SNPs were shown in **Figure 5.1**. It had one LD block of *CYP2D6* that consist of two tagSNPs including 100C>T (rs1065852) and 1039C>T (rs1081003), which had length of linkage disequilibrium block approximately 0.9 kilo base (kb). No linkage disequilibrium block of polymorphisms in *CYP2C19*.

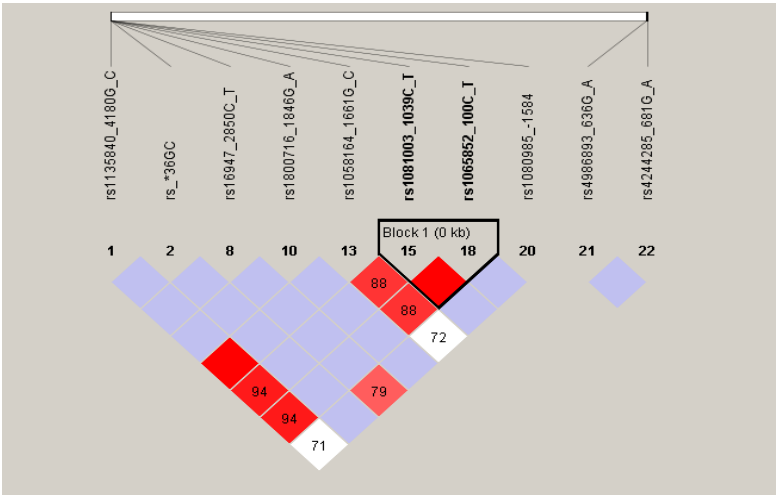



**Figure 5.1 Linkage disequilibrium maps of *CYP2D6* and *CYP2C19***

## 5.4 Haplotype analysis of *CYP2D6*

Haplotype analysis showed that *CYP2D6* SNPs 100C>T and 1039C>T are in linkage disequilibrium (D′= 1.0, r-squared = 0.959), list in Table 5.3. Haplotype frequencies comprised of *CC* (51.0%), *TT* (47.9%), and *CT* (1.0%), respectively.

**Table 5.3 Haplotype frequencies of *CYP2D6***

| Haplotype | Non-recurrence | | Recurrence | | Chi-square | *P* |
|---|---|---|---|---|---|---|
| ***CYP2D6*** | **Number** | **Freq.** | **Number** | **Freq.** | | |
| *CC* | 26 | 0.54 | 23 | 0.48 | 0.375 | 0.540 |
| *TT* | 22 | 0.46 | 24 | 0.50 | 0.167 | 0.683 |
| *CT* | 0 | 0.00 | 1 | 0.02 | 1.011 | 0.315 |

## 5.5 *CYP2D6* and *CYP2C19* polymorphisms profile

This study was determined *CYP2D6* and *CYP2C19* genotypes of these 48 patients. Allele frequencies and summarized genotypes and phenotypes are shown in **Table 5.4**. The *CYP2D6* allele frequencies were **10* (45.90%), **1* (34.40%), **2* (10.40%), **5* (4.20%), **41* (2.10%), **4* (1.00%), **35* (1.00%) and **36* (1.00%), respectively. There were seven mutant SNPs and one gene conversion of *CYP2D6* from 29 polymorphisms found in this study including -1584C>G, 100C>T, 1039C>T, 1661G>C, 1846G>A, 2850C>T, 4180G>C, and *CYP2D6*36 gene conversion* (**Table 5.5**), and one gene deletion is *CYP2D6*5*. The most variant alleles is *CYP2D6*10* (100C>T) that similar frequencies in Asian population. It is rare frequencies of *CYP2D6* null alleles (**4, *5*) in this study compared with Caucasian population (**Table 5.4**). The frequencies of *CYP2D6* genotype were **1/*1* (16.70%), **1/*2* (2.10%), **1/*5* (2.10%), **1/*10* (27.00%), **1/*36* (2.10%), **1/*41* (2.10%), **2/*2* (4.20%), **2/*4* (2.10%), **2/*10* (8.30%), **5/*10* (6.20%), **10/*10* (22.90%), **10/*35* (2.10%) and **10/*41* (2.10%), respectively, listed in **Table 5.7**. The most genotype is *CYP2D6*1/*10* (27.00%). There are two *CYP2D6* predicted phenotypes (**Table 5.8**) including EM (**1/*1, *1/*2, *1/*5, *1/*10, *1/*36, *1/*41, *2/*2, *2/*4*, **2/*10* and

**10/*35*) and IM (**5/*10* and **10/*10*). However, no homozygous PM and gene duplication was observed in this study. The frequencies of homozygous EM patients with two functional alleles (EM/EM) were 11 (23.00%) and heterozygous EM patients with one reduced or non-functional alleles (EM/IM, EM/PM, and EM/PM) were 22 (45.80%). There were 12 (25.00%) patients had homozygous IM (IM/IM) with two reduced functional alleles and 3 (6.20%) patients had heterozygous IM (IM/PM) with one reduced functional allele and one non-functional allele (**Table 5.7**).

The *CYP2C19* allele frequencies were *CYP2C19*1* (68.80%), **2* (25.00%), and **3* (6.20%), respectively. There were two mutant SNPs of *CYP2C19* including 681G>A (*CYP2C19*2*) and 636G>A (*CYP2C19*3*). The most variant alleles were *CYP2C19*2* (rs4244285) and *CYP2C19*3* (rs4986893), respectively. The frequencies of *CYP2C19* variant alleles were similar frequencies in South East Asian population but it were higher frequencies than Caucasian and African-American population (**Table 5.4**). The frequencies of *CYP2C19* genotype were **1/*1* (43.80%), **1/*2* (37.50%), **1/*3* (12.50%), and **2/*2,* respectively, listed in **Table 5.6**. The most variant *CYP2C19* genotype is **1/*2*. There are two *CYP2C19* predicted phenotypes (**Table 5.8**) including EM (**1/*1, *1/*2, *1/*3*) and PM (**2/*2*). The frequencies of homozygous EM patients with two functional alleles (EM/EM) were 21 (43.80%) and heterozygous EM patients with one reduced or non-functional alleles (EM/PM) were 24 (50.00%). There were 3 (6.20%) patients had homozygous PM (PM/PM) with two non-functional allele (**Table 5.7**).

The *CYP2D6* and *CYP2C19* polymorphisms including SNPs, genotypes and phenotypes were compared the different between cases versus control group by Chi- square test and Fisher's exact test. All analyses were performed in STATA version 12.0.

**Table 5.4 Frequencies of *CYP2D6* [55, 56, 104-107] and *CYP2C19* [108] allele in different ethnic groups**

| Alleles | Major genetic variant | Enzyme activity | SNP ID | Current study n (%) | Asian | Caucasian | African-American |
|---|---|---|---|---|---|---|---|
| ***CYP2D6*** (n= 96) | | | | | | | |
| **1* | None | Normal | | 33 (34.40) | 20-40 | 30-40 | 28-50 |
| **2* | 2850C>T, 4180G>C | Normal | rs16947, rs1135840 | 10 (10.40) | 9-20 | 20-35 | 10-80 |
| **4* | 1846G>A | None | rs3892097 | 1 (1.00) | 0.5-3 | 12-23 | 2-7 |
| **5* | Gene deletion | None | | 4 (4.20) | 4-6 | 1.5-7 | 0.5-6 |
| **10* | 100C>T | Decrease | rs1065852 | 44 (45.90) | 40-70 | 2-8 | 3-8 |
| **35* | 31G>A | Normal | | 1 (1.00) | 1 | 4-6 | - |
| **36* | Gene conversion | Decrease | | 1 (1.00) | - | - | 1 |
| **41* | 1661G>C, 2850C>T, 4180G>C | Decrease | rs1058164 | 2 (2.10) | 1.4-2.6 | 8 | 15 |
| ***CYP2C19*** (n= 96) | | | | | **Southeast Asian** | **Caucasian** | **African-American** |
| **1* | None | Normal | | 66 (68.80) | 63.12 | 86.4 | 81 |
| **2* | 681G>A | None | rs4244285 | 24 (25.00) | 31.2 | 12.7 | 18.2 |
| **3* | 636G>A | None | rs4986893 | 6 (6.20) | 5.7 | 0.9 | 0.8 |

The rs numbers in parentheses are the accession numbers in the National Center for Biotechnology information single nucleotide polymorphism (SNP) database, dbSNP.

**Table 5.5 Allelic frequencies of *CYP2D6* and *CYP2C19* among two groups**

| Alleles | Total No. | Non-recurrence n (%) | Recurrence n (%) | *P* |
|---|---|---|---|---|
| ***CYP2D6*** | (n=48) | (n=24) | (n= 24) | |
| -1584C>G, rs1080985 | | | | |
| *CC* | 39 | 19 (79.17) | 20 (83.33) | 1.000[b] |
| *CG* | 7 | 3 (12.50) | 4 (16.67) | 1.000[b] |
| *GG* | 2 | 2 (8.33) | 0 (0.00) | 0.489[b] |
| 100C>T, rs1065852 | | | | |
| *CC* | 13 | 7 (29.17) | 6 (25.00) | 0.745[c] |
| *CT* | 23 | 12 (50.00) | 11 (45.83) | 0.773[c] |
| *TT* | 12 | 5 (20.83) | 7 (29.17) | 0.740[c] |
| 1039C>T, rs1081003 | | | | |
| *CC* | 14 | 7 (29.17) | 7 (29.17) | 1.000[c] |
| *CT* | 22 | 12 (54.55) | 10 (45.83) | 0.562[c] |
| *TT* | 12 | 5 (20.83) | 7 (25.00) | 0.505[c] |
| 1661G>C, rs1058164 | | | | |
| *GG* | 11 | 5 (20.83) | 6 (25.00) | 0.731[c] |
| *GC* | 18 | 10 (41.67) | 8 (33.33) | 0.551[c] |
| *CC* | 19 | 9 (37.50) | 10 (41.67) | 0.768[c] |
| 1846G>A, rs3892097 | | | | |
| *GG* | 47 | 24 (100) | 23 (95.83) | 1.000[b] |
| *GA* | 1 | 0 (0.00) | 1 (4.17) | 1.000[b] |
| *AA* | 0 | 0 (0.00) | 0 (0.00) | - |
| 2850C>T, rs16947 | | | | |
| *CC* | 38 | 18 (75.00) | 20 (83.33) | 0.724[b] |
| *CT* | 9 | 5 (20.83) | 4 (16.67) | 1.000[b] |
| *TT* | 1 | 1 (4.17) | 0 (0.00) | 1.000[b] |
| 4180G>C, rs1135840 | | | | |
| *GG* | 10 | 4 (16.67) | 6 (25.00) | 0.477[c] |
| *GC* | 19 | 11 (45.83) | 8 (33.33) | 0.376[c] |
| *CC* | 19 | 9 (37.50) | 10 (41.67) | 0.768[c] |
| ***CYP2C19*** | | | | |
| 681G>A, rs4244285 | | | | |
| *GG* | 26 | 12 (50.00) | 14 (58.33) | 0.562[c] |
| *GA* | 19 | 9 (37.50) | 10 (41.67) | 0.768[c] |
| *AA* | 3 | 3 (12.50) | 0 (0.00) | 0.234 |
| 636G>A, rs4986893 | | | | |
| *GG* | 42 | 21 (87.50) | 21 (87.50) | 1.000[b] |
| *GA* | 6 | 3 (12.50) | 3 (12.50) | 1.000[b] |
| *AA* | 0 | 0 (0.00) | 0 (0.00) | - |

[b] Fisher's exact test; [c] Chi-square test; The rs numbers in parentheses are the accession numbers in the National Center for Biotechnology information single nucleotide polymorphism (SNP) database,dbSNP.

**Table 5.6 *CYP2D6* and *CYP2C19* allele frequencies compared between groups**

| Alleles | Total No. | Non-recurrence n (%) | Recurrence n (%) | *P* |
|---|---|---|---|---|
| ***CYP2D6*** | (n=96) | (n=48) | (n=48) | |
| **1* | 33 | 17 (35.42) | 16 (33.33) | 0.830[c] |
| **2* | 10 | 7 (14.58) | 3 (6.26) | 0.181[c] |
| **4* | 1 | 0 (0.00) | 1 (2.08) | 1.000[b] |
| **5* | 4 | 2 (4.17) | 2 (4.17) | 1.000[b] |
| **10* | 44 | 20 (41.67) | 24 (50.00) | 0.413[c] |
| **35* | 1 | 0 (0.00) | 1 (2.08) | 1.000[b] |
| **36* | 1 | 1 (2.08) | 0 (0.00) | 1.000[b] |
| **41* | 2 | 1 (2.08) | 1 (2.08) | 1.000[b] |
| ***CYP2C19*** | | | | |
| **1* | 66 | 31 (64.58) | 35 (72.91) | 0.378[c] |
| **2* | 24 | 14 (29.16) | 10 (20.83) | 0.346[c] |
| **3* | 6 | 3 (6.26) | 3 (6.26) | 1.000[b] |

[b] Fisher's exact test
[c] Chi-square test

**Table 5.7 *CYP2D6* and *CYP2C19* genotype frequencies and associated predicted phenotypes**

| Genotypes | Total No. | Non-recurrence n (%) | Recurrence n (%) | Predicted Phenotypes | *P* |
|---|---|---|---|---|---|
| ***CYP2D6*** | (n=48) | (n=24) | (n=24) | | |
| **1/*1* | 8 | 4 (16.67) | 4 (16.67) | EM/EM | 1.000[b] |
| **1/*2* | 1 | 1 (4.16) | 0 (0.00) | EM/EM | 1.000[b] |
| **1/*5* | 1 | 0 (0.00) | 1 (4.16) | EM/PM | 1.000[b] |
| **1/*10* | 13 | 7 (29.17) | 6 (25.00) | EM/IM | 0.745[c] |
| **1/*36* | 1 | 1 (4.16) | 0 (0.00) | EM/IM | 1.000[b] |
| **1/*41* | 1 | 0 (0.00) | 1 (4.16) | EM/IM | 1.000[b] |
| **2/*2* | 2 | 2 (8.33) | 0 (0.00) | EM/EM | 0.489[b] |
| **2/*4* | 1 | 0 (0.00) | 1 (4.16) | EM/PM | 1.000[b] |
| **2/*10* | 4 | 2 (8.33) | 2 (8.33) | EM/IM | 1.000[b] |
| **5/*10* | 3 | 2 (8.33) | 1 (4.16) | PM/IM | 1.000[b] |
| **10/*10* | 11 | 4 (16.67) | 7 (29.17) | IM/IM | 0.303[c] |
| **10/*35* | 1 | 0 (0.00) | 1 (4.16) | IM/EM | 1.000[b] |
| **10/*41* | 1 | 1 (4.16) | 0 (0.00) | IM/IM | 1.000[b] |
| ***CYP2C19*** | | | | | |
| **1/*1* | 21 | 10 (41.67) | 11 (45.83) | EM/EM | 0.771[c] |
| **1/*2* | 18 | 8 (33.33) | 10 (41.67) | EM/PM | 0.551[c] |
| **1/*3* | 6 | 3 (12.50) | 3 (12.50) | EM/PM | 1.000[b] |
| **2/*2* | 3 | 3 (12.50) | 0 (0.00) | PM/PM | 0.234[b] |

Abbreviations: EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer.
[b] Fisher's exact test; [c] Chi-square test

**Table 5.8 *CYP2D6* and *CYP2C19* predicted phenotypes / genotypes according to two groups**

| Predicted Phenotype | Genotype | Non-recurrence n (%) | Recurrence n (%) | *P* |
|---|---|---|---|---|
| ***CYP2D6*** | | (n=24) | (n=24) | |
| EM/EM | **1/*1, *1/*2, *2/*2* | 7 (29.20) | 4 (16.70) | 0.303[c] |
| EM/IM | **1/*10, *2/*10, *10/*35, *1/*36, *1/*41* | 10 (41.70) | 10 (41.70) | 1.000[c] |
| EM/PM | **1/*5, *2/*4* | 0 (0.00) | 2 (8.30) | 0.489[b] |
| IM/IM | **10/*10, *10/*41* | 5 (20.80) | 7 (29.20) | 0.505[c] |
| IM/PM | **5/*10* | 2 (8.30) | 1 (4.10) | 1.000[b] |
| ***CYP2C19*** | | | | |
| EM/EM | **1/*1* | 10 (41.70) | 11 (45.80) | 0.771[c] |
| EM/PM | **1/*2, *1/*3* | 11 (45.80) | 13 (54.20) | 0.564[c] |
| PM/PM | **2/*2* | 3 (12.50) | 0 (0.00) | 0.234[b] |

Abbreviation: EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer.
[b] Fisher 's exact test
[c] Chi-square test

**Table 5.9 Allele frequencies of *CYP2D6* and *CYP2C19* among pre- and post-menopause breast cancer patients**

| SNPs Genotype | Pre-menopause n (%) | Post-menopause n (%) | *P* |
|---|---|---|---|
| ***CYP2D6*** | (n= 30) | (n= 18) | |
| -1584C>G,rs1080985 | | | |
| *CC* | 25 (83.33) | 14 (77.78) | 0.711[b] |
| *CG* | 4 (13.33) | 3 (16.67) | 1.000[b] |
| *GG* | 1 (3.33) | 1 (5.56) | 1.000[b] |
| 100C>T,rs1065852 | | | |
| *CC* | 6 (20.00) | 7 (38.89) | 0.190[b] |
| *CT* | 18 (60.00) | 5 (27.78) | 0.031[c] |
| *TT* | 6 (20.00) | 6 (33.33) | 0.325[b] |
| 1039C>T,rs1081003 | | | |
| *CC* | 7 (23.33) | 7 (38.89) | 0.251[c] |
| *CT* | 17 (56.67) | 5 (27.78) | 0.052[c] |
| *TT* | 6 (20.00) | 6 (33.33) | 0.325[b] |
| 1661G>C,rs1058164 | | | |
| *GG* | 7 (23.33) | 4 (22.22) | 1.000[b] |
| *GC* | 11 (36.67) | 7 (38.89) | 0.878[c] |
| *CC* | 12 (40.00) | 7 (38.89) | 0.939[c] |
| 1846G>A,rs3892097 | | | |
| *GG* | 29 (96.67) | 18 (100.00) | 1.000[b] |
| *GA* | 1 (3.33) | 0 (0.00) | 1.000[b] |
| *AA* | 0 (0.00) | 0 (0.00) | - |
| 2850C>T,rs16947 | | | |
| *CC* | 24 (80.00) | 14 (77.78) | 1.000[b] |
| *CT* | 5 (16.67) | 4 (22.22) | 0.711[b] |
| *TT* | 1 (3.33) | 0 (0.00) | 1.000[b] |
| 4180G>C,rs1135840 | | | |
| *GG* | 6 (20.00) | 4 (22.22) | 1.000[b] |
| *GC* | 12 (40.00) | 7 (38.89) | 0.939[c] |
| *CC* | 12 (40.00) | 7 (38.89) | 0.939[c] |
| ***CYP2C19*** | | | |
| 681G>A,rs4244285 | | | |
| *GG* | 17 (56.67) | 9 (50.00) | 0.654[c] |
| *GA* | 11 (36.67) | 8 (44.44) | 0.594[c] |
| *AA* | 2 (6.67) | 1 (5.56) | 1.000[b] |
| 636G>A,rs4986893 | | | |
| *GG* | 26 (86.67) | 16 (88.89) | 1.000[b] |
| *GA* | 4 (13.33) | 2 (11.11) | 1.000[b] |
| *AA* | 0 (0.00) | 0 (0.00) | - |

The rs numbers in parentheses are the accession numbers in the National Center for Biotechnology information single nucleotide polymorphism (SNP) database, dbSNP.
[b] Fisher's exact tes, [c] Chi-square test

**Table 5.10 *CYP2D6* and *CYP2C19* allele frequencies among two groups**

| Alleles | Pre-menopause n (%) | Post-menopause n (%) | *P* |
|---|---|---|---|
| ***CYP2D6*** | (n=60) | (n=36) | |
| **1* | 20 (33.33) | 13 (36.11) | 0.781[c] |
| **2* | 6 (10.00) | 4 (11.11) | 1.000[b] |
| **4* | 1 (1.67) | 0 (0.00) | 1.000[b] |
| **5* | 2 (3.33) | 2 (5.55) | 0.629 |
| **10* | 29 (48.33) | 15 (41.67) | 0.526[c] |
| **35* | 0 (0.00) | 1 (2.78) | 0.375[b] |
| **36* | 1 (1.67) | 0 (0.00) | 1.000[b] |
| **41* | 1 (1.67) | 1 (2.78) | 1.000[b] |
| ***CYP2C19*** | | | |
| **1* | 42 (70.00) | 24 (66.67) | 0.733[c] |
| **2* | 14 (23.33) | 10 (27.77) | 0.626[c] |
| **3* | 4 (6.67) | 2 (5.56) | 1.000[b] |

[b] Fisher's exact test
[c] Chi-square test

**Table 5.11 *CYP2D6* and *CYP2C19* genotype frequencies and associated predicted phenotypes among two groups**

| Genotypes | Pre-menopause n (%) | Post-menopause n (%) | Predicted Phenotype | *P* |
|---|---|---|---|---|
| ***CYP2D6*** | (n=30) | (n=18) | | |
| **1/*1* | 4 (13.34) | 4 (22.22) | EM/EM | 0.451[b] |
| **1/*2* | 0 (0.00) | 1 (5.56) | EM/EM | 0.375[b] |
| **1/*5* | 1 (3.33) | 0 (0.00) | EM/PM | 1.000[b] |
| **1/*10* | 10 (33.34) | 3 (16.66) | EM/IM | 0.317[b] |
| **1/*36* | 1 (3.33) | 0 (0.00) | EM/IM | 1.000[b] |
| **1/*41* | 0 (0.00) | 1 (5.56) | EM/IM | 0.375[b] |
| **2/*2* | 1 (3.33) | 1 (5.56) | EM/EM | 1.000[b] |
| **2/*4* | 1 (3.33) | 0 (0.00) | EM/PM | 1.000[b] |
| **2/*10* | 3 (10.00) | 1 (5.56) | EM/IM | 1.000[b] |
| **5/*10* | 1 (3.33) | 2 (11.11) | PM/IM | 0.547[b] |
| **10/*10* | 7 (23.34) | 4 (22.22) | IM/IM | 1.000[b] |
| **10/*35* | 0 (0.00) | 1 (5.56) | IM/EM | 0.375[b] |
| **10/*41* | 1 (3.33) | 0 (0.00) | IM/IM | 1.000[b] |
| ***CYP2C19*** | | | | |
| **1/*1* | 14 (46.67) | 7 (38.89) | EM/EM | 0.765[b] |
| **1/*2* | 10 (33.33) | 8 (44.44) | EM/PM | 0.543[b] |
| **1/*3* | 4 (13.33) | 2 (11.11) | EM/PM | 1.000[b] |
| **2/*2* | 2 (6.67) | 1 (5.56) | PM/PM | 1.000[b] |

Abbreviation: EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer.
[b] Fisher's exact test. [c] Chi-square test

**Table 5.12 *CYP2D6* and *CYP2C19* predicted phenotypes / genotypes among two groups**

| Predicted Phenotype | Genotype | Pre-menopause n (%) | Post-menopause n (%) | *P* |
|---|---|---|---|---|
| ***CYP2D6*** | | (n=30) | (n=18) | |
| EM/EM | **1/*1, *1/*2, *2/*2,*1/*10, *2/*10* | 5 (16.70) | 6 (33.30) | 0.288[b] |
| EM/IM | **10/*35, *1/*36, *1/*41* | 14 (46.70) | 6 (33.30) | 0.364[c] |
| EM/PM | **1/*5, *2/*4* | 2 (6.60) | 0 (0.00) | 0.521[b] |
| IM/IM | **10/*10, *10/*41* | 8 (26.70) | 4 (22.20) | 1.000[b] |
| IM/PM | **5/*10* | 1 (3.30) | 2 (11.10) | 0.547[b] |
| ***CYP2C19*** | | | | |
| EM/EM | **1/*1* | 14 (46.70) | 7 (38.90) | 0.599[c] |
| EM/PM | **1/*2, *1/*3* | 14 (46.70) | 10 (55.60) | 0.551[c] |
| PM/PM | **2/*2* | 2 (6.60) | 1 (5.50) | 1.000[b] |

Abbreviation: EM, extensive metabolizer; IM, intermediate metabolizer; PM, poor metabolizer
[b] Fisher's exact test; [c] Chi-square test

## 5.6 Disease free survival analysis

### 5.6.1 *CYP2D6* polymorphisms and clinical outcomes

Time to develop breast cancer recurrence was evaluated using the Kaplan-Meier analysis. The disease free survival was calculated from surgery to the time of breast cancer recurrence or beginning of breast cancer event (local, regional, or distant occurrence, or contralateral breast cancer) or death from any cause. Patients carrying heterozygous *GA* at nucleotide 1846G>A (*CYP2D6*4*) had significantly shorter disease free survival than patients with homozygous *G/A* in overall and pre-menopause group (Log-rank test; $P$ = 0.031 and $P$ = 0.019, respectively). No statistically significant difference DFS was detectable for others nucleotide (**Table 5.16**). Furthermore, Kaplan-Meier estimates showed significant shorter disease free survival in patients with homozygous variant (*TT*) compared those with heterozygous *CT* or homozygous wild type (*CC*) at nucleotides 100C>T and 1039C>T (*CYP2D6*10*) in post-menopause group (Log-rank test; $P$ = 0.046, 0.046), which two SNPs are in linkage disequilibrium.

**Table 5.13 Log-rank test of *CYP2D6* genotypes, predicted phenotypes and others model**

| ***CYP2D6* genotypes** | **No.** | **Log-rank Test; *P* value** | | |
|---|---|---|---|---|
| | | **Overall** | **Pre-menopause** | **Post-menopause** |
| (Wt/Wt vs. Wt/V vs. V/V) | 48 | | | |
| -1584C>G | | 0.439 | 0.356 | 0.744 |
| 100C>T | | 0.658 | 0.671 | 0.135 |
| 1039C>T | | 0.579 | 0.361 | 0.135 |
| 1661G>C | | 0.613 | 0.376 | 0.341 |
| 1846G>A | | 0.031 | 0.020 | - |
| 2850C>T | | 0.640 | 0.616 | 0.448 |
| 4180G>C | | 0.400 | 0.078 | 0.341 |
| Wt/Wt vs. (Wt/V + V/V) | 48 | | | |
| -1584C>G | | 0.787 | 0.697 | 0.558 |
| 100C>T | | 0.844 | 0.398 | 0.384 |
| 1039C>T | | 0.847 | 0.154 | 0.384 |
| 1661G>C | | 0.560 | 0.163 | 0.521 |
| 1846G>A | | 0.031 | 0.019 | - |
| 2850C>T | | 0.502 | 0.870 | 0.448 |
| 4180G>C | | 0.321 | 0.025 | 0.521 |
| (Wt/Wt + Wt/V) vs. V/V | 48 | | | |
| -1584C>G | | 0.225 | 0.349 | 0.472 |
| 100C>T | | 0.363 | 0.720 | 0.046 |
| 1039C>T | | 0.363 | 0.720 | 0.046 |
| 1661G>C | | 0.608 | 0.667 | 0.143 |
| 1846G>A | | - | - | - |
| 2850C>T | | 0.398 | 0.349 | - |
| 4180G>C | | 0.608 | 0.667 | 0.143 |
| **1/*1* vs. **1/*10* vs. **10/*10* | 32 | 0.447 | 0.569 | 0.099 |
| *Wt/Wt* vs. *Wt/*10* vs. **10/*10* | 40 | 0.355 | 0.957 | 0.074 |
| *EM/EM* vs. *EM/IM* vs. *IM/IM* | 43 | 0.529 | 0.881 | 0.103 |
| *Wt/Wt* vs. *Wt/V* vs. *V/V* | 48 | 0.655 | 0.747 | 0.123 |

### 5.6.2 *CYP2C19* polymorphisms and clinical outcomes

There was no significant differences disease free survival of *CYP2C19* genotypes between breast cancers cases versus breast cancers controls in the overall, pre-menopause, and post-menopause groups (**Table 5.14**).

**Table 5.14 Log-rank test of *CYP2C19* genotypes and others model**

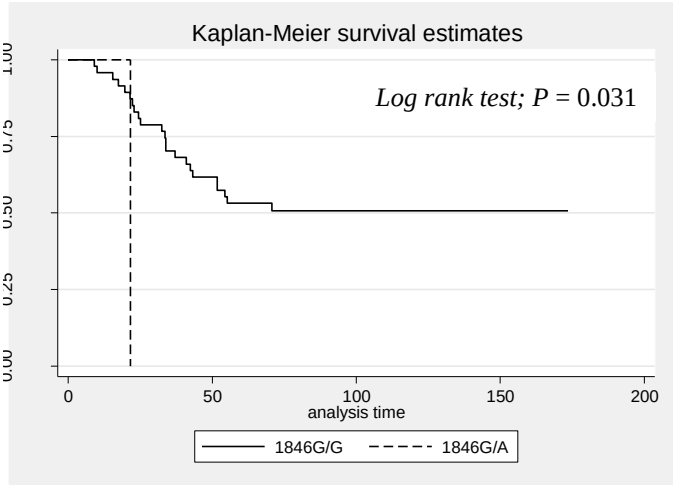
| ***CYP2C19* genotypes** | **No.** | **Log-rank Test; *P* value** | | |
|---|---|---|---|---|
| | | **Overall** | **Pre-menopause** | **Post-menopause** |
| Wt/Wt vs. Wt/V vs. V/V | 48 | | | |
| 681G>A | | 0.317 | 0.270 | 0.618 |
| 636G>A | | 0.979 | 0.267 | 0.291 |
| Wt/Wt vs. (Wt/V + V/V) | 48 | | | |
| 681G>A | | 0.474 | 0.205 | 0.656 |
| 636G>A | | 0.979 | 0.267 | 0.291 |
| (Wt/Wt + Wt/V) vs. V/V | 48 | | | |
| 681G>A | | 0.136 | 0.174 | 0.472 |
| 636G>A | | - | - | - |
| homo**1* vs. het**1* vs. homo**2* | 48 | 0.329 | 0.388 | 0.765 |
| homoEM vs. hetEM vs. homoPM | 48 | 0.329 | 0.388 | 0.765 |

## 5.7 Kaplan-Meier analysis

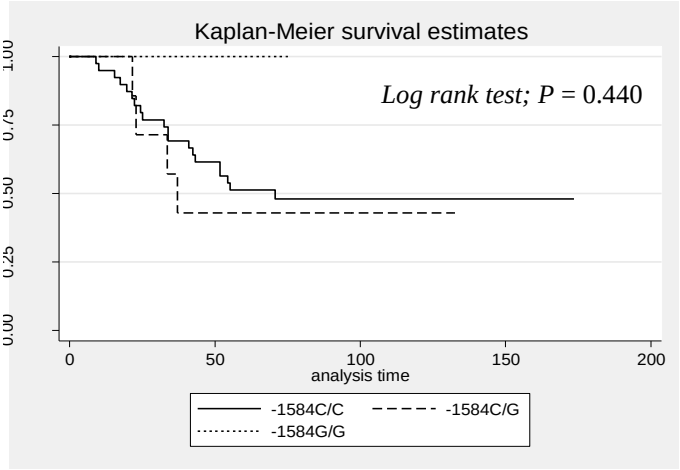
### 5.7.1 *CYP2D6* genotypes and disease free survival comparison among homozygous variant, heterozygous and homozygous wild type in overall group

There was significant differences disease free survival of *CYP2D6* genotype in patients with heterozygous *GA* at nucleotide 1846G>A compared with homozygous *GG* between breast cancers cases versus breast cancers controls (Log-rank test, $P$ = 0.031). No statistically significant differences disease free survivals were found in other SNPs (-1584C>G, 100C>T, 1039C>T, 1661G>C, 2850C>T and 4180G>C).

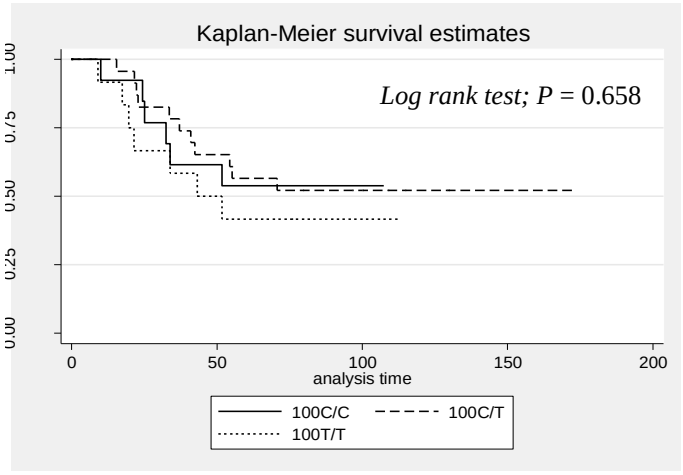
A) 1846G>A



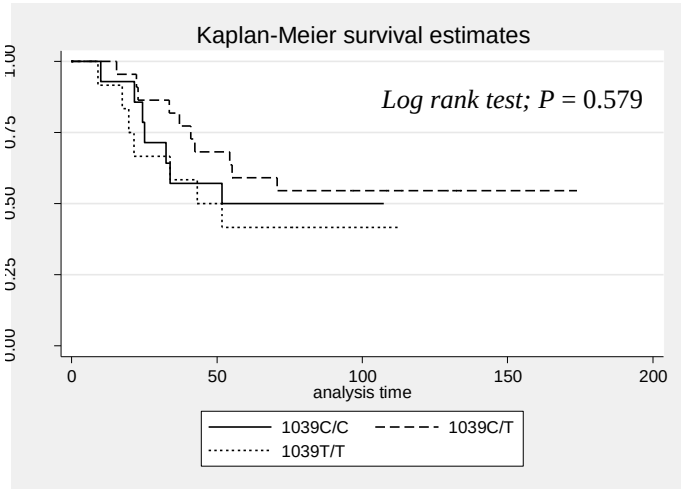

B) -1584C>G



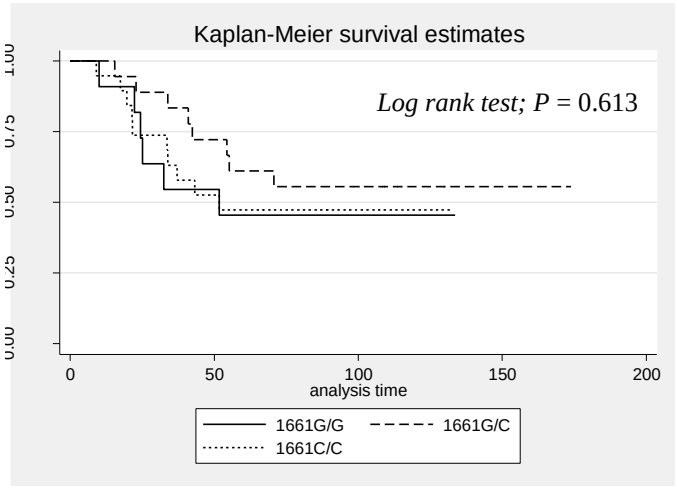
C) 100C>T



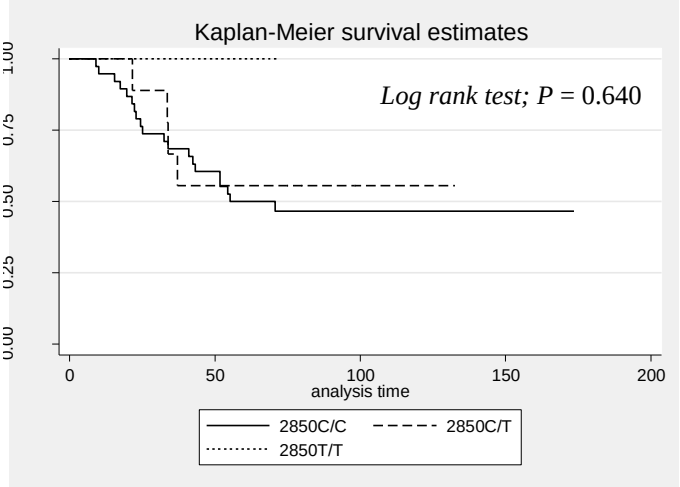

D) 1039C>T




E) 1661G>C



F) 2850C>T




G) 4180G>C

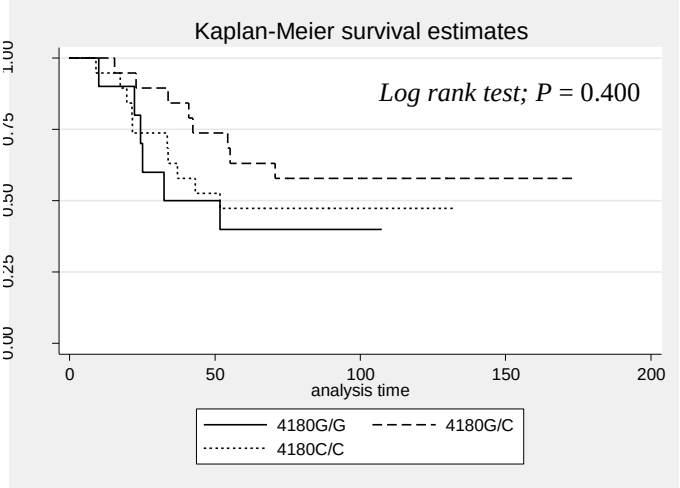
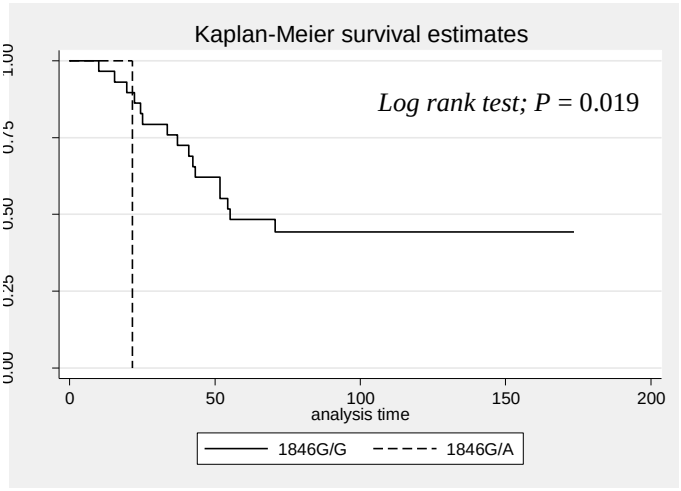



**Figure 5.2 Kaplan-Meier probabilities of disease free survival of patients with *CYP2D6* genotypes in overall group (comparison among homozygous variant, heterozygous and homozygous wild type). A) 1846G>A, B) -1584C>G, C) 100C>T, D) 1039C>T, E) 1661G>C, F) 2850C>T and G) 4180G>C**

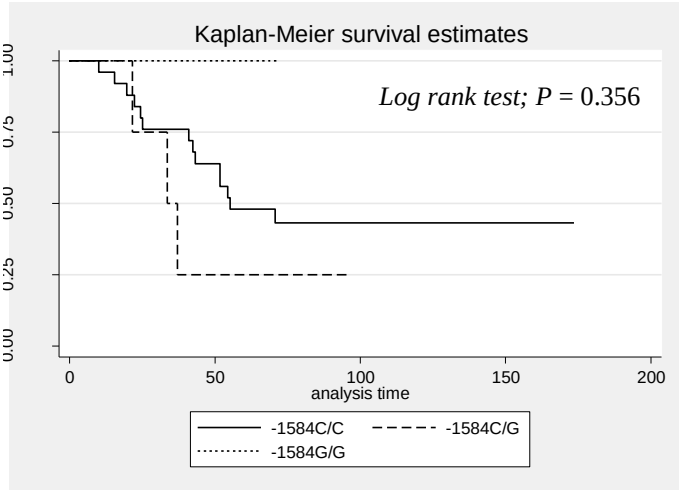
### 5.7.2 *CYP2D6* genotypes and disease free survival comparison among homozygous variant, heterozygous and homozygous wild type in pre-menopause group

There were significant differences disease free survival of *CYP2D6* genotypes in patients with heterozygous *GA* at nucleotide 1846G>A compared with homozygous wild type (*GG*) between breast cancers cases versus breast cancers controls (Log-rank test, $P = 0.019$). No statistically significant differences disease free survivals were found in other SNPs (-1584C>G, 100C>T, 1039C>T, 1661G>C, 2850C>T and 4180G>C).

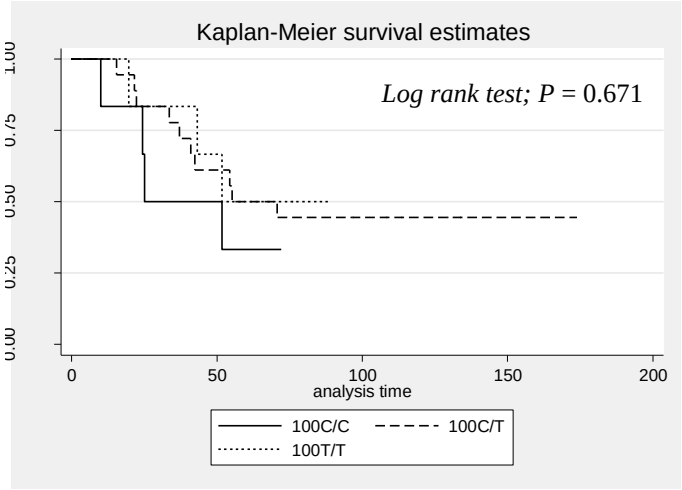
A) 1846G>A



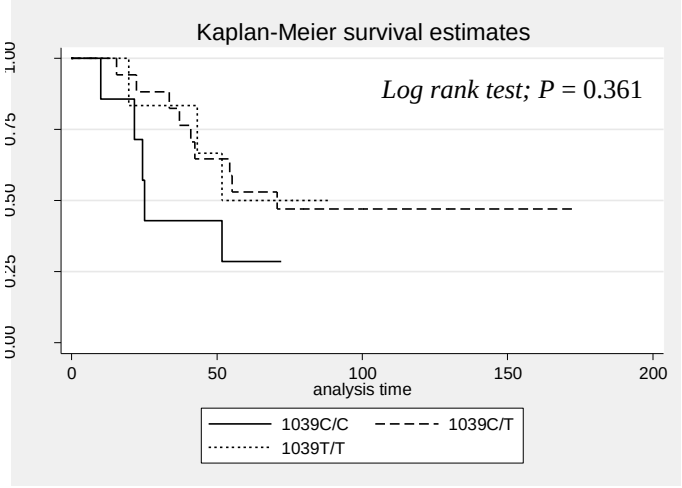

B) -1584C>G



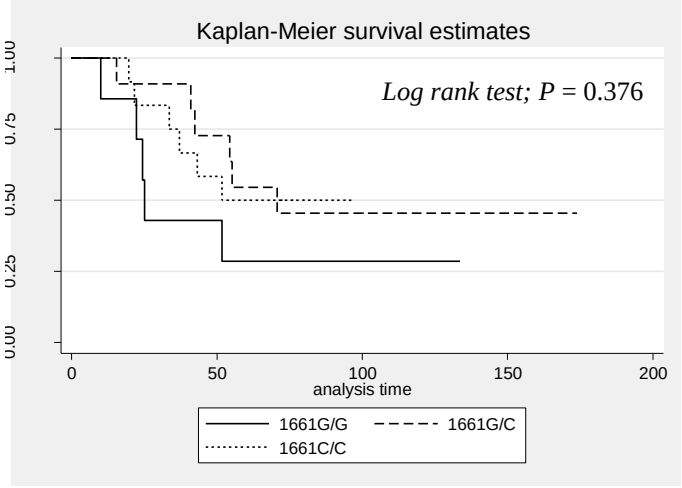
C) 100C>T



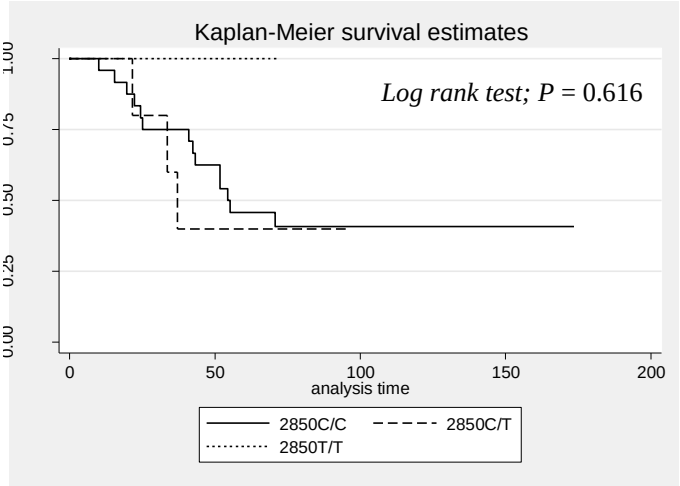

D) 1039C>T




E) 1661G>C



F) 2850C>T




G) 4180G>C

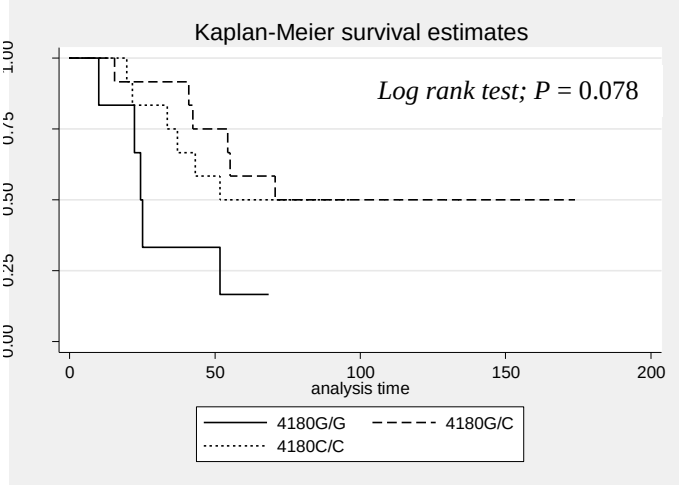
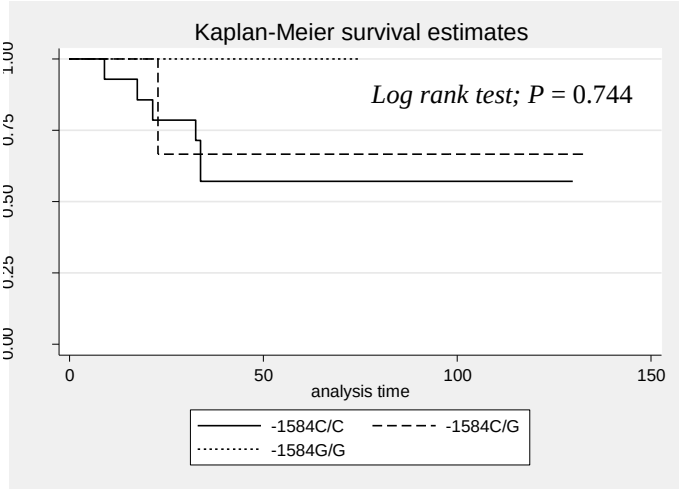



**Figure 5.3 Kaplan-Meier probabilities of disease free survival of patients with *CYP2D6* genotypes in pre-menopause group (comparison among homozygous variant, heterozygous and homozygous wild type). A) 1846G>A, B) -1584C>G, C) 100C>T, D) 1039C>T, E) 1661G>C, F) 2850C>T and G) 4180G>C**

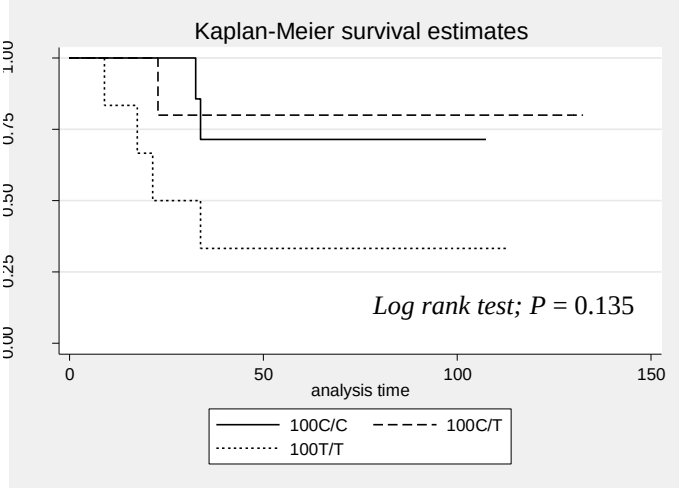
### 5.7.3 *CYP2D6* genotypes and disease free survival comparison among homozygous variant, heterozygous and homozygous wild type in post-menopause group

There was no significant differences disease free survival of *CYP2D6* genotype in patients with homozygous variant at nucleotide -1584C>G, 100C>T, 1039C>T, 1661G>C, 1846G>A, 2850C>T and 4180G>C compared with homozygous wild type and heterozygous compared between breast cancers cases versus breast cancers controls in post-menopause group (Log-rank test, $P > 0.05$).

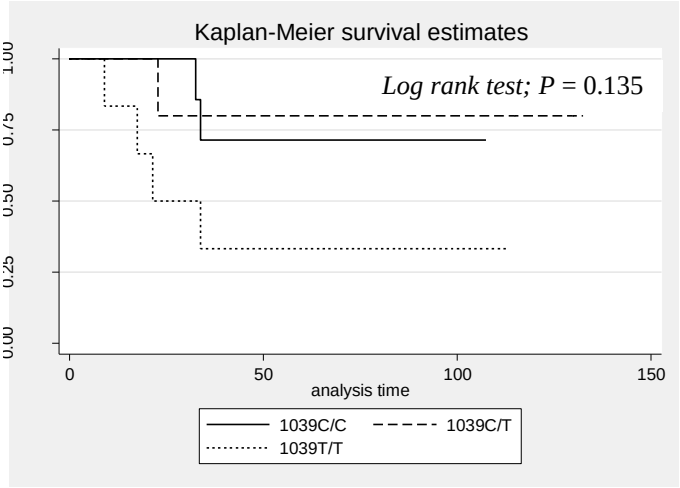
A) -1584C>G



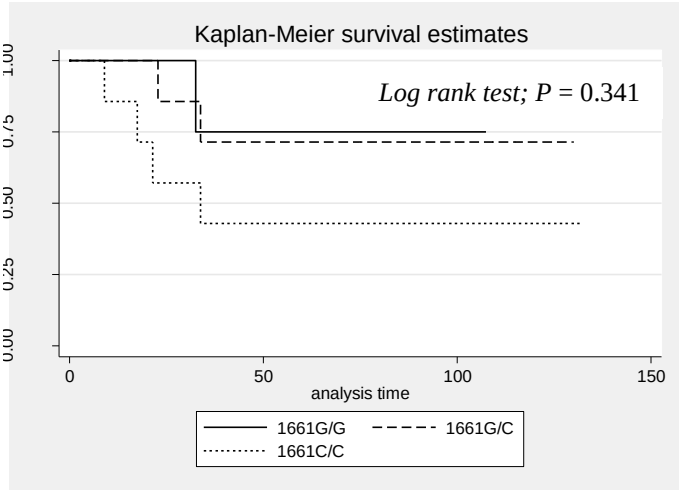

B) 100C>T



C) 1039C>T



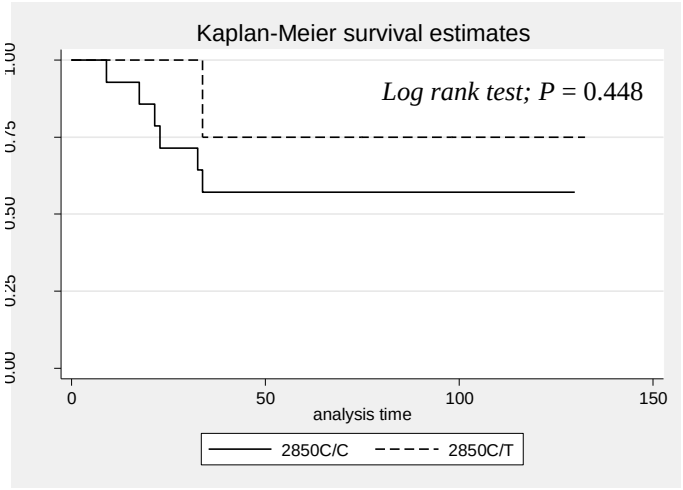

D) 1661G>C




E) 1846G>A

No comparison analysis is performed because the factor variable has only one value for every stratum

F) 2850C>T




G) 4180G>C

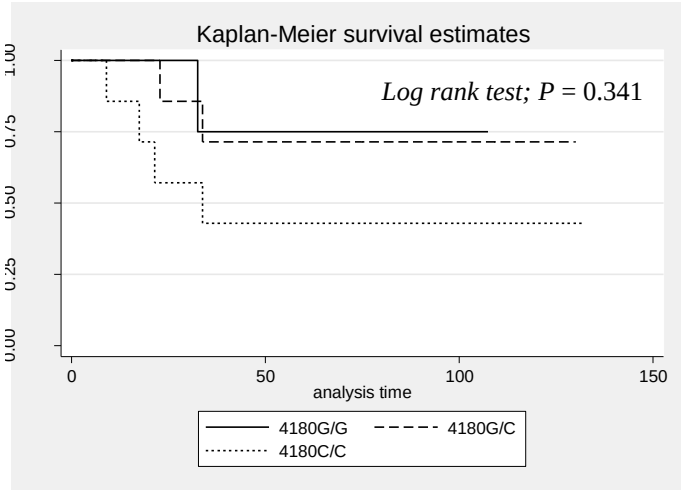
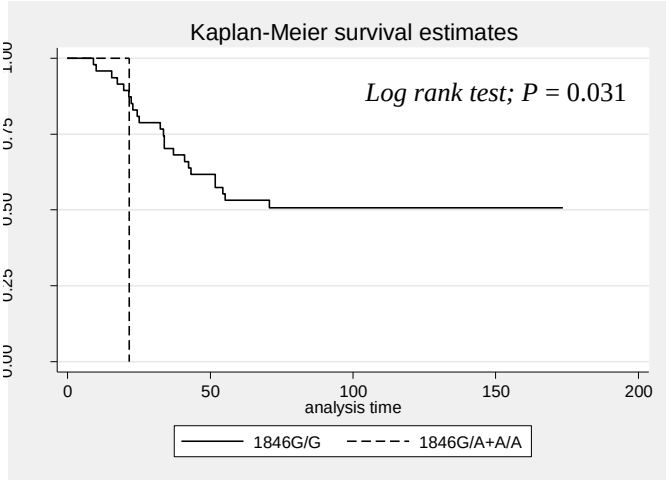



**Figure 5.4 Kaplan-Meier probabilities of disease free survival of patients with *CYP2D6* genotypes in pre-menopause group (comparison among homozygous variant, heterozygous and homozygous wild type). A) -1584C>G, B) 100C>T, C) 1039C>T, D) 1661G>C, E) 1846G>A, F) 2850C>T and G) 4180G>C**

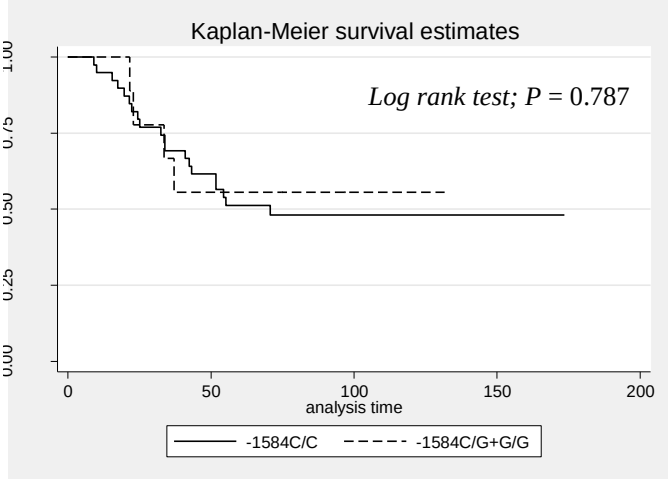
### 5.7.4 *CYP2D6* genotypes and disease free survival comparison among homozygous wild and those with homozygous variant and heterozygous in overall group

There was significant differences disease free survival of *CYP2D6* genotype in patients with homozygous wild type (*GG*) at nucleotide 1846G>A compared those with homozygous variant (*AA*) and heterozygous (*GA*) compared between breast cancers cases versus breast cancers controls in overall group (Log-rank test, $P$ = 0.031). No statistically significant differences disease free survivals were found in other SNPs (-1584C>G, 100C>T, 1039C>T, 1661G>C, 2850C>T and 4180G>C).

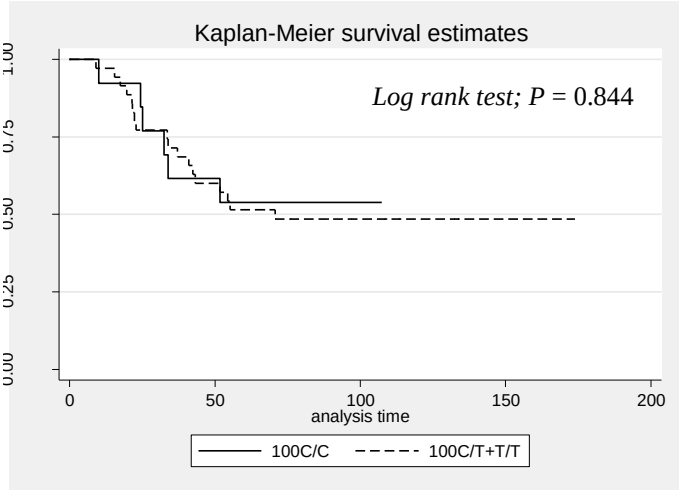
A) 1846G>A



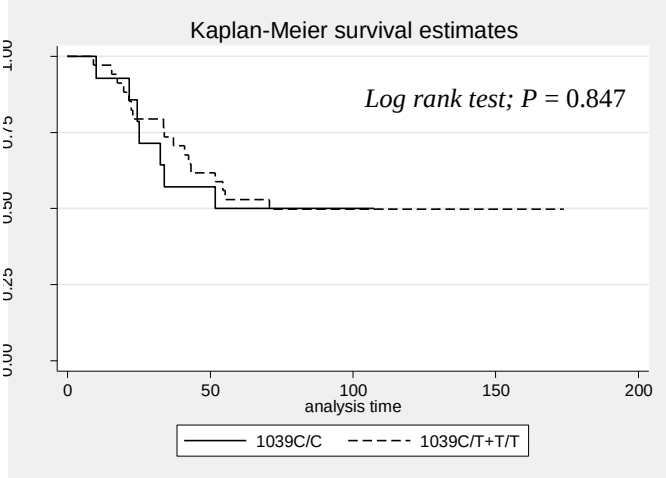

B) -1584C>G



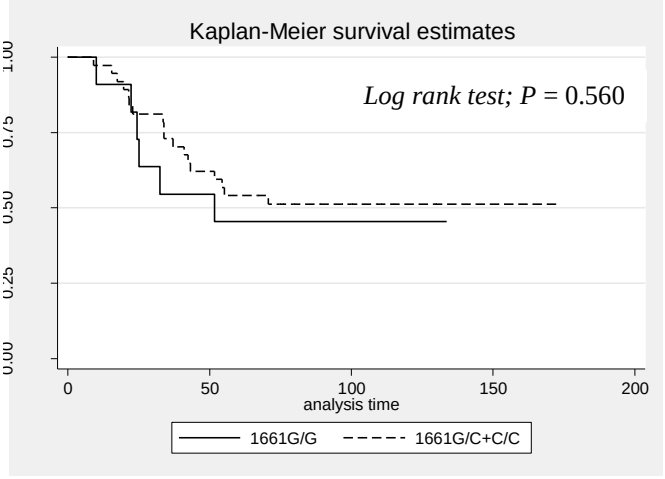
C) 100C>T



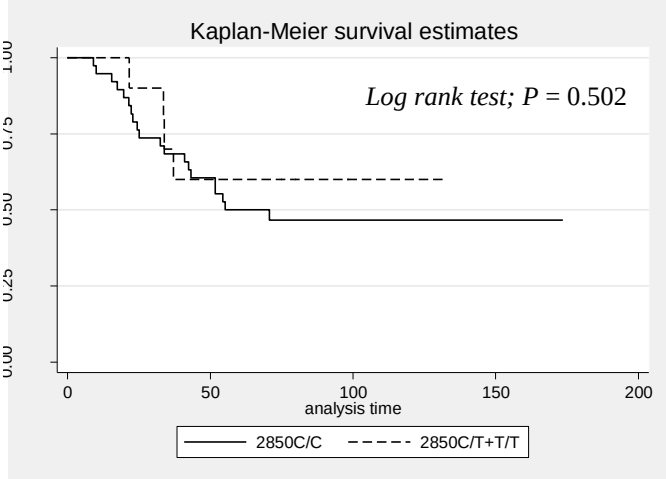

D) 1039C>T




E) 1661G>C



F) 2850C>T




G) 4180G>C

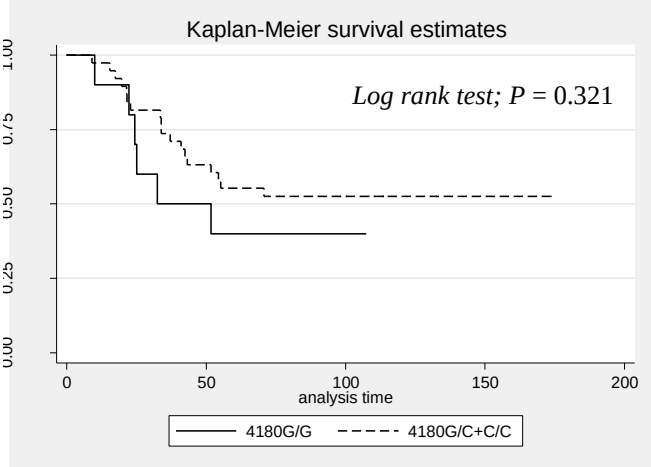
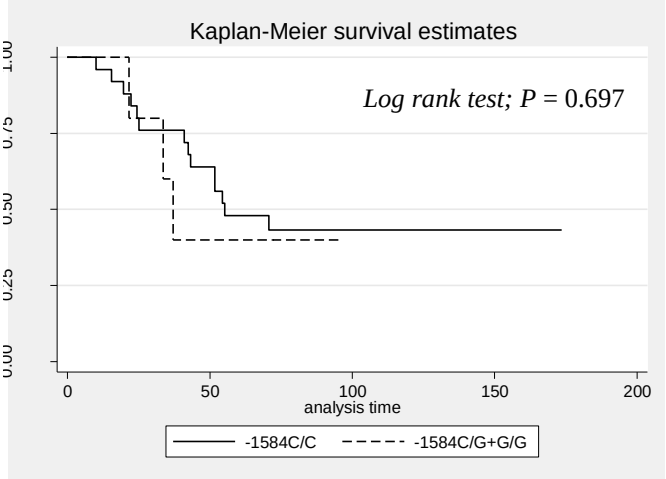



**Figure 5.5 Kaplan-Meier probabilities of disease free survival of patients with *CYP2D6* genotypes in overall group (comparison among homozygous wild type and those with homozygous variant and heterozygous). A) 1846G>A, B) -1584C>G, C) 100C>T, D) 1039C>T, E) 1661G>C, F) 2850C>T and G) 4180G>C**

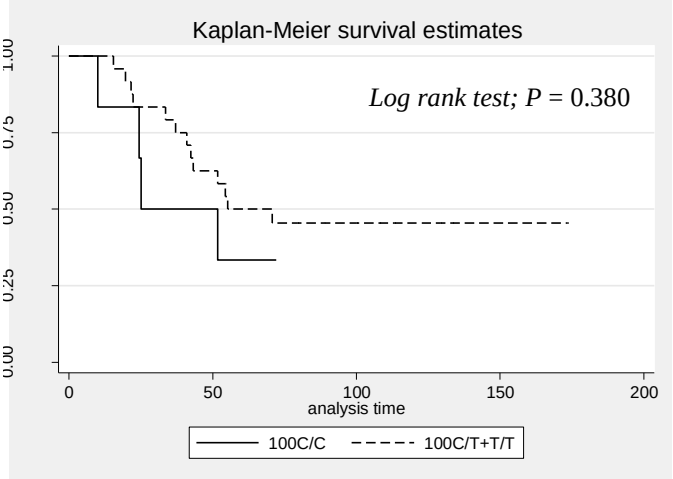
### 5.7.5 *CYP2D6* genotypes and disease free survival comparison among homozygous wild type and those with homozygous variant and heterozygous in pre-menopause group

There was significant differences disease free survival of *CYP2D6* genotype in patients with homozygous wild type (*GG*) at nucleotide 1846G>A compared those with homozygous variant and heterozygous compared between breast cancers cases versus breast cancers controls (Log-rank test, $P = 0.020$). No statistically significant differences disease free survivals were found in other SNPs (-1584C>G, 100C>T, 1039C>T, 1661G>C, 2850C>T and 4180G>C).

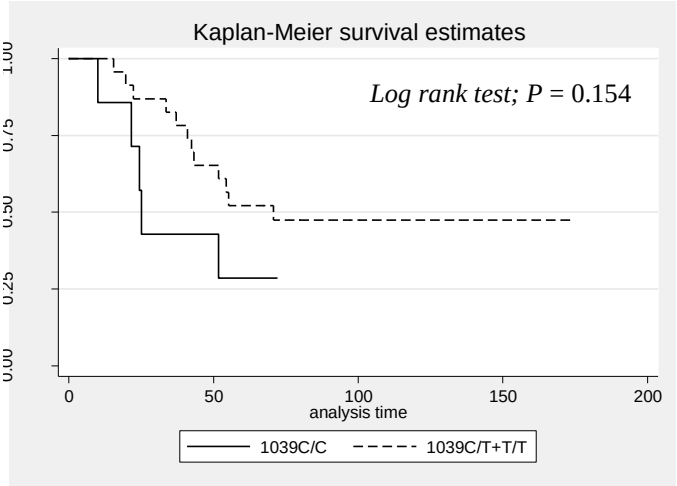
A) -1584C>G



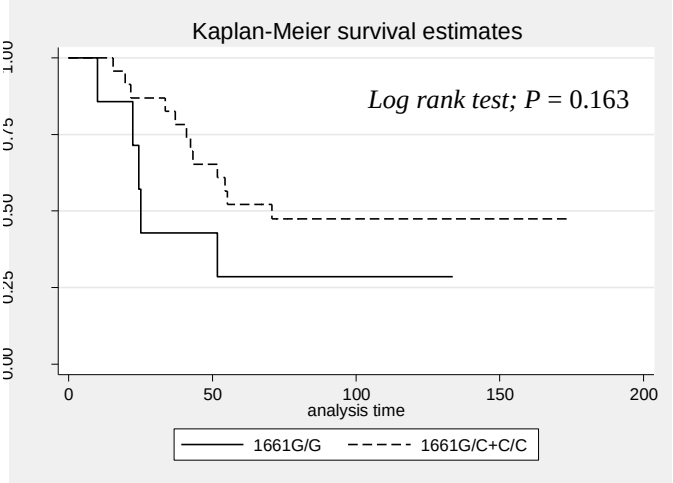

B) 100C>T



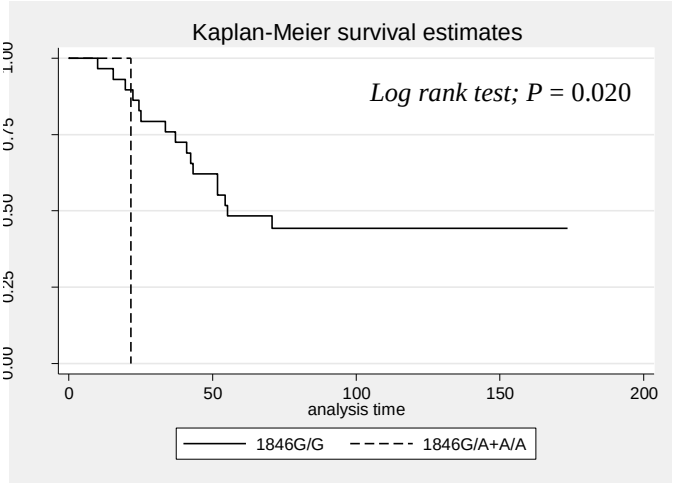
C) 1039C>T



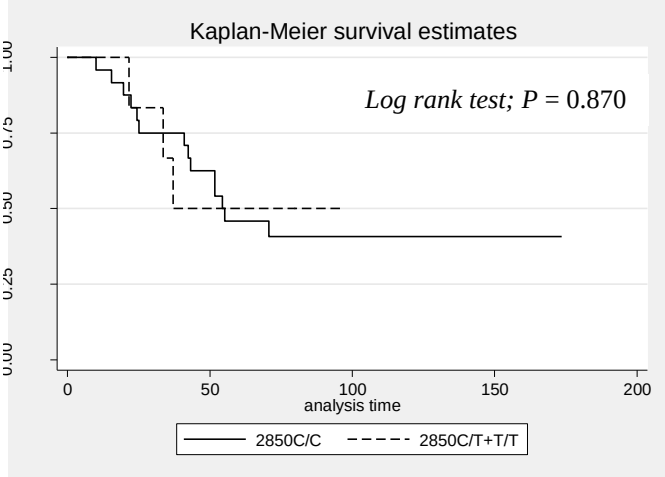

D) 1661G>C




E) 1846G>A



F) 2850C>T




G) 4180G>C

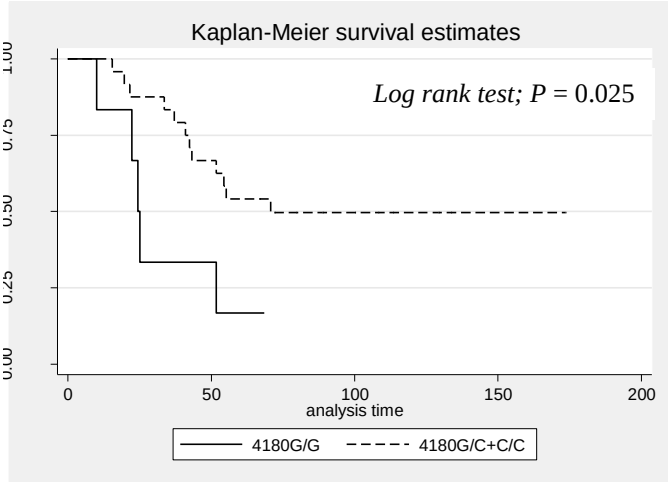
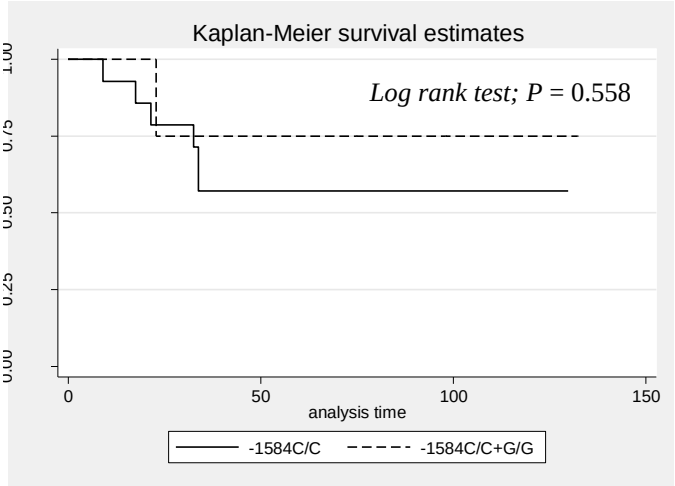



**Figure 5.6 Kaplan-Meier probabilities of disease free survival of patients with *CYP2D6* genotypes in pre-menopause group (comparison among homozygous wild type and those with homozygous variant and heterozygous). A) -1584C>G, B) 100C>T, C) 1039C>T, D) 1661G>C, E) 1846G>A, F) 2850C>T and G) 4180G>C**

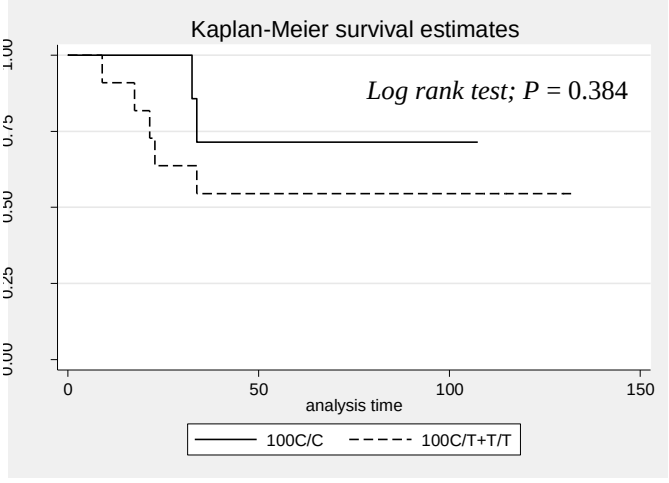
**5.7.6 *CYP2D6* genotypes and disease free survival comparison among homozygous wild type and those with homozygous variant and heterozygous in post-menopause group**

There was no significant differences disease free survival of *CYP2D6* genotype in patients with homozygous wild type at nucleotide -1584C>G, 100C>T, 1039C>T, 1661G>C, 1846G>A, 2850C>T and 4180G>C compared those with homozygous variant and heterozygous compared between breast cancers cases versus breast cancers controls (Log-rank test, $P > 0.05$).

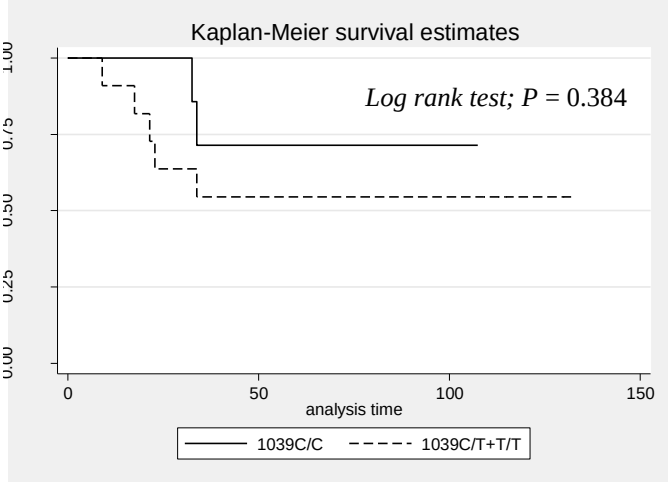
A) -1584C>G



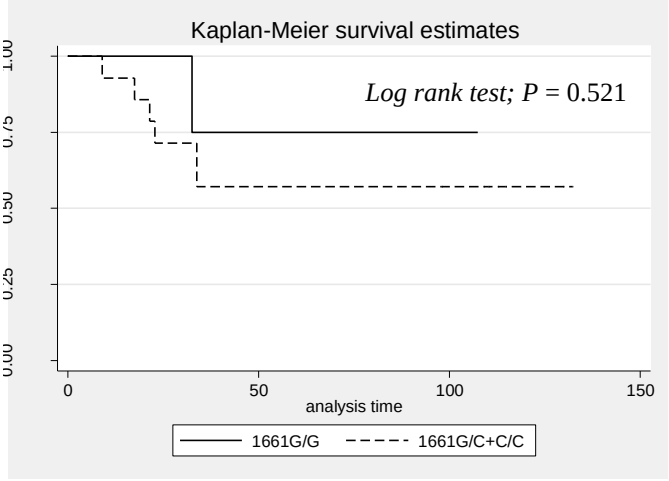

B) 100C>T



C) 1039C>T



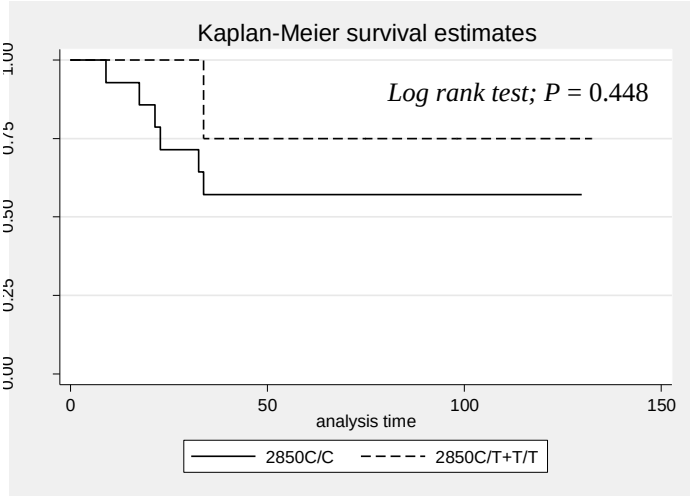

D) 1661G>C




E) 1846G>A

No comparison analysis is performed because the factor variable has only one value for every stratum

F) 2850C>T




G) 4180G>C

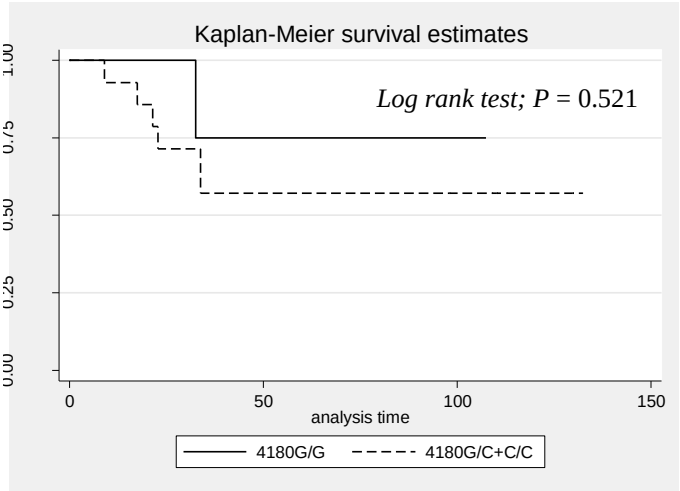
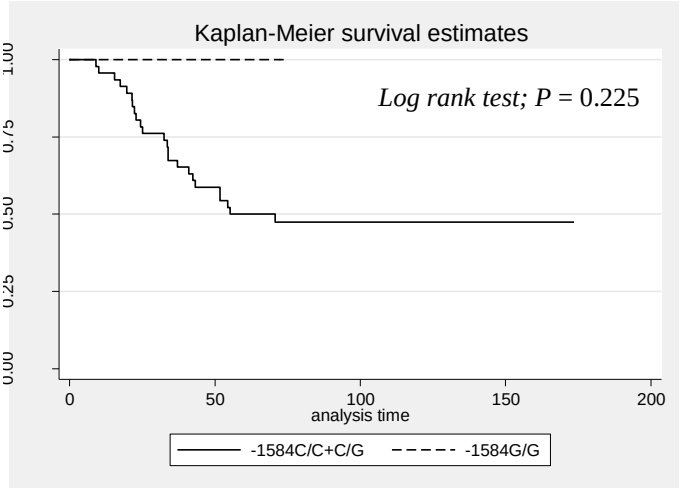



**Figure 5.7 Kaplan-Meier probabilities of disease free survival of patients with *CYP2D6* genotypes in post-menopause group (comparison among homozygous wild type and those with homozygous variant and heterozygous). A) -1584C>G, B) 100C>T, C) 1039C>T, D) 1661G>C, E) 1846G>A, F) 2850C>T and G) 4180G>C**

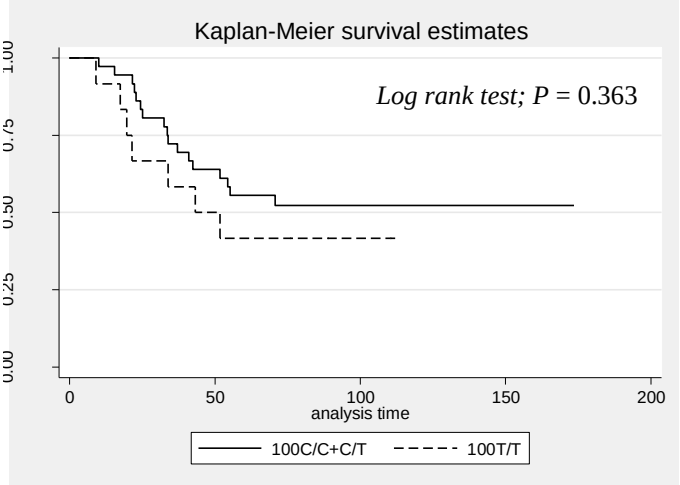
### 5.7.7 *CYP2D6* genotypes and disease free survival comparison among homozygous variant and those with homozygous wild and heterozygous type in overall group

There was no significant differences disease free survival of *CYP2D6* genotype in patients with homozygous variant at nucleotide -1584C>G, 100C>T, 1039C>T, 1661G>C, 1846G>A, 2850C>T and 4180G>C compared those with homozygous wild type and heterozygous compared between breast cancers cases versus breast cancers controls (Log-rank test, $P > 0.05$).

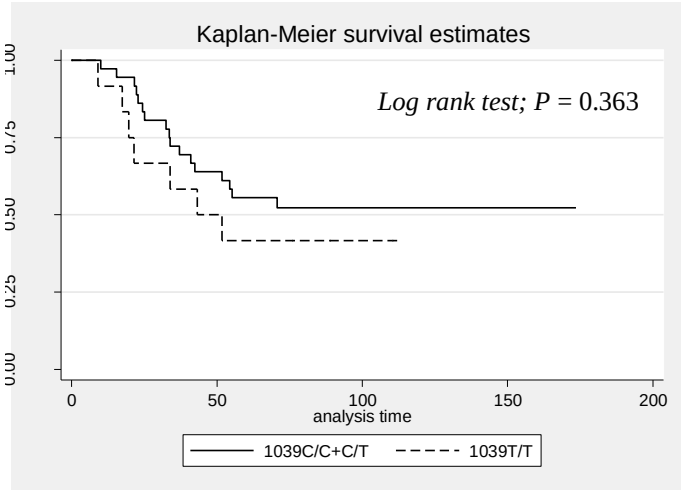
A) -1584C>G



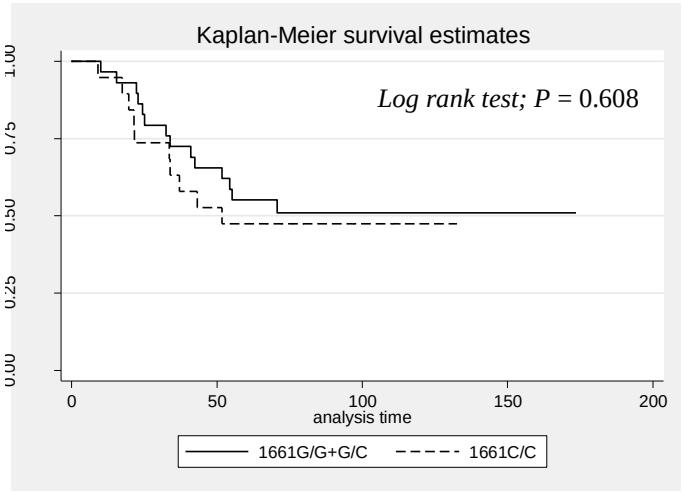

B) 100C>T



C) 1039C>T



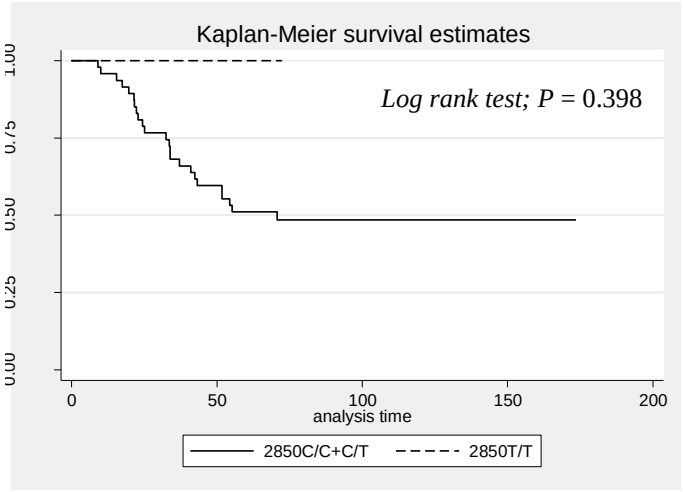

D) 1661G>C




E) 1846G>A

No comparison analysis is performed because the factor variable has only one value for every stratum

F) 2850C>T




G) 4180G>C

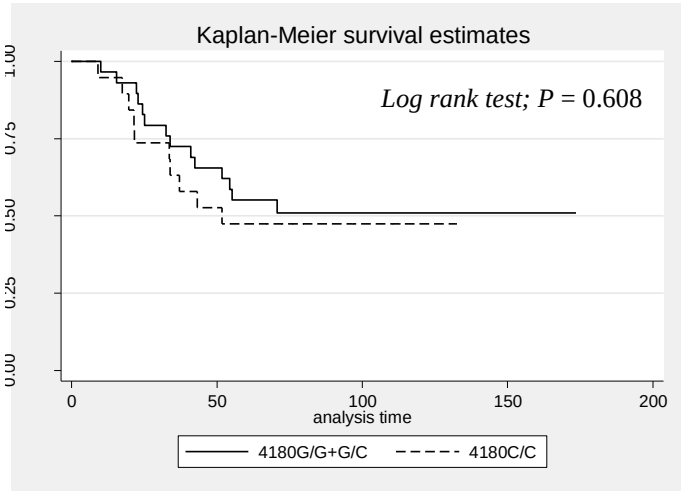
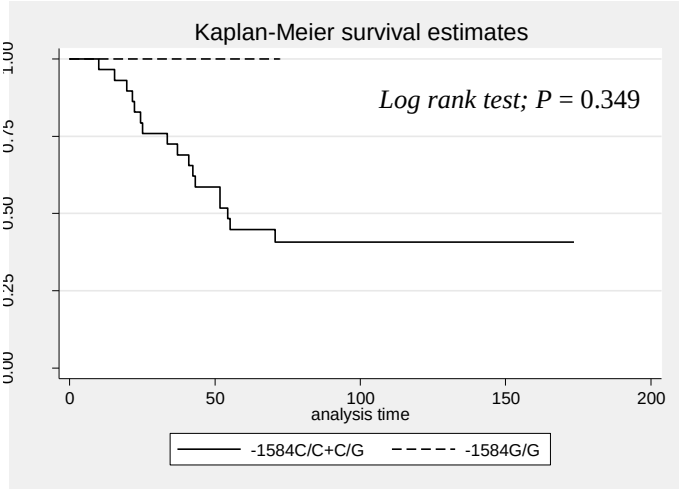



**Figure 5.8 Kaplan-Meier probabilities of disease free survival of patients with *CYP2D6* genotypes in overall group (comparison among homozygous variant and those with homozygous wild type and heterozygous). A) -1584C>G, B) 100C>T, C) 1039C>T, D) 1661G>C, E) 1846G>A, F) 2850C>T and G) 4180G>C**

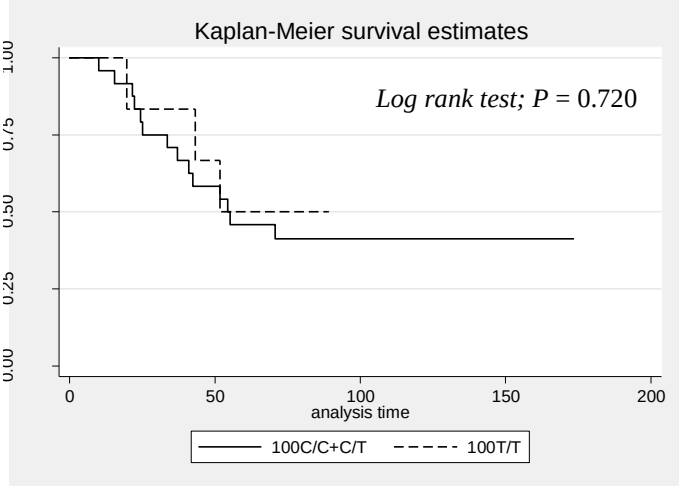
**5.7.8 *CYP2D6* genotypes and disease free survival comparison among homozygous variant and those with homozygous wild and heterozygous type in pre-menopause group**

There were no significant differences disease free survival of *CYP2D6* genotypes in patients with homozygous variant at nucleotide -1584C>G, 100C>T, 1039C>T, 1661G>C, 1846G>A, 2850C>T and 4180G>C compared those with homozygous wild type and heterozygous compared between breast cancers cases versus breast cancers controls (Log-rank test, $P > 0.05$).

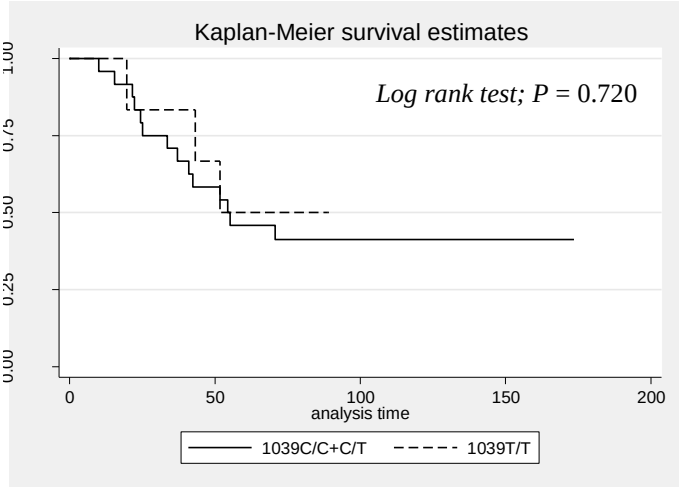
A) -1584C>G



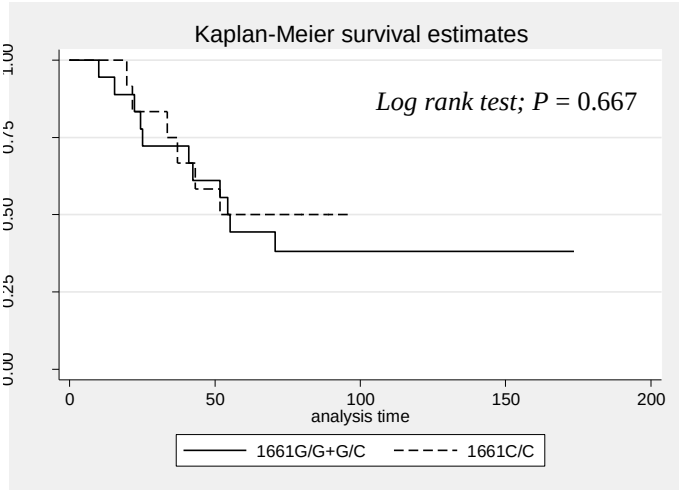

B) 100C>T



C) 1039C>T



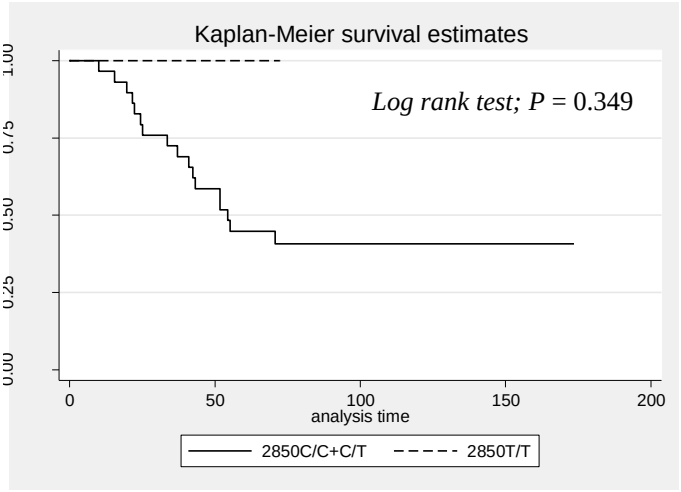

D) 1661G>C




E) 1846G>A

No comparison analysis is performed because the factor variable has only one value for every stratum

F) 2850C>T




G) 4180G>C

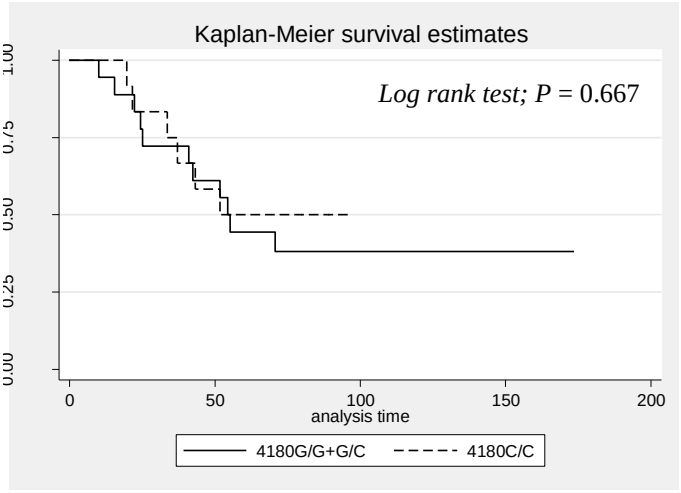
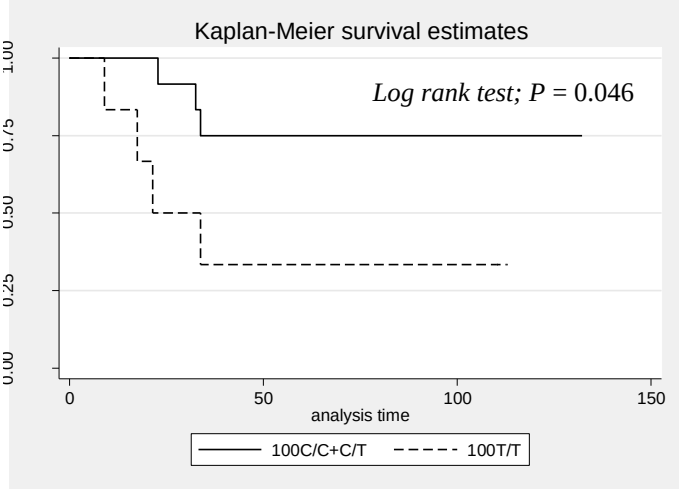



**Figure 5.9 Kaplan-Meier probabilities of disease free survival of patients with *CYP2D6* genotypes in pre-menopause group (comparison among homozygous variant and those with homozygous wild type and heterozygous). A) -1584C>G, B) 100C>T, C) 1039C>T, D) 1661G>C, E) 1846G>A, F) 2850C>T and G) 4180G>C**

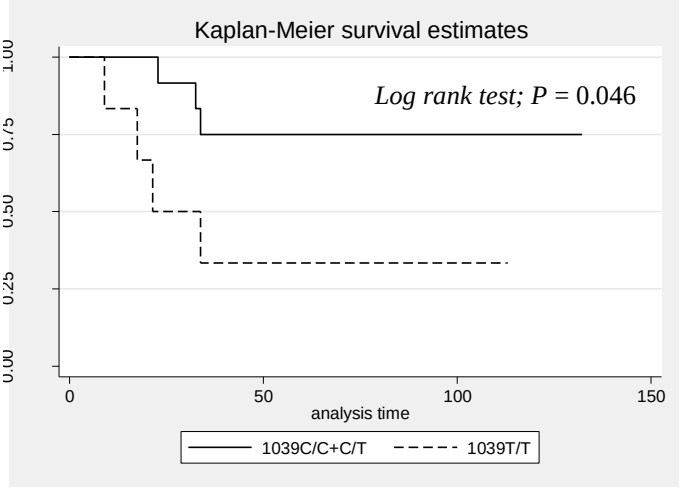
### 5.7.9 *CYP2D6* genotypes and disease free survival comparison among homozygous variant and those with homozygous wild and heterozygous type in post-menopause group

There was significant differences disease free survival of *CYP2D6* genotype in patients with homozygous variant at nucleotide 100C>T and 1039C>T compared those with homozygous wild type and heterozygous compared between breast cancers cases versus breast cancers controls in post-menopause group (Log-rank test, $P$ = 0.046). No statistically significant differences disease free survivals were found in other SNPs (-1584C>G, 1661G>C, 2850C>T and 4180G>C).

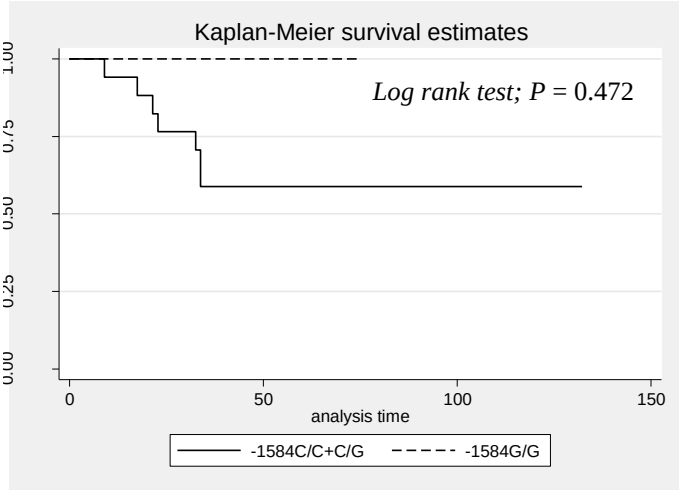
A) 100C>T



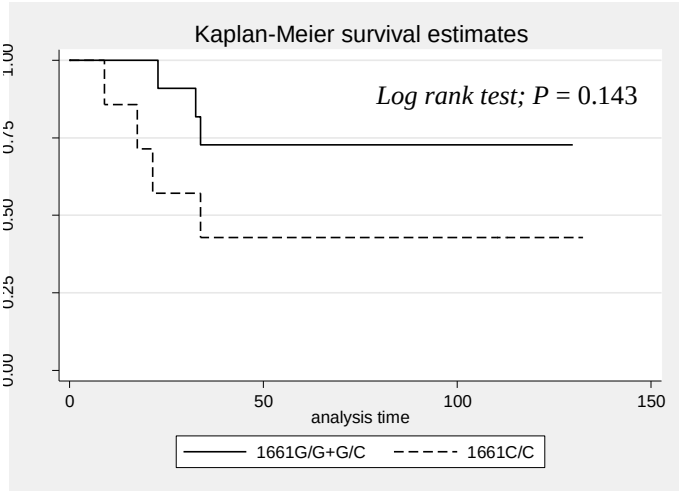

B) 1039C>T



C) -1584C>G




D) 1661G>C




E) 1846G>A

No comparison analysis is performed because the factor variable has only one value for every stratum

F) 2850C>T

No comparison analysis is performed because the factor variable has only one value for every stratum

G) 4180G>C

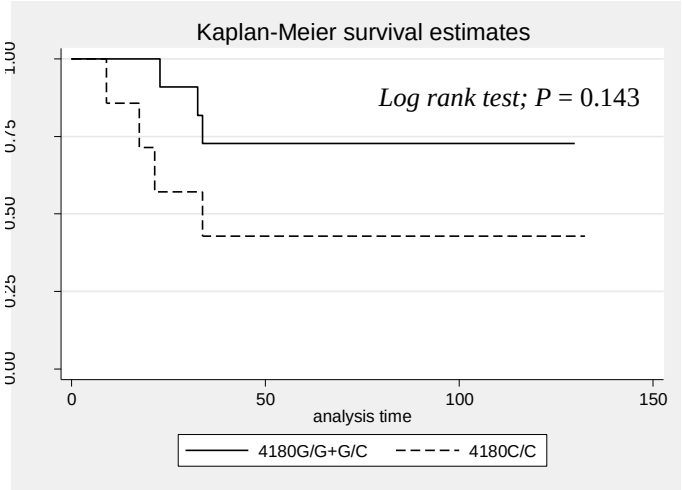
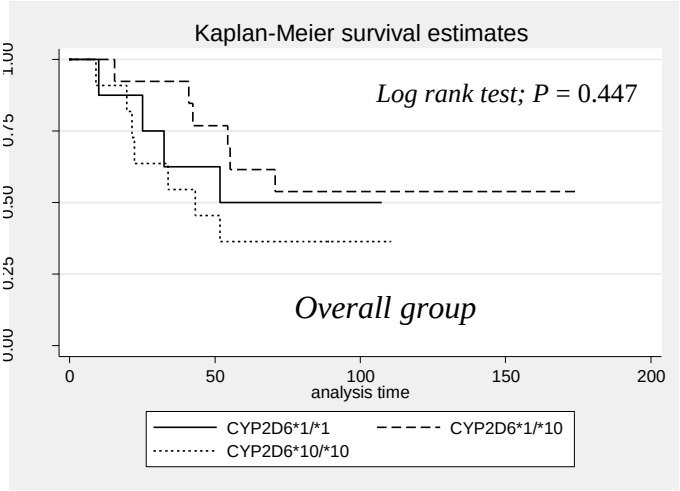



**Figure 5.10 Kaplan-Meier probabilities of disease free survival of patients with *CYP2D6* genotypes in post-menopause group (comparison among homozygous variant and those with homozygous wild type and heterozygous). A) 100C>T, B) 1039C>T, C) -1584C>G, D) 1661G>C, E) 1846G>A, F) 2850C>T and G) 4180G>C**

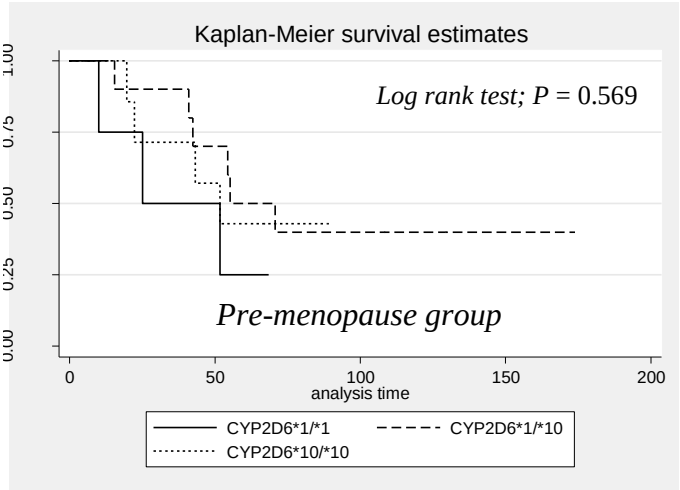
### 5.7.10 *CYP2D6* genotypes and disease free survival comparison among **1/*1, *1/*10* and **10/*10*

There was no significant differences disease free survival of *CYP2D6* genotypes in patients with homozygous variant allele (*CYP2D6*10/*10*) compared with heterozygous (*CYP2D6*1/*10*) and homozygous wild type (*CYP2D6*1/*1*) between breast cancers cases versus breast cancers controls among three groups.

A) Overall group




B) Pre-menopause group



C) Post-menopause group

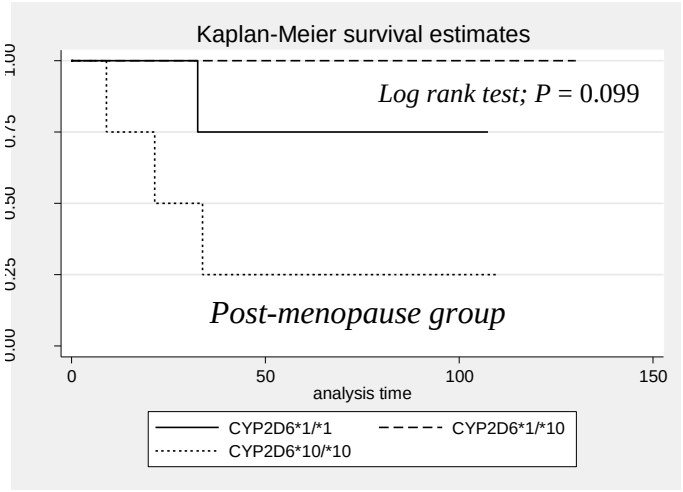
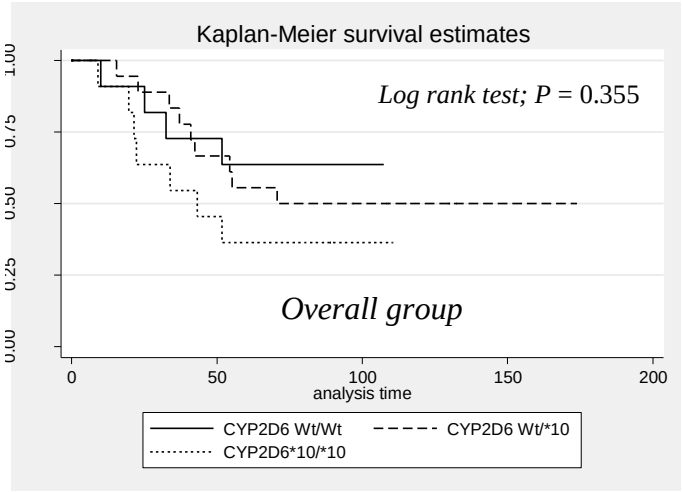



**Figure 5.11 Kaplan-Meier probabilities of disease free survival of patients with *CYP2D6* genotypes in difference group (comparison among **1/*1, *1/*10* and **10/*10*). A) Overall group, B) Pre-menopause, C) Post-menopause**

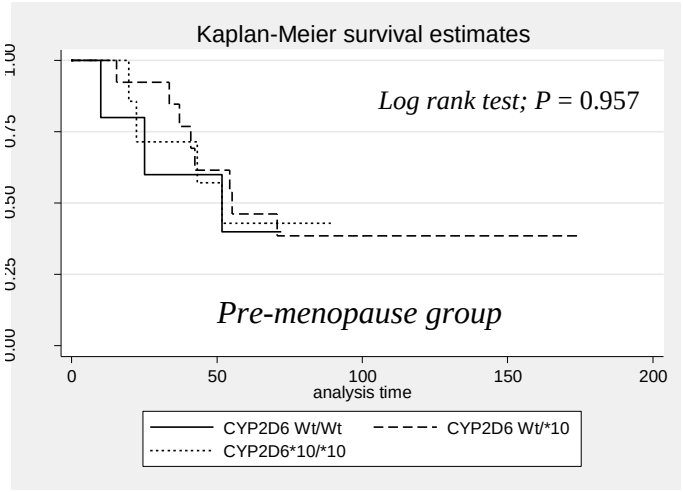
### 5.7.11 *CYP2D6* genotypes and disease free survival comparison among *Wt/Wt , Wt/*10* and **10/*10*

There was no significant differences disease free survival of *CYP2D6* genotypes in patients with homozygous variant allele (*CYP2D6*10/*10*) compared with heterozygous (*CYP2D6 Wt/*10*) and homozygous wild type (*CYP2D6 Wt/Wt*) between breast cancers cases versus breast cancers controls among three groups.

A) Overall group




B) Pre-menopause group



C) Post-menopause group

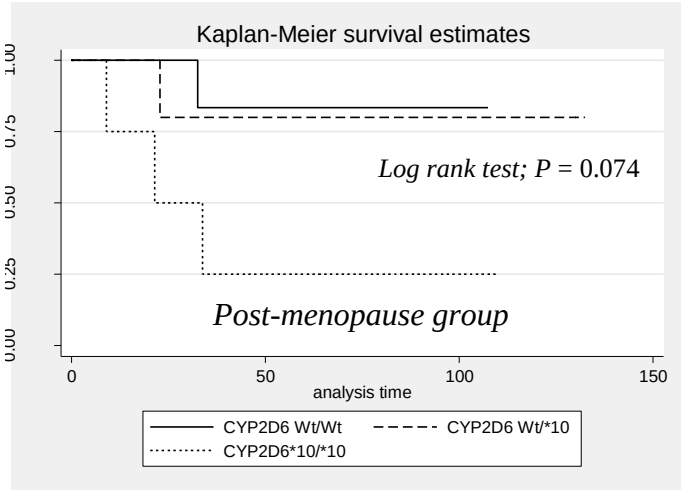
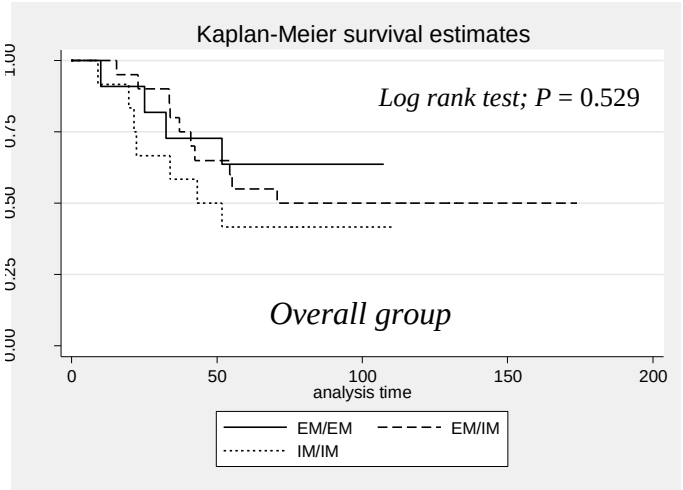



**Figure 5.12 Kaplan-Meier probabilities of disease free survival of patients with *CYP2D6* genotypes in difference group (comparison among *Wt/Wt, Wt/*10* and **10/*10*). A) Overall group, B) Pre-menopause, C) Post-menopause**

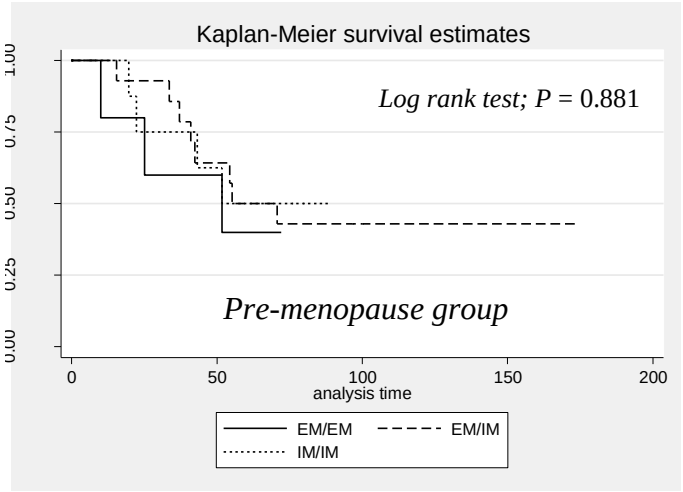
### 5.7.12 *CYP2D6* predicted phenotypes and disease free survival comparison among *EM/EM*, *EM/IM* and *IM/IM*

There were no significant differences disease free survival of *CYP2D6* genotypes in patients with homozygous IM allele (IM/IM) compared with heterozygous EM allele (EM/IM) and homozygous EM allele (EM/EM) between breast cancers cases versus breast cancers controls among three groups.

A) Overall group



B) Pre-menopause group




C) Post-menopause group

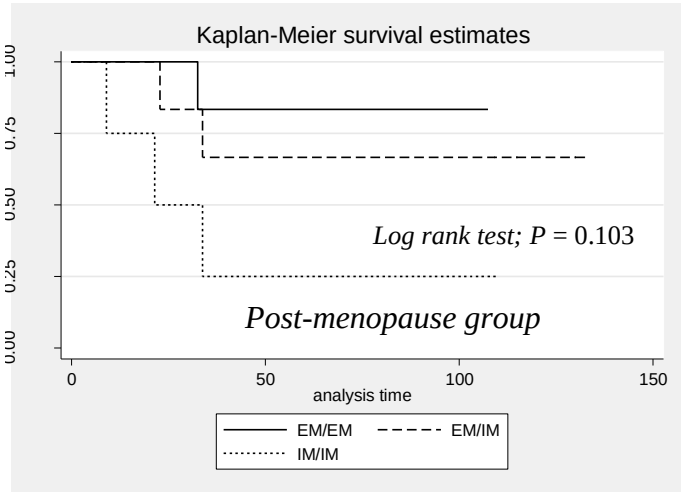
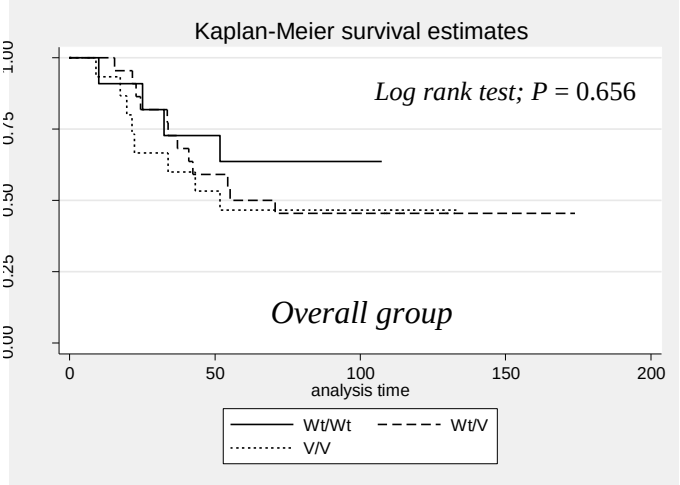



**Figure 5.13 Kaplan-Meier probabilities of disease free survival of patients with *CYP2D6* genotypes in difference group (comparison among *EM*/*EM*, *EM*/*IM* and *IM*/*IM*). A) Overall group, B) Pre-menopause, C) Post-menopause**

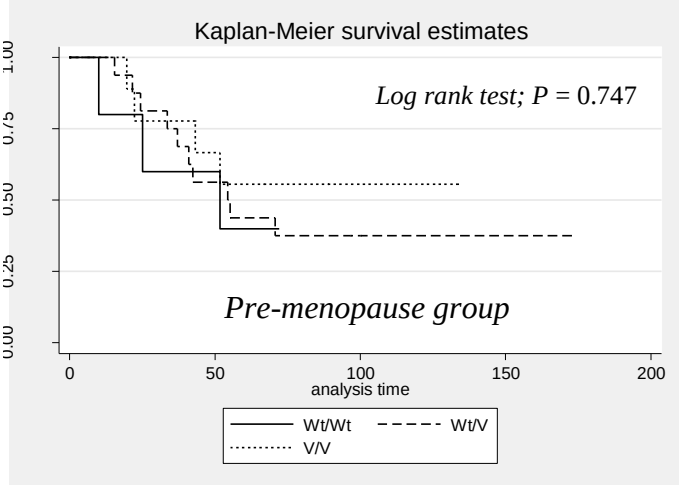
### 5.7.13 *CYP2D6* genotypes and disease free survival comparison among *Wt/Wt, Wt/V* and *V/V*

There was no significant differences disease free survival of *CYP2D6* genotypes in patients with homozygous variant allele compared with heterozygous allele (*Wt/V*) and homozygous wild type (*Wt/Wt*) between breast cancers cases versus breast cancers controls in among three groups.

A) Overall group




B) Pre-menopause group



C) Post-menopause group

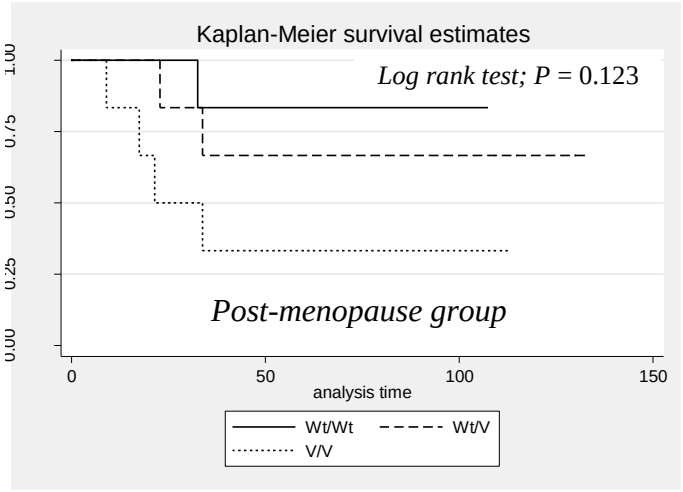
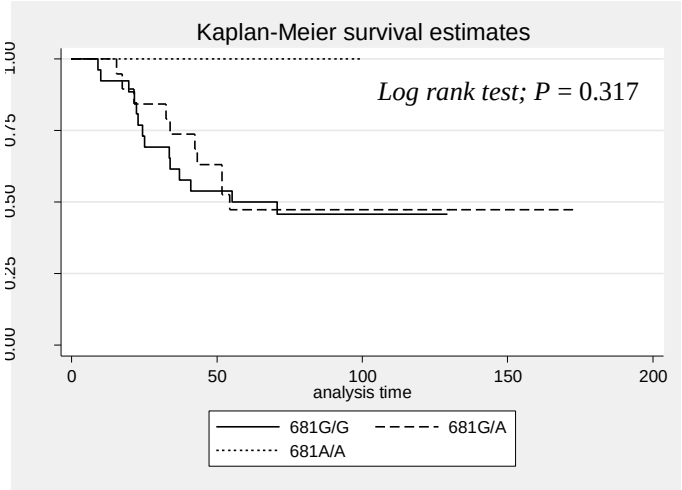



**Figure 5.14 Kaplan-Meier probabilities of disease free survival of patients with *CYP2D6* genotypes in difference group (comparison among *Wt*/*Wt*, *Wt*/*V* and *V*/*V*). A) Overall group, B) Pre-menopause, C) Post-menopause**

### 5.7.14 *CYP2C19* genotypes and disease free survival comparison among homozygous wild type, heterozygous and homozygous variant in overall group

There was no significant differences disease free survival of *CYP2C19* genotypes in patients with homozygous variant allele compared with heterozygous and homozygous wild type between breast cancers cases versus breast cancers controls in overall group.

A) 681G>A




B) 636G>A

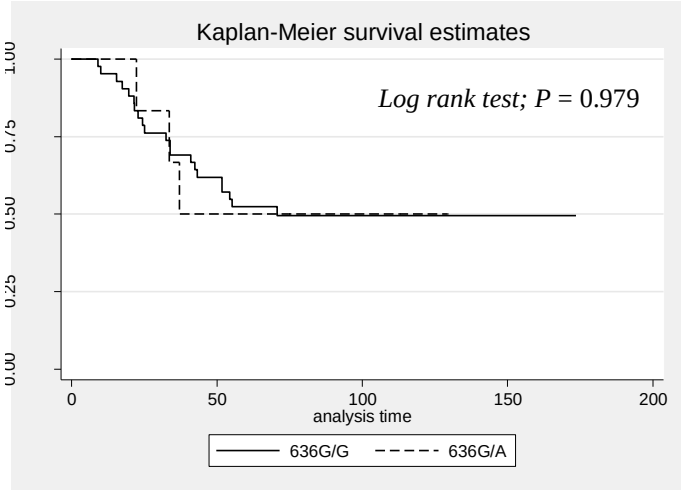
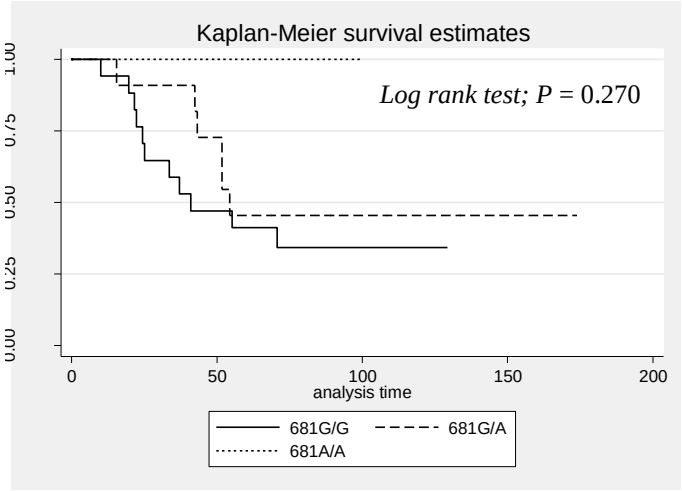



**Figure 5.15 Kaplan-Meier probabilities of disease free survival of patients with *CYP2D6* genotypes in overall group (comparison among homozygous wild type, heterozygous and homozygous variant). A) 681G>A, B) 636G>A**

### 5.7.15 *CYP2C19* genotypes and disease free survival comparison among homozygous wild type, heterozygous and homozygous variant in pre-menopause group

There was no significant differences disease free survival of *CYP2C19* genotypes in patients with homozygous variant allele compared with heterozygous and homozygous wild type between breast cancers cases versus breast cancers controls in pre-menopausal group.

A) 681G>A




B) 636G>A

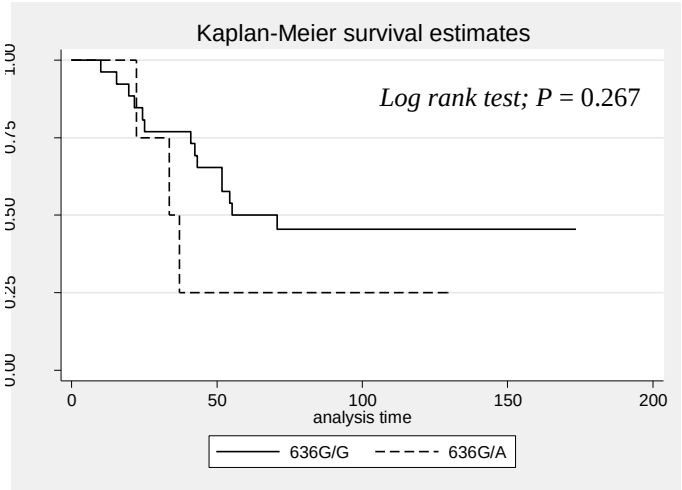
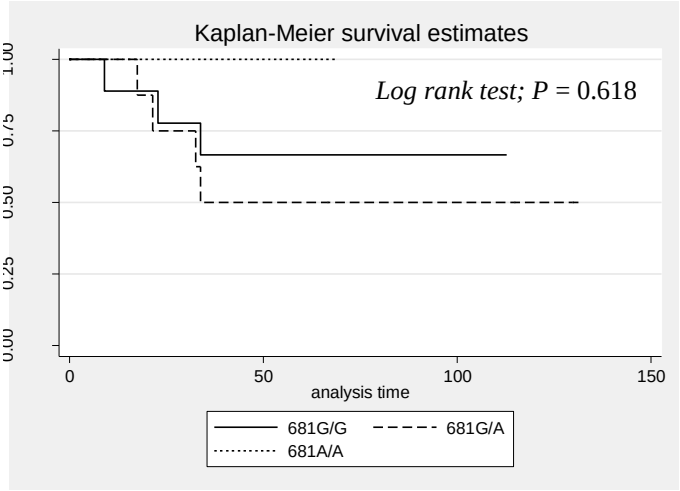



**Figure 5.16 Kaplan-Meier probabilities of disease free survival of patients with *CYP2D6* genotypes in pre-menopause group (comparison among homozygous wild type, heterozygous and homozygous variant). A) 681G>A, B) 636G>A**

### 5.7.16 *CYP2C19* genotypes and disease free survival comparison among homozygous wild type, heterozygous and homozygous variant in post-menopause group

There was no significant differences disease free survival of *CYP2C19* genotypes in patients with homozygous variant allele compared with heterozygous and homozygous wild type between breast cancers cases versus breast cancers controls in post-menopausal group.

A) 681G>A




B) 636G>A

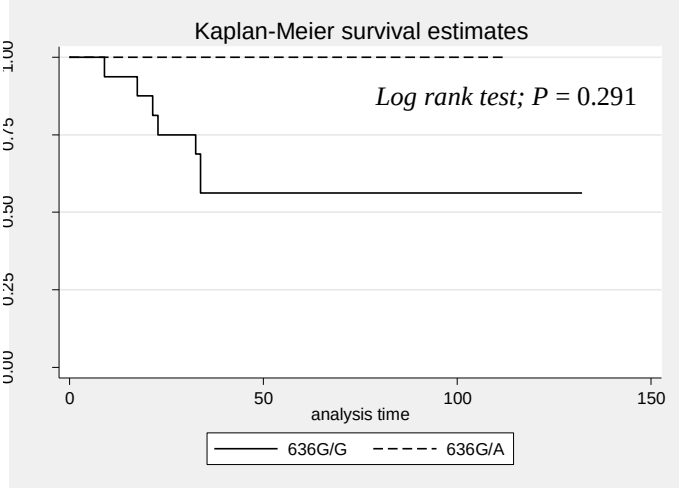
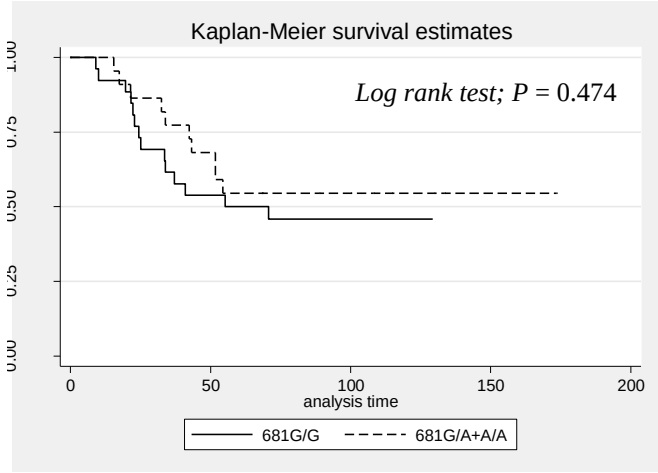



**Figure 5.17 Kaplan-Meier probabilities of disease free survival of patients with *CYP2D6* genotypes in post-menopause group (comparison among homozygous wild type, heterozygous and homozygous variant). A) 681G>A, B) 636G>A**

**5.7.17 *CYP2C19* genotypes and disease free survival comparison among homozygous wild type and those with heterozygous and homozygous variant in overall group**

There was no significant differences disease free survival of *CYP2C19* genotypes in patients with homozygous variant allele compared with heterozygous and homozygous wild type between breast cancers cases versus breast cancers controls in overall group.

A) 681G>A




B) 636G>A

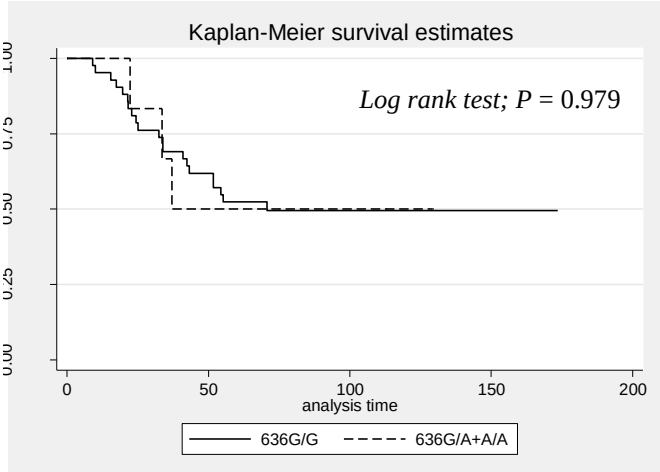
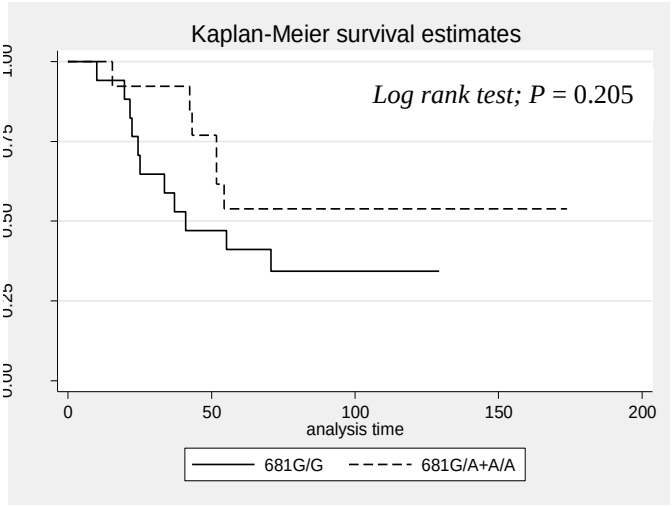



**Figure 5.18 Kaplan-Meier probabilities of disease free survival of patients with *CYP2D6* genotypes in overall group (comparison among homozygous wild type and those with heterozygous and homozygous variant). A) 681G>A, B) 636G>A**

### 5.7.18 *CYP2C19* genotypes and disease free survival comparison among homozygous wild type and those with heterozygous and homozygous variant in pre-menopause group

There was no significant differences disease free survival of *CYP2C19* genotypes in patients with homozygous variant allele compared with heterozygous and homozygous wild type between breast cancers cases versus breast cancers controls in pre-menopause group.

A) 681G>A




B) 636G>A

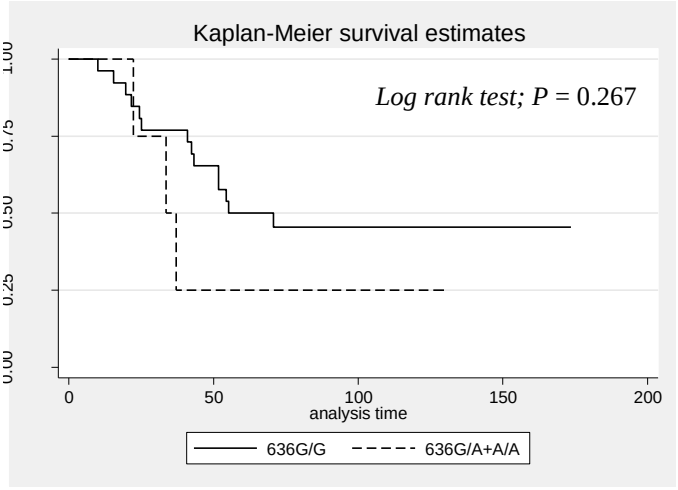
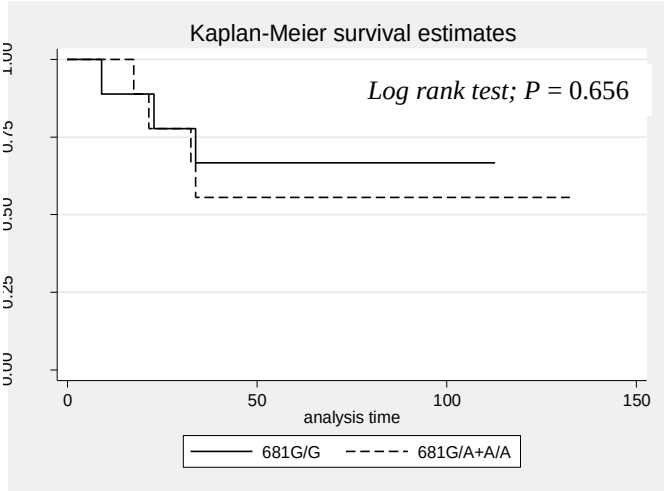



**Figure 5.19 Kaplan-Meier probabilities of disease free survival of patients with *CYP2D6* genotypes in pre-menopause group (comparison among homozygous wild type and those with heterozygous and homozygous variant). A) 681G>A, B) 636G>A**

**5.7.19 *CYP2C19* genotypes and disease free survival comparison among homozygous wild type and those with heterozygous and homozygous variant in post-menopause group**

There was no significant differences disease free survival of *CYP2C19* genotypes in patients with homozygous variant allele compared with heterozygous and homozygous wild type between breast cancers cases versus breast cancers controls in post-menopause group.

A) 681G>A




B) 636G>A

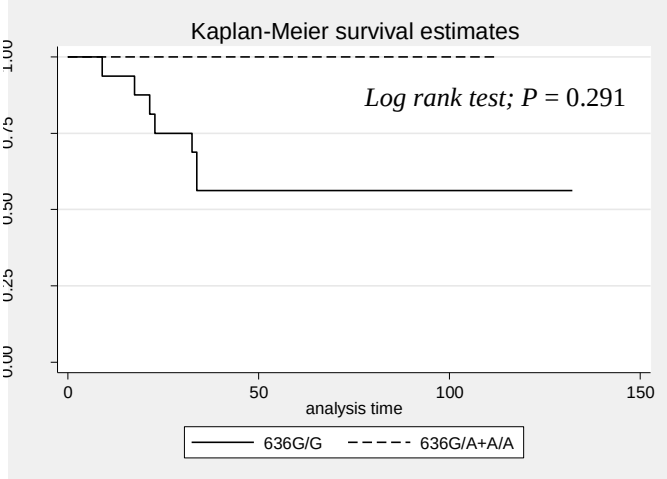
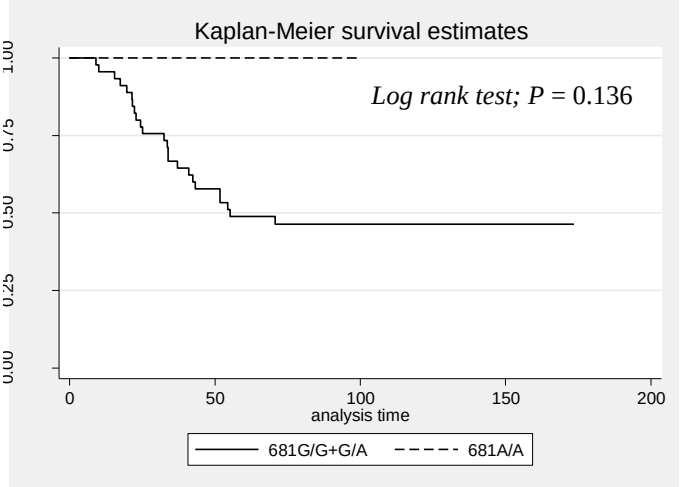



**Figure 5.20 Kaplan-Meier probabilities of disease free survival of patients with *CYP2D6* genotypes in post-menopause group (comparison among homozygous wild type and those with heterozygous and homozygous variant). A) 681G>A, B) 636G>A**

### 5.7.20 *CYP2C19* genotypes and disease free survival comparison among homozygous variant and those with heterozygous and homozygous wild type in overall group

There was no significant differences disease free survival of *CYP2C19* genotypes in patients with homozygous variant allele compared with heterozygous plus homozygous wild type between breast cancers cases versus breast cancers controls in overall group.

A) 681G>A




B) 636G>A

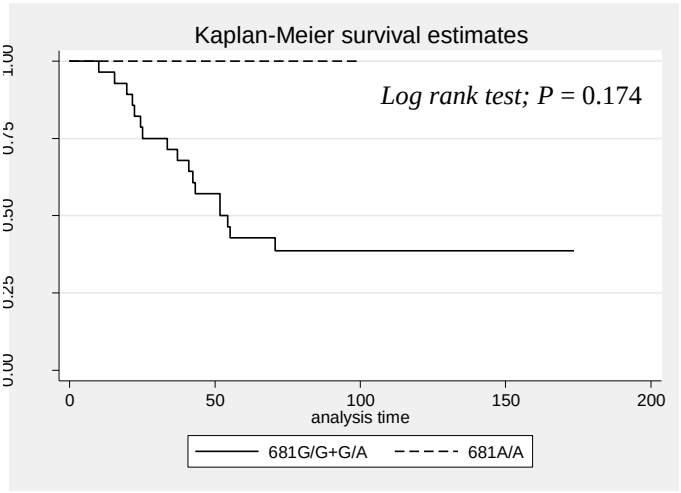
No comparison analysis is performed because the factor variable has only one value for every stratum.

**Figure 5.21 Kaplan-Meier probabilities of disease free survival of patients with *CYP2D6* genotypes in overall group (comparison among homozygous variant and those with heterozygous and homozygous wild type). A) 681G>A, B) 636G>A**

**5.7.21 *CYP2C19* genotypes and disease free survival comparison among homozygous variant and those with heterozygous and homozygous wild type in pre-menopause group**

There was no significant differences disease free survival of *CYP2C19* genotypes in patients with homozygous variant allele compared with heterozygous plus homozygous wild type between breast cancers cases versus breast cancers controls in pre-menopause group.

A) 681G>A




B) 636G>A

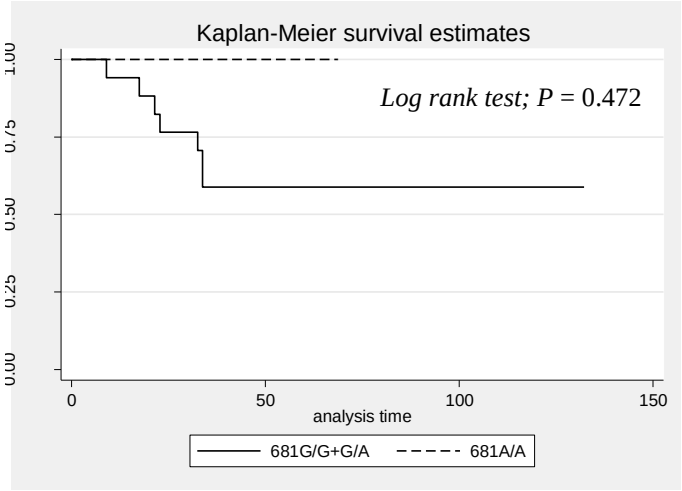
No comparison analysis is performed because the factor variable has only one value for every stratum

**Figure 5.22 Kaplan-Meier probabilities of disease free survival of patients with *CYP2D6* genotypes in pre-menopause group (comparison among homozygous variant and those with heterozygous and homozygous wild type). A) 681G>A, B) 636G>A**

### 5.7.22 *CYP2C19* genotypes and disease free survival comparison among homozygous variant and those with heterozygous and homozygous wild type in post-menopause group

There was no significant differences disease free survival (DFS) of *CYP2C19* genotypes in patients with homozygous variant allele compared with heterozygous plus homozygous wild type between breast cancers cases versus breast cancers controls in post-menopause group.

A) 681G>A




B) 636G>A

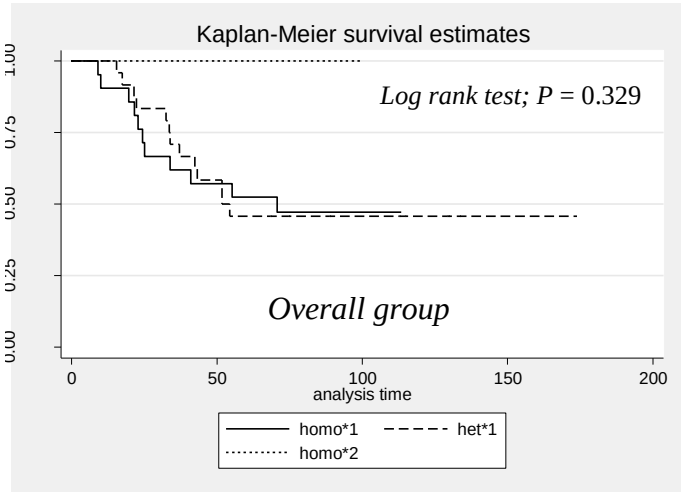
No comparison analysis is performed because the factor variable has only one value for every stratum

**Figure 5.23 Kaplan-Meier probabilities of disease free survival of patients with *CYP2D6* genotypes in post-menopause group (comparison among homozygous variant and those with heterozygous and homozygous wild type). A) 681G>A, B) 636G>A**

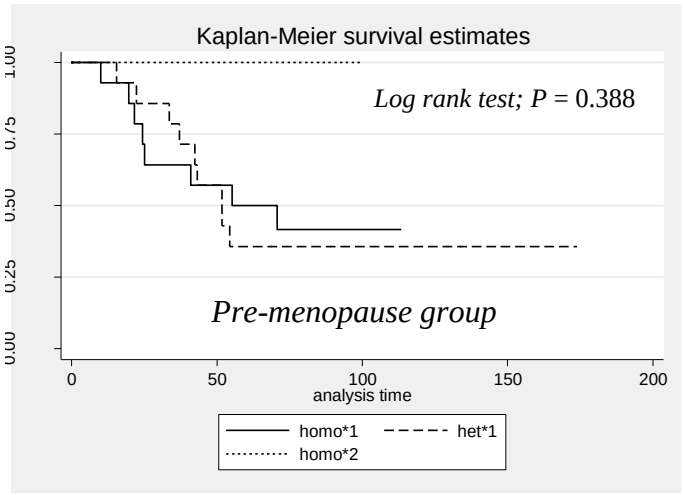
### 5.7.23 *CYP2C19* genotypes and disease free survival comparison among Homo**1*, Het**1* and Homo**2*

There was no significant differences disease free survival (DFS) of *CYP2C19* genotypes in patients with homozygous **2* (*CYP2C19*2/*2*) compared with heterozygous **1* (*CYP2C19*1/*2* and **1/*3*) and homozygous **1* (*CYP2C19*1/*1*) between breast cancers cases versus breast cancers controls among three groups.

A) Overall group




B) Pre-menopause group



C) Post-menopause group

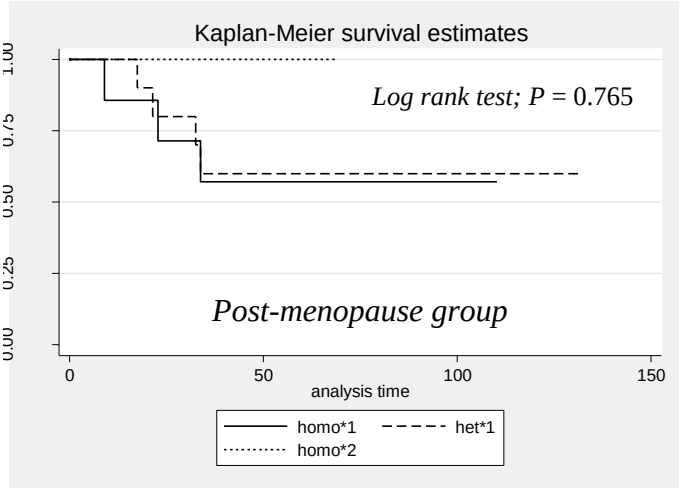
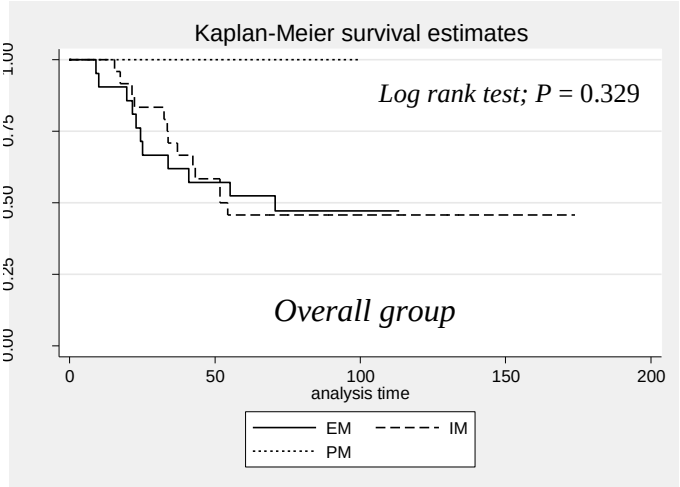



**Figure 5.24 Kaplan-Meier probabilities of disease free survival of patients with *CYP2C19* genotypes in difference groups (comparison among Homo**1*, Het**1* and Homo**2*). A) overall group, B) pre-menopause, C) post-menopause**

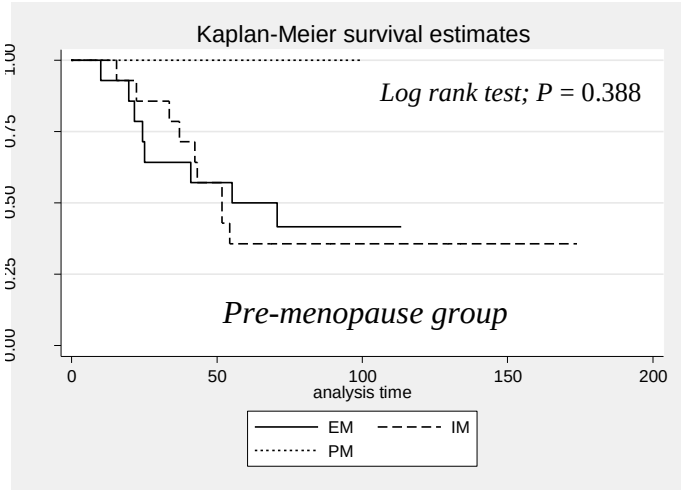
### 5.7.24 *CYP2C19* predicted phenotypes and disease free survival comparison among EM, IM and PM

There were no significant differences disease free survival of *CYP2C19* predicted phenotypes in patients with PM compared with IM and EM between breast cancers cases versus breast cancers controls among three groups.

A) Overall group



B) Pre-menopause group




C) Post-menopause group

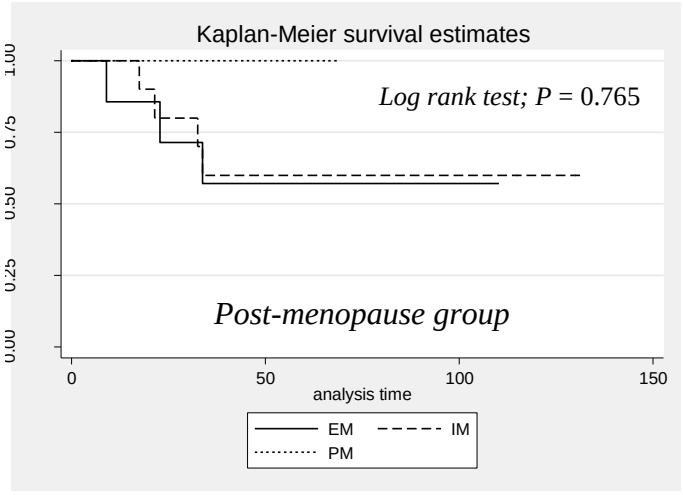



**Figure 5.25 Kaplan-Meier probabilities of disease free survival of patients with *CYP2C19* genotypes in difference groups (comparison among EM, IM and PM). A) Overall group, B) Pre-menopause, C) Post-menopause**

## 5.8 Relationship between risk of breast cancer recurrence and *CYP2D6* and *CYP2C19* genetic polymorphisms

Risk estimates were calculated by logistic regression. Patients with heterozygous *GA* at nucleotide 1846G>A showed an increased risk of developing recurrence but the difference was not statistically significant was observed in overall and pre-menopause group (HR, 7.32; 95% CI, 0.88-60.82; *P* = 0.065 and HR, 9.15; 95% CI, 0.95-88.05; *P* = 0.055, respectively). On the other hand, in pre-menopause group, the result showed that patients with heterozygous *GC* at nucleotide 4180G>C in pre-menopause women had significantly decreased risk of developing recurrence compared to patients with homozygous *GG* (HR, 0.29; 95% CI, 0.08-0.97; *P* = 0.045), listed in **Table 5.15**. In overall and post-menopause group, patients with homozygous *TT* at nucleotide 100C>T and 1039C>T had tend to increased risk of developing recurrence but no statistically significant association was observed (HR, 2.01; 95% CI, 0.95-4.28; *P* = 0.068). On the other hand, patients with homozygous *TT* at nucleotides 100C>T and 1039C>T in pre-menopause group had tend to decreased risk but no significant association was observed, list in **Table 5.15**. The *CYP2D6* and *CYP2C19* genotype were estimated by using a univariate Cox proportion hazard model. The hazard ratio presented in **Table 5.16** shows tend to increased risk of disease recurrence but it was not statistically significant. In the overall and post-menopause group, patients with homozygous *CYP2D6*10*, homozygous IM or homozygous variant had increased risk for disease recurrence compared to patients with homozygous or heterozygous wild type. In contrast, pre-menopause women had decreased risk for disease recurrence compared to patients with homozygous wild type but it was not statistically significant.

**Table 5.15. Risk estimation between genotypes and recurrences in breast cancer patients among three groups**

| Genotype | Overall | | | Pre-menopause | | | Post-menopause | | |
|---|---|---|---|---|---|---|---|---|---|
| ***CYP2D6*** | **N** | **HR (95% CI)** | ***P*** | **N** | **HR (95% CI)** | ***P*** | **N** | **HR (95% CI)** | ***P*** |
| **-1584C>G** | **48** | | | **30** | | | **18** | | |
| *CC* | 39 | 1.0 (ref) | | 25 | 1.0 (ref) | | 14 | 1.0 (ref) | |
| *CG* | 7 | 1.24 (0.42-3.65) | 0.690 | 4 | 1.96 (0.55-6.94) | 0.296 | 3 | 0.87 (0.30-2.51) | 0.795 |
| *GG* | 2 | 5.88e-16 | 1.000 | 1 | 6.03e-16 | 1.000 | 1 | 1.40e-8 | 1.000 |
| *CG + GG* | 9 | 0.86 (0.29-2.53) | 0.788 | 5 | 1.28 (0.37-4.48) | 0.699 | 4 | 0.54 (0.06-4.49) | 0.569 |
| And | | | | | | | | | |
| *CC + CG* | 46 | 1.0 (ref) | | 29 | 1.0 (ref) | | 17 | 1.0 (ref) | |
| *GG* | 2 | 5.71e-16 | 1.000 | 1 | 5.79e-16 | 1.000 | 1 | 1.44e-8 | 1.000 |
| **100C>T** | | | | | | | | | |
| *CC* | 13 | 1.0 (ref) | | 6 | 1.0 (ref) | | 7 | 1.0 (ref) | |
| *CT* | 23 | 0.95 (0.35-2.57) | 0.919 | 18 | 0.62 (0.19-2.00) | 0.429 | 5 | 0.86 (0.26-2.86) | 0.806 |
| *TT* | 12 | 1.45 (0.49-4.32) | 0.505 | 6 | 0.56 (0.12-2.50) | 0.446 | 6 | 1.91 (0.81-4.47) | 0.139 |
| *CT + TT* | 35 | 1.10 (0.43-2.77) | 0.845 | 24 | 0.61 (0.20-1.87) | 0.386 | 11 | 2.03 (0.39-10.47) | 0.400 |
| and | | | | | | | | | |
| *CC + CT* | 36 | 1.0 (ref) | | 24 | 1.0 (ref) | | 12 | 1.0 (ref) | |
| *TT* | 12 | 1.50 (0.62-3.62) | 0.367 | 6 | 0.80 (0.23-2.78) | 0.721 | 6 | 2.01 (0.95-4.28) | 0.068 |
| **1039C>T** | | | | | | | | | |
| *CC* | 14 | 1.0 (ref) | | 7 | 1.0 (ref) | | 7 | 1.0 (ref) | |
| *CT* | 22 | 0.76 (0.29-2.02) | 0.590 | 17 | 0.48 (0.16-1.44) | 0.188 | 5 | 0.86 (0.26-2.86) | 0.806 |
| *TT* | 12 | 1.27 (0.45-3.63) | 0.653 | 6 | 0.47 (0.11-1.97) | 0.299 | 6 | 1.91 (0.81-4.47) | 0.139 |
| *CT + TT* | 34 | 0.92 (0.38-2.22) | 0.848 | 23 | 0.47 (0.17-1.36) | 0.164 | 11 | 2.03 (0.39-10.47) | 0.400 |
| and | | | | | | | | | |
| *CC + CT* | 36 | 1.0 (ref) | | 24 | 1.0 (ref) | | 12 | 1.0 (ref) | |
| *TT* | 12 | 1.50 (0.62-3.62) | 0.367 | 6 | 0.80 (0.23-2.78) | 0.721 | 6 | 2.01 (0.95-4.28) | 0.068 |

To continue

| Genotype | | Overall | | | Pre-menopause | | | Post-menopause | |
|---|---|---|---|---|---|---|---|---|---|
| ***CYP2D6*** | **N** | **HR (95% CI)** | ***P*** | **N** | **HR (95% CI)** | ***P*** | **N** | **HR (95% CI)** | ***P*** |
| **1661G>C** | | | | | | | | | |
| *GG* | 11 | 1.0 (ref) | | 7 | 1.0 (ref) | | 4 | 1.0 (ref) | |
| *GC* | 18 | 0.62 (0.21-1.80) | 0.384 | 11 | 0.47 (0.14-1.55) | 0.213 | 7 | 1.06 (0.32-3.53) | 0.922 |
| *CC* | 19 | 0.92 (0.33-2.53) | 0.871 | 12 | 0.50 (0.15-1.64) | 0.250 | 7 | 1.77 (0.59-5.30) | 0.309 |
| *GC + CC* | 37 | 0.76 (0.30-1.92) | 0.562 | 23 | 0.48 (0.17-1.38) | 0.174 | 14 | 1.96 (0.24-16.30) | 0.534 |
| and | | | | | | | | | |
| *GG + GC* | 29 | 1.0 (ref) | | 18 | 1.0 (ref) | | 11 | 1.0 (ref) | |
| *CC* | 19 | 1.24 (0.55-2.78) | 0.610 | 12 | 0.80 (0.30-2.18) | 0.669 | 7 | 1.70 (0.80-3.60) | 0.166 |
| **1846G>A** | | | | | | | | | |
| *GG* | 47 | 1.0 (ref) | | 29 | 1.0 (ref) | | 18 | 1.0 (ref) | |
| *GA* | 1 | 7.32 (0.88-60.82) | 0.065 | 1 | 9.15 (0.95-88.05) | 0.055 | 0 | - | - |
| *AA* | 0 | - | - | 0 | - | - | 0 | - | - |
| *GA + AA* | 1 | 7.32 (0.88-60.82) | 0.065 | 1 | 9.15 (0.95-88.05) | 0.055 | 0 | - | - |
| and | | | | | | | | | |
| *GG + GA* | 48 | 1.0 (ref) | | 30 | 1.0 (ref) | | 18 | 1.0 (ref) | |
| *AA* | 0 | - | - | 0 | - | - | 0 | - | - |
| **2850C>T** | | | | | | | | | |
| *CC* | 38 | 1.0 (ref) | | 24 | 1.0 (ref) | | 14 | 1.0 (ref) | |
| *CT* | 9 | 0.79 (0.27-2.33) | 0.678 | 5 | 1.21 (0.35-4.23) | 0.767 | 4 | 0.67 (0.23-1.95) | 0.465 |
| *TT* | 1 | 4.24e-15 | 1.000 | 1 | 5.95e-16 | 1.000 | 0 | - | - |
| *CT + TT* | 10 | 0.69 (0.24-2.03) | 0.505 | 6 | 0.90 (0.26-3.14) | 0.870 | 4 | 0.45 (0.05-3.78) | 0.465 |
| and | | | | | | | | | |
| *CC + CT* | 47 | 1.0 (ref) | | 29 | 1.0 (ref) | | 18 | 1.0 (ref) | |
| *TT* | 1 | 4.44e-15 | 1.000 | 1 | 5.79e-16 | 1.000 | 0 | - | - |

To continue

| Genotype | | Overall | | | Pre-menopause | | | Post-menopause | |
|---|---|---|---|---|---|---|---|---|---|
| ***CYP2D6*** | **N** | **HR (95% CI)** | ***P*** | **N** | **HR (95% CI)** | ***P*** | **N** | **HR (95% CI)** | ***P*** |
| **4180G>C** | | | | | | | | | |
| *GG* | 10 | 1.0 (ref) | | 6 | 1.0 (ref) | | 4 | 1.0 (ref) | |
| *GC* | 19 | 0.49 (0.17-1.45) | 0.202 | 12 | 0.29 (0.08-0.97) | 0.045 | 7 | 1.06 (0.32-3.53) | 0.922 |
| *CC* | 19 | 0.79 (0.28-2.17) | 0.643 | 12 | 0.34 (0.10-1.15) | 0.082 | 7 | 1.77 (0.59-5.30) | 0.309 |
| *GC + CC* | 38 | 0.63 (0.25-1.59) | 0.326 | 24 | 0.31 (0.11-0.92 | 0.034 | 14 | 1.96 (0.24-16.30) | 0.534 |
| and | | | | | | | | | |
| *GG + GC* | 29 | 1.0 (ref) | | 18 | 1.0 (ref) | | 11 | 1.0 (ref) | |
| *CC* | 19 | 1.24 (0.55-2.78) | 0.610 | 12 | 0.80 (0.30-2.18) | 0.669 | 7 | 1.70 (0.80-3.60) | 0.166 |
| ***CYP2C19*** | | | | | | | | | |
| **681G>A** | | | | | | | | | |
| *GG* | 26 | 1.0 (ref) | | 17 | 1.0 (ref) | | 9 | 1.0 (ref) | |
| *GA* | 19 | 0.90 (0.39-2.03) | 0.798 | 11 | 0.66 (0.24-1.79) | 0.410 | 8 | 1.27 (0.60-2.70) | 0.527 |
| *AA* | 3 | 5.20e-16 | 1.000 | 2 | 5.91e-17 | 1.000 | 1 | 2.64e-8 | 1.000 |
| *GA + AA* | 22 | 0.74 (0.33-1.68) | 0.477 | 13 | 0.53 (0.19-1.44) | 0.214 | 9 | 1.39 (0.31-6.25) | 0.662 |
| and | | | | | | | | | |
| *GG + GA* | 45 | 1.0 (ref) | | 8 | 1.0 (ref) | | 17 | 1.0 (ref) | |
| *AA* | 3 | 2.01e-16 | 1.000 | 2 | 7.16e-17 | 1.000 | 1 | 1.44e-8 | 1.000 |
| **636G>A** | | | | | | | | | |
| *GG* | 42 | 1.0 (ref) | | 26 | 1.0 (ref) | | 16 | 1.0 (ref) | |
| *GA* | 6 | 1.02 (0.30-3.41) | 0.979 | 4 | 2.02 (0.57-7.15) | 0.277 | 2 | 2.23e-8 | 1.000 |
| *AA* | 0 | - | - | 0 | - | - | 0 | - | - |
| *GA + AA* | 6 | 1.02 (0.30-3.41) | 0.979 | 4 | 2.02 (0.57-7.15) | 0.277 | 2 | 4.99e-16 | 1.000 |
| and | | | | | | | | | |
| *GG + GA* | 48 | | | 30 | | | 18 | | |
| *AA* | 0 | - | - | 0 | - | - | 0 | - | - |

All *P* value were test by Chi-square test; HR, hazard ratio; 95% CI, 95% confidence intervals

**Table 5.16. Risk estimation between *CYP2D6* and *CYP2C19* genotypes and recurrences in breast cancer patients among three groups**

| Genotypes | Overall | | | Pre-menopause | | | Post-menopause | | |
|---|---|---|---|---|---|---|---|---|---|
| ***CYP2D6*** | **N** | **HR (95% CI)** | ***P*** | **N** | **HR (95% CI)** | ***P*** | **N** | **HR (95% CI)** | ***P*** |
| No. of Patients | 32 | | | 21 | | | 11 | | |
| **1/*1* | 8 | ref | | 4 | ref | | 4 | ref | |
| **1/*10* | 13 | 0.71 (0.20-2.54) | 0.599 | 10 | 0.48 (0.12-1.97) | 0.308 | 3 | 4.94e-09 (0) | 1.000 |
| **10/*10* | 11 | 1.43 (0.42-4.89) | 0.573 | 7 | 0.55 (0.13-2.49) | 0.438 | 4 | 2.08 (0.67-6.48) | 0.209 |
| No. of Patients | 40 | | | 25 | | | 15 | | |
| *Wt/Wt* | 11 | ref | | 5 | ref | | 6 | ref | |
| *Wt/*10* | 18 | 1.31 (0.40-4.26) | 0.654 | 13 | 0.82 (0.22-3.10) | 0.769 | 5 | 1.13 (0.28-4.51) | 0.865 |
| **10/*10* | 11 | 2.26 (0.66-7.74) | 0.194 | 7 | 0.86 (0.19-3.83) | 0.839 | 4 | 2.65 (0.85-8.27) | 0.093 |
| No. of Patients | 43 | | | 27 | | | 16 | | |
| *EM/EM* | 11 | ref | | 5 | ref | | 6 | ref | |
| *EM/IM* | 20 | 1.31(0.41-4.19) | 0.645 | 14 | 0.74 (0.20-2.81) | 0.662 | 6 | 1.44 (0.43-4.78) | 0.553 |
| *IM/IM* | 12 | 1.94 (0.57-6.65) | 0.194 | 8 | 0.70 (0.19-3.83) | 0.642 | 4 | 2.66 (0.85-8.28) | 0.091 |
| No. of Patients | 48 | | | 30 | | | 18 | | |
| *Wt/Wt* | 11 | ref | | 5 | ref | | 6 | ref | |
| *Wt/V* | 22 | 1.53(0.49-4.74) | 0.469 | 16 | 0.90 (0.25-3.29) | 0.878 | 6 | 1.43 (0.43-4.77) | 0.555 |
| *V/V* | 15 | 1.74(0.52-5.76) | 0.370 | 9 | 0.62 (0.14-2.71) | 0.512 | 6 | 2.49 (0.83-7.46 | 0.104 |

To continue.

| Genotypes | | Overall | | | Pre-menopause | | | Post-menopause | |
|---|---|---|---|---|---|---|---|---|---|
| ***CYP2C19*** | **N** | **HR (95% CI)** | ***P*** | **N** | **HR (95% CI)** | ***P*** | **N** | **HR (95% CI)** | ***P*** |
| No. of Patients | 48 | | | 30 | | | 18 | | |
| Homo **1* | 21 | ref | | 14 | ref | | 7 | ref | |
| Het**1* | 24 | 0.98 (0.44-2.20) | 0.970 | 14 | 1.10 (0.42-2.87) | 0.843 | 10 | 0.95 (0.45-2.01) | 0.898 |
| Homo**2* | 3 | 1.99e-16 | 1.000 | 2 | 1.02e-17 | 1.000 | 1 | 2.31e-08 | 1.000 |
| No. of Patients | 48 | | | 30 | | | 18 | | |
| Homo EM | 21 | 1.0 (ref) | | 14 | 1.0 (ref) | | 7 | 1.0 (ref) | |
| Het EM | 24 | 0.98 (0.44-2.20) | 0.970 | 14 | 1.10 (0.42-2.87) | 0.843 | 10 | 0.95 (0.45-2.01) | 0.898 |
| Homo PM | 3 | 1.99e-16 | 1.000 | 2 | 1.02e-17 | 1.000 | 1 | 2.31e-08 | 1.000 |

All *P* value were test by Chi-square test; HR, hazard ratio; 95% CI, 95% confidence intervals

# CHAPTER VI
# DISCUSSION

This study is the first to investigate the association between host genetic and breast cancer outcomes in tamoxifen treated Thai women breast cancer patients. The present study aimed to identify the polymorphisms in *CYP2D6* and *CYP2C19* may influence on the tamoxifen efficacy and clinical outcomes during the five years after breast cancer surgery and treated with adjuvant tamoxifen. Previous studies have been reported that tamoxifen metabolisms (*CYP2D6, CYP2C19, CYP3A5, and SULT1A1*) or tamoxifen metabolites (4-OHtam and endoxifen) influence on breast cancer outcomes but our results did not support the previous studies. The variant alleles of two genes were not involved in tamoxifen efficacy. Our results shown that no significant difference in the polymorphisms in *CYP2D6* and *CYP2C19* between breast cancer cases who had disease recurrence during five years treated with adjuvant tamoxifen compared with breast cancer control who had not disease recurrence. However, there was significant difference in allelic frequencies of *CYP2D6* (heterozygous *CT* at nucleotide *100, CYP2D6*10*) between pre-menopause breast cancers and post-menopause breast cancers (*P* value = 0.031). In addition, the presence of variant alleles of *CYP2D6* and *CYP2C19* were found that no significant differences disease free survival (DFS) between breast cancers cases and breast cancers controls but we could found that Kaplan-Meier estimates showed significant difference DFS of *CYP2D6* genotypes in patients with heterozygous *GA* at nucleotide *1846G>A* (*CYP2D6*4*) compared with homozygous wild type (*GG*) in overall and pre-menopause groups (Log-rank test, *P* = 0.031 and 0.019, respectively). There was no homozygous variant (*AA*) at nucleotide *1846G>A*. This result seemed to agree with Schroth and colleagues [25] that patients with carrying *CYP2D6*4* had significantly worse in both event free survival rates and shorter relapse free time. Because of the heterozygous *CYP2D6*4* genotype establishes a small number of patients, result should therefore be confirmed in a larger patient population. Furthermore, Kaplan-Meier estimates showed significant difference disease free survival (DFS) in patients

with homozygous variant (*TT*) compared those with heterozygous *CT* or homozygous wild type (*CC*) at nucleotides *100* and *1039* (*CYP2D6*10*) in post-menopause group (Log-rank test, *P* = 0.046 and 0.046), which two SNPs were associated in linkage disequilibrium.

The characteristics of breast cancer patients may affect to the clinical outcome. We found no association between patient's characteristics and tamoxifen efficacy in patients with breast cancer who receiving adjuvant tamoxifen. However, the method for breast cancer therapy, chemotherapy was related to DFS in overall and pre-menopausal group (Log-rank test, *P* = 0.003 and 0.002, respectively).

The metabolism of tamoxifen is complex and mediated by multiple CYP enzymes and genetic polymorphisms of these enzymes may influence the plasma concentrations of tamoxifen and its metabolites. Tamoxifen is metabolized to its active metabolites, such as 4-hydroxytamoxifen and endoxifen, by multiple CYP enzymes including CYP2D6, CYP3A4/5, CYP2C9, CYP2C19, CYP1A2, CYP2B6 and it is eliminated by other enzymes in part of pharmacokinetic excretion including sulfotransferase (SULT)1A1 and UDP-glucuronosyltransferase (UGT)2B15 and UGT1A4 [43]. Nowell S *et al*. [43] reported that breast cancer patients with *SULT* homozygous variant allele (*SULT1A1*2*/*2*) had approximately three times the risk of death (HR, 2.9; 95% CI, 1.1 - 7.6) compared with breast cancer patients who were homozygous for the common allele of *SULT1A1* (*SULT1A1*1/*1*) or breast cancer patients who were heterozygous variant allele of *SULT1A1* (*SULT1A1*1/*2*). Several previous studies have been investigated the association between the polymorphisms in *CYP2D6* and tamoxifen efficacy and clinical outcomes in patients receiving adjuvant tamoxifen. Goetz *et al*. [110] reported that breast cancer patients with decreased *CYP2D6* metabolism had significantly shorter time to recurrence (HR, 1.91; 95% CI, 1.05-3.45; *P* = 0.034) and worse relapse-free survival (HR, 1.74; 95%CI, 1.10-2.74; *P* = 0.017) compared to patients with extensive *CYP2D6* metabolism. In addition, patients with PM phenotype (*CYP2D6*4/*4*) had significant higher risk of breast cancer relapse approximately three times compared to extensive metabolizers (*CYP2D6*1/*1* and **1/*4*) (HR, 3.12; *P* = 0.007). It has been also reported that patients who carrying at least one decrease *CYP2D6* alleles (*CYP2D6*4, *5, *10, *41*) had significantly shorter relapse free time (RFTs) (HR, 2.24; 95%CI, 1.16 - 4.33; *P* =

0.02) and worse event free survival (EFS) rates (HR = 1.89; 95%CI, 1.10 - 3.25; *P* = 0.02) compared to patients with at least one functional allele (*CYP2D6*1/*1*). In addition, patients with heterozygous or homozygous *CYP2C19*17* had significantly better RFTs (HR, 0.45; 95% CI, 0.21- 0.92; *P* = 0.03) than the carriers of *CYP2C19*1*, **2*, and **3* alleles but there was no associations between polymorphisms in *CYP2C19* (**2* and **3*), *CYP3A5*, *CYP2B6*, *CYP2C9* and treatment outcome or survival time [68]. Teh L *et al*. [111] demonstrated that patients carrying *CYP2D6*10/*10* and heterozygous null allele had higher risk of breast cancer relapse and metastasis (OR, 13.14; 95%CI, 1.57-109.94; *P* = 0.004) compared to patients with *CYP2D6*1/*1* and **1/*10* genotype. Furthermore, breast cancer patients with homozygous *CC* genotypes at nucleotide *C3435T* of *ABCB1* had a shorter time to relapse compared to *CT* and *TT* genotypes, but it was not statistically significant. Xu *et al*. [76] showed that patients with *CYP2D6*10* homozygous *TT* genotype had significant worse DFS than patients with heterozygous *CT* and homozygous *CC* genotype (HR, 4.7; 95%CI, 1.1-20.0; *P* = 0.004). Lim *et al*. [112] reported that patients with *CYP2D6*10/*10* genotype had significantly higher risk of breast cancer relapse within 10 years after surgery compared with the other genotypes (time to disease progression,TTP: 5.03 v 21.8 months, *P* = 0.0032). Kiyotani *et al*. reported that patients with *CYP2D6*10/*10* and *CYP2D6*1/*10* showed significantly shorter recurrence free survival compared to patients with *CYP2D6*1/*1* (HR, 9.52; 95% CI, 2.79-32.45; *P* = 0.000036).

There are some arguments about the effect of *CYP2D6* genetic polymorphisms on tamoxifen efficacy. Some previous studies from both European and Asian population showed that no significant association between polymorphisms in *CYP2D6* and tamoxifen treatment outcomes. Okishiro *et al*. [78] reported that breast cancer patients with *CYP2D6*10/*10* genotypes showed no significantly different RFS rates compared with homozygous *wt/wt* or *wt/*10* genotypes and no significantly different RFS rates between *CYP2C19* PM genotypes (*CYP2C19*2/*2*, **2/*3*, or **3/*3*) compared to patients with *CYP2C19* EM genotypes (*CYP2C19 wt/wt*, *wt/*2*, or *wt/*3*). Toyama *et al*. [80] demonstrated that no significantly correlation between patients with *CYP2D6*10/*10* genotype and survival time (DFS, DDFS, BCSS, and OS) compared with *CYP2D6 wt/wt* and *wt/*10* genotypes. In contrast, the result from Wegman *et al*. [79] showed that patients with *CYP2D6*4/*4* genotype had

significantly better DFS than patients with heterozygous or homozygous *CYP2D6*1* ($P$ = 0.004 and $P$ = 0.005, respectively). Furthermore, they found that patients with homozygous *CYP3A5*3* had significant higher RFS than the other genotypes in tamoxifen treatment for 5 years (HR, 0.20; 95%CI, 0.07-0.55; $P$ = 0.002) and there was no correlation between RFS and *SULT1A1* or *UGT2B15* genotypes. Our data support the conclusion of these studies that *CYP2D6* and *CYP2C19* variants are not significantly associated with clinical outcome in breast cancer patients with adjuvant tamoxifen.

The Amplichip CYP450 Test can identify 33 allelic variants in *CYP2D6* and 3 allelic variants in *CYP2C19* in one sample. The use of an automatic system such as the AmpliChip *CYP450* may help to speed up the analytical process with minor limitations in specificity, such as misinterpretation between two SNPs, one EM and the other IM. However, several *CYP2D6* variant alleles are not detected by the Amplichip CYP450 Test i.e. *CYP2D6*12, *13, *16, *18, *21, *22, *23, *24, *27, *28, *32, *33, *34, *37, *38, *39, *42-*59, *61-*67* and for *CYP2C19*, which several alleles are not detected by the Amplichip CYP450 Test i.e. *CYP2C19*4-*9*, and **17* . In addition, Justenhoven *et al*. [62] reported that patients with heterozygous or homozygous *CYP2C19*17* had reduced breast cancer risk (OR, 0.77, 95% CI; 0.65–0.93; $P$ = 0.005).

This study has some limitations. First of all, the retrospective nature of the design is a weakness shared with all other available studies. The design study with retrospective method is lack data of correlation between polymorphisms in *CYP2D6* and plasma concentration of tamoxifen metabolites. The small sample size and low PM phenotype number gives a low statistical power. It is possible that one or more of these variants are associated with a specific subgroup of breast cancer patients. The data showed that high frequency of *CYP2D6*10* (100C>T) similar previous reported in Asian population [24, 111]. The result demonstrated that only 8 from 29 polymorphisms could be found in this study including -1584C>G, 100C>T, 1039C>T, 1661G>C, 1846G>A, 2850C>T, 4180G>C and **36GC*. This study had no homozygous *CYP2D6* PM allele (*CYP2D6*3*, **4,* and **5*) or no homozygous UM allele (*CYP2D6*1XN*, **2XN* and **35XN*), it is possible that there was small sample size.

# CHAPTER VII
# CONCLUSION

Genetic polymorphisms of *CYP2D6* and *CYP2C19* may be used for help physician plan for treatment patient with breast cancer. This study presented that *CYP2D6*10* (100C>T and 1039C>T) was significantly associated with shorter DFS in subgroup post-menopausal group but no statistically difference in other groups. Therefore that genetic polymorphisms *CYP2D6* might be necessary in post-menopausal breast cancer patients receiving tamoxifen. However, the metabolism of tamoxifen is complex. The combination of several mechanisms might be explained the relationship between host genetic and tamoxifen outcomes. This is a small retrospective study. The large numbers of patients will be needed to be verified by the future studies.

# REFERENCES


1 Institute, N.C., Hospital-based cancer registry, 2009: Bangkok,Thailand.

2 Fisher, B., Costantino, J.P., Wickerham, D.L., Cecchini, R.S., Cronin, W.M., Robidoux, A., Bevers, T.B., Kavanah, M.T., Atkins, J.N., Margolese, R.G. et al. Tamoxifen for the prevention of breast cancer: current status of the National Surgical Adjuvant Breast and Bowel Project P-1 study. *J Natl Cancer Inst*. 2005; 97(22): 1652-1662.

3 Fisher, B., Bryant, J., Dignam, J.J., Wickerham, D.L., Mamounas, E.P., Fisher, E.R., Margolese, R.G., Nesbitt, L., Paik, S., Pisansky, T.M. et al. Tamoxifen, radiation therapy, or both for prevention of ipsilateral breast tumor recurrence after lumpectomy in women with invasive breast cancers of one centimeter or less. *J Clin Oncol*. 2002; 20(20): 4141-4149.

4 Colleoni, M., Gelber, S., Goldhirsch, A., Aebi, S., Castiglione-Gertsch, M., Price, K.N., Coates, A.S. and Gelber, R.D. Tamoxifen after adjuvant chemotherapy for premenopausal women with lymph node-positive breast cancer: International Breast Cancer Study Group Trial 13-93. *J Clin Oncol*. 2006; 24(9): 1332-1341.

5 Desta, Z., Ward, B.A., Soukhova, N.V. and Flockhart, D.A. Comprehensive evaluation of tamoxifen sequential biotransformation by the human cytochrome P450 system in vitro: prominent roles for CYP3A and CYP2D6. *J Pharmacol Exp Ther*. 2004; 310(3): 1062-1075.

6 Stearns, V., Johnson, M.D., Rae, J.M., Morocho, A., Novielli, A., Bhargava, P., Hayes, D.F., Desta, Z. and Flockhart, D.A. Active tamoxifen metabolite plasma concentrations after coadministration of tamoxifen and the selective serotonin reuptake inhibitor paroxetine. *J Natl Cancer Inst*. 2003; 95(23): 1758-1764.

7 Johnson, M.D., Zuo, H., Lee, K.H., Trebley, J.P., Rae, J.M., Weatherman, R.V., Desta, Z., Flockhart, D.A. and Skaar, T.C. Pharmacological

characterization of 4-hydroxy-N-desmethyl tamoxifen, a novel active metabolite of tamoxifen. *Breast Cancer Res Treat*. 2004; 85(2): 151-159.

8 Lien, E.A., Solheim, E., Lea, O.A., Lundgren, S., Kvinnsland, S. and Ueland, P.M. Distribution of 4-hydroxy-N-desmethyltamoxifen and other tamoxifen metabolites in human biological fluids during tamoxifen treatment. *Cancer Res*. 1989; 49(8): 2175-2183.

9 Daly, A.K. Pharmacogenetics of the major polymorphic metabolizing enzymes. *Fundam Clin Pharmacol*. 2003; 17(1): 27-41.

10 Michalets, E.L. Update: clinically significant cytochrome P-450 drug interactions. *Pharmacotherapy*. 1998; 18(1): 84-112.

11 www.cypalleles.ki.se/cyp2d6.htm.

12 Stamer, U.M. and Stuber, F. Genetic factors in pain and its treatment. *Curr Opin Anaesthesiol*. 2007; 20(5): 478-484.

13 Griese, E.U., Zanger, U.M., Brudermanns, U., Gaedigk, A., Mikus, G., Morike, K., Stuven, T. and Eichelbaum, M. Assessment of the predictive power of genotypes for the in-vivo catalytic function of CYP2D6 in a German population. *Pharmacogenetics*. 1998; 8(1): 15-26.

14 Johansson, I., Lundqvist, E., Bertilsson, L., Dahl, M.L., Sjoqvist, F. and Ingelman-Sundberg, M. Inherited amplification of an active gene in the cytochrome P450 CYP2D locus as a cause of ultrarapid metabolism of debrisoquine. *Proc Natl Acad Sci U S A*. 1993; 90(24): 11825-11829.

15 Sistonen, J., Sajantila, A., Lao, O., Corander, J., Barbujani, G. and Fuselli, S. CYP2D6 worldwide genetic variation shows high frequency of altered activity variants and no continental structure. *Pharmacogenet Genomics*. 2007; 17(2): 93-101.

16 Solus, J.F., Arietta, B.J., Harris, J.R., Sexton, D.P., Steward, J.Q., McMunn, C., Ihrie, P., Mehall, J.M., Edwards, T.L. and Dawson, E.P. Genetic variation in eleven phase I drug metabolism genes in an ethnically diverse population. *Pharmacogenomics*. 2004; 5(7): 895-931.

17 Bjornsson, T.D., Wagner, J.A., Donahue, S.R., Harper, D., Karim, A., Khouri, M.S., Murphy, W.R., Roman, K., Schneck, D., Sonnichsen, D.S. et al. A

review and assessment of potential sources of ethnic differences in drug responsiveness. *J Clin Pharmacol*. 2003; 43(9): 943-967.

18 Ingelman-Sundberg, M. Genetic polymorphisms of cytochrome P450 2D6 (CYP2D6): clinical consequences, evolutionary aspects and functional diversity. *Pharmacogenomics J*. 2005; 5(1): 6-13.

19 Goetz, M.P., Kamal, A. and Ames, M.M. Tamoxifen pharmacogenomics: the role of CYP2D6 as a predictor of drug response. *Clin Pharmacol Ther*. 2008; 83(1): 160-166.

20 Ingelman-Sundberg, M. Duplication, multiduplication, and amplification of genes encoding drug-metabolizing enzymes: evolutionary, toxicological, and clinical pharmacological aspects. *Drug Metab Rev*. 1999; 31(2): 449-459.

21 Goldstein, J.A. Clinical relevance of genetic polymorphisms in the human CYP2C subfamily. *Br J Clin Pharmacol*. 2001; 52(4): 349-355.

22 Streetman, D.S., Bertino, J.S., Jr. and Nafziger, A.N. Phenotyping of drug-metabolizing enzymes in adults: a review of in-vivo cytochrome P450 phenotyping probes. *Pharmacogenetics*. 2000; 10(3): 187-216.

23 Jin, Y., Desta, Z., Stearns, V., Ward, B., Ho, H., Lee, K.H., Skaar, T., Storniolo, A.M., Li, L., Araba, A. et al. CYP2D6 genotype, antidepressant use, and tamoxifen metabolism during adjuvant breast cancer treatment. *J Natl Cancer Inst*. 2005; 97(1): 30-39.

24 Lim, Y.C., Li, L., Desta, Z., Zhao, Q., Rae, J.M., Flockhart, D.A. and Skaar, T.C. Endoxifen, a secondary metabolite of tamoxifen, and 4-OH-tamoxifen induce similar changes in global gene expression patterns in MCF-7 breast cancer cells. *J Pharmacol Exp Ther*. 2006; 318(2): 503-512.

25 Goetz, M.P., Rae, J.M., Suman, V.J., Safgren, S.L., Ames, M.M., Visscher, D.W., Reynolds, C., Couch, F.J., Lingle, W.L., Flockhart, D.A. et al. Pharmacogenetics of tamoxifen biotransformation is associated with clinical outcomes of efficacy and hot flashes. *J Clin Oncol*. 2005; 23(36): 9312-9318.

26 http://www.nlm.nih.gov/medlineplus/breastcancer.html.

27 Society, A.C. Cancer Facts and Figures 2010. Atlanta, Ga: *American Cancer Society*; 2010.

28 http://www.cancer.gov/cancertopics/wyntk/breast/WYNTK_breast.pdf.

29 http://www.cancer.gov/cancertopics/pdq/treatment/breast/Patient/page1.

30 Jordan, V.C. New insights into the metabolism of tamoxifen and its role in the treatment and prevention of breast cancer. *Steroids*. 2007; 72(13): 829-842.

31 Smith, I.E. and Dowsett, M. Aromatase Inhibitors in Breast Cancer. *New England Journal of Medicine*. 2003; 348(24): 2431-2442.

32 Letrozole Therapy Alone or in Sequence with Tamoxifen in Women with Breast Cancer. *New England Journal of Medicine*. 2009; 361(8): 766-776.

33 Martinez Guisado, A., Sanchez Munoz, A., de la Cabeza Lomas Garrido, M., Ruiz Borrego, M., Bayo Calero, J., de Toro Salas, R., Gonzalez Mancha, R., de la Haba Rodriguez, J. and Alba Conejo, E. Initialization of adjuvant hormonal treatment for breast cancer. *Adv Ther*. 2011; 28 Suppl 666-84.

34 Smith, I.E. New drugs for breast cancer. *Lancet*. 2002; 360(9335): 790-792.

35 Ingelman-Sundberg, M., Sim, S.C., Gomez, A. and Rodriguez-Antona, C. Influence of cytochrome P450 polymorphisms on drug therapies: pharmacogenetic, pharmacoepigenetic and clinical aspects. *Pharmacol Ther*. 2007; 116(3): 496-526.

36 Ingelman-Sundberg, M. Human drug metabolising cytochrome P450 enzymes: properties and polymorphisms. *Naunyn Schmiedebergs Arch Pharmacol*. 2004; 369(1): 89-104.

37 Vallance, P. and Smart, T.G. The future of pharmacology. *Br J Pharmacol*. 2006; 147 Suppl 1S304-307.

38 Guo, Y., Shafer, S., Weller, P., Usuka, J. and Peltz, G. Pharmacogenomics and drug development. *Pharmacogenomics*. 2005; 6(8): 857-864.

39 Pugsley, M.K., Authier, S. and Curtis, M.J. Principles of Safety Pharmacology. *Br J Pharmacol*. 2008; 154(7): 1382-1399.

40 Zhou, S.F. Polymorphism of human cytochrome P450 2D6 and its clinical significance: Part I. *Clin Pharmacokinet*. 2009; 48(11): 689-723.

41 Rendic, S. Summary of information on human CYP enzymes: human P450 metabolism data. Drug Metab Rev. 2002; 34(1-2): 83-448.

42 Eichelbaum, M., Baur, M.P., Dengler, H.J., Osikowska-Evers, B.O., Tieves, G., Zekorn, C. and Rittner, C. Chromosomal assignment of human cytochrome P-450 (debrisoquine/sparteine type) to chromosome 22. *Br J Clin Pharmacol*. 1987; 23(4): 455-458.

43 Heim, M. and Meyer, U.A. Genotyping of poor metabolisers of debrisoquine by allele-specific PCR amplification. *Lancet*. 1990; 336(8714): 529-532.

44 Kimura, S., Umeno, M., Skoda, R.C., Meyer, U.A. and Gonzalez, F.J. The human debrisoquine 4-hydroxylase (CYP2D) locus: sequence and identification of the polymorphic CYP2D6 gene, a related gene, and a pseudogene. *Am J Hum Genet*. 1989; 45(6): 889-904.

45 Brauch, H., Schroth, W., Eichelbaum, M., Schwab, M. and Harbeck, N. Clinical Relevance of CYP2D6 Genetics for Tamoxifen Response in Breast Cancer. *Breast Care (Basel).* 2008; 3(1): 43-50.

46 http://medicine.iupui.edu/flockhart.

47 Zanger, U.M., Raimundo, S. and Eichelbaum, M. Cytochrome P450 2D6: overview and update on pharmacology, genetics, biochemistry. *Naunyn Schmiedebergs Arch Pharmacol*. 2004; 369(1): 23-37.

48 Borges, S., Desta, Z., Li, L., Skaar, T.C., Ward, B.A., Nguyen, A., Jin, Y., Storniolo, A.M., Nikoloff, D.M., Wu, L. et al. Quantitative effect of CYP2D6 genotype and inhibitors on tamoxifen metabolism: implication for optimization of breast cancer treatment. *Clin Pharmacol Ther*. 2006; 80(1): 61-74.

49 Griese, E.U., Asante-Poku, S., Ofori-Adjei, D., Mikus, G. and Eichelbaum, M. Analysis of the CYP2D6 gene mutations and their consequences for enzyme function in a West African population. *Pharmacogenetics*. 1999; 9(6): 715-723.

50 Kubota, T., Yamaura, Y., Ohkawa, N., Hara, H. and Chiba, K. Frequencies of CYP2D6 mutant alleles in a normal Japanese population and metabolic activity of dextromethorphan O-demethylation in different CYP2D6 genotypes. *Br J Clin Pharmacol*. 2000; 50(1): 31-34.

51 Sachse, C., Brockmoller, J., Bauer, S. and Roots, I. Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequences. *Am J Hum Genet.* 1997; 60(2): 284-295.

52 Muralidharan, G., Hawes, E.M., McKay, G., Korchinski, E.D. and Midha, K.K. Quinidine but not quinine inhibits in man the oxidative metabolic routes of methoxyphenamine which involve debrisoquine 4-hydroxylase. *Eur J Clin Pharmacol*. 1991; 41(5): 471-474.

53 Abraham, B.K., Adithan, C., Mohanasundaram, J., Shashindran, C.H., Koumaravelou, K. and Asad, M. Genetic polymorphism of CYP2D6 in Tamil population. *Eur J Clin Pharmacol*. 2001; 56(11): 849-850.

54 Paine, M.F., Hart, H.L., Ludington, S.S., Haining, R.L., Rettie, A.E. and Zeldin, D.C. The human intestinal cytochrome P450 "pie". *Drug Metab Dispos*. 2006; 34(5): 880-886.

55 http://www.cypalleles.ki.se/cyp2c19.htm.

56 Rosemary, J. and Adithan, C. The pharmacogenetics of CYP2C9 and CYP2C19: ethnic variation and clinical significance. *Curr Clin Pharmacol*. 2007; 2(1): 93-109.

57 Sim, S.C., Risinger, C., Dahl, M.L., Aklillu, E., Christensen, M., Bertilsson, L. and Ingelman-Sundberg, M. A common novel CYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. *Clin Pharmacol Ther*. 2006; 79(1): 103-113.

58 Justenhoven, C., Hamann, U., Pierl, C.B., Baisch, C., Harth, V., Rabstein, S., Spickenheuer, A., Pesch, B., Bruning, T., Winter, S. et al. CYP2C19*17 is associated with decreased breast cancer risk. *Breast Cancer Res Treat*. 2009; 115(2): 391-396.

59 Santos, P.C., Soares, R.A., Santos, D.B., Nascimento, R.M., Coelho, G.L., Nicolau, J.C., Mill, J.G., Krieger, J.E. and Pereira, A.C. CYP2C19 and ABCB1 gene polymorphisms are differently distributed according to ethnicity in the Brazilian general population. *BMC Med Genet*. 2011; 1213.

60 Shuldiner, A.R., O'Connell, J.R., Bliden, K.P., Gandhi, A., Ryan, K., Horenstein, R.B., Damcott, C.M., Pakyz, R., Tantry, U.S., Gibson, Q. et al. Association of cytochrome P450 2C19 genotype with the antiplatelet effect and clinical efficacy of clopidogrel therapy. *JAMA*. 2009; 302(8): 849-857.

61 Simon, T., Verstuyft, C., Mary-Krause, M., Quteineh, L., Drouet, E., Meneveau, N., Steg, P.G., Ferrieres, J., Danchin, N. and Becquemont, L. Genetic determinants of response to clopidogrel and cardiovascular events. *N Engl J Med*. 2009; 360(4): 363-375.

62 Jose, R., Chandrasekaran, A., Sam, S.S., Gerard, N., Chanolean, S., Abraham, B.K., Satyanarayanamoorthy, K., Peter, A. and Rajagopal, K. CYP2C9 and CYP2C19 genetic polymorphisms: frequencies in the south Indian population. *Fundam Clin Pharmacol*. 2005; 19(1): 101-105.

63 Schroth, W., Antoniadou, L., Fritz, P., Schwab, M., Muerdter, T., Zanger, U.M., Simon, W., Eichelbaum, M. and Brauch, H. Breast cancer treatment outcome with adjuvant tamoxifen relative to patient CYP2D6 and CYP2C19 genotypes. *J Clin Oncol*. 2007; 25(33): 5187-5193.

64 Okishiro, M., Taguchi, T., Jin Kim, S., Shimazu, K., Tamaki, Y. and Noguchi, S. Genetic polymorphisms of CYP2D6 10 and CYP2C19 2, 3 are not associated with prognosis, endometrial thickness, or bone mineral density in Japanese breast cancer patients treated with adjuvant tamoxifen. *Cancer*. 2009; 115(5): 952-961.

65 Brauch, H., Murdter, T.E., Eichelbaum, M. and Schwab, M. Pharmacogenomics of tamoxifen therapy. *Clin Chem*. 2009; 55(10): 1770-1782.

66 Nowell, S., Sweeney, C., Winters, M., Stone, A., Lang, N.P., Hutchins, L.F., Kadlubar, F.F. and Ambrosone, C.B. Association between sulfotransferase 1A1 genotype and survival of breast cancer patients receiving tamoxifen therapy. *J Natl Cancer Inst*. 2002; 94(21): 1635-1640.

67 Mouridsen, H., Gershanovich, M., Sun, Y., Perez-Carrion, R., Boni, C., Monnier, A., Apffelstaedt, J., Smith, R., Sleeboom, H.P., Janicke, F. et al. Superior efficacy of letrozole versus tamoxifen as first-line therapy for postmenopausal women with advanced breast cancer: results of a phase III

study of the International Letrozole Breast Cancer Group. *J Clin Oncol. 2001*; 19(10): 2596-2606.

68 Mouridsen, H., Gershanovich, M., Sun, Y., Perez-Carrion, R., Boni, C., Monnier, A., Apffelstaedt, J., Smith, R., Sleeboom, H.P., Jaenicke, F. et al. Phase III study of letrozole versus tamoxifen as first-line therapy of advanced breast cancer in postmenopausal women: analysis of survival and update of efficacy from the International Letrozole Breast Cancer Group. *J Clin Oncol*. 2003; 21(11): 2101-2109.

69 Bonneterre, J., Buzdar, A., Nabholtz, J.M., Robertson, J.F., Thurlimann, B., von Euler, M., Sahmoud, T., Webster, A. and Steinberg, M. Anastrozole is superior to tamoxifen as first-line therapy in hormone receptor positive advanced breast carcinoma. *Cancer*. 2001; 92(9): 2247-2258.

70 Buck, M.B., Coller, J.K., Murdter, T.E., Eichelbaum, M. and Knabbe, C. TGFbeta2 and TbetaRII are valid molecular biomarkers for the antiproliferative effects of tamoxifen and tamoxifen metabolites in breast cancer cells. *Breast Cancer Res Treat*. 2008; 107(1): 15-24.

71 Schroth, W., Goetz, M.P., Hamann, U., Fasching, P.A., Schmidt, M., Winter, S., Fritz, P., Simon, W., Suman, V.J., Ames, M.M. et al. Association between CYP2D6 polymorphisms and outcomes among women with early stage breast cancer treated with tamoxifen. *JAMA*. 2009; 302(13): 1429-1436.

72 Serrano, D., Lazzeroni, M., Zambon, C.F., Macis, D., Maisonneuve, P., Johansson, H., Guerrieri-Gonzaga, A., Plebani, M., Basso, D., Gjerde, J. et al. Efficacy of tamoxifen based on cytochrome P450 2D6, CYP2C19 and SULT1A1 genotype in the Italian Tamoxifen Prevention Trial. *Pharmacogenomics J*. 2011; 11(2): 100-107.

73 Abraham, J.E., Maranian, M.J., Driver, K.E., Platte, R., Kalmyrzaev, B., Baynes, C., Luccarini, C., Shah, M., Ingle, S., Greenberg, D. et al. CYP2D6 gene variants: association with breast cancer specific survival in a cohort of breast cancer patients from the United Kingdom treated with adjuvant tamoxifen. *Breast Cancer Res*. 2010; 12(4): R64.

74 Madlensky, L., Natarajan, L., Tchu, S., Pu, M., Mortimer, J., Flatt, S.W., Nikoloff, D.M., Hillman, G., Fontecha, M.R., Lawrence, H.J. et al.

Tamoxifen metabolite concentrations, CYP2D6 genotype, and breast cancer outcomes. *Clin Pharmacol Ther*. 2011; 89(5): 718-725.

75 Kiyotani, K., Mushiroda, T., Imamura, C.K., Hosono, N., Tsunoda, T., Kubo, M., Tanigawara, Y., Flockhart, D.A., Desta, Z., Skaar, T.C. et al. Significant effect of polymorphisms in CYP2D6 and ABCC2 on clinical outcomes of adjuvant tamoxifen therapy for breast cancer patients. *J Clin Oncol*. 2010; 28(8): 1287-1293.

76 Xu, Y., Sun, Y., Yao, L., Shi, L., Wu, Y., Ouyang, T., Li, J., Wang, T., Fan, Z., Fan, T. et al. Association between CYP2D6 *10 genotype and survival of breast cancer patients receiving tamoxifen treatment. *Ann Oncol*. 2008; 19(8): 1423-1429.

77 Lim, J.S.L., Chen, X.A., Singh, O., Yap, Y.S., Ng, R.C.H., Wong, N.S., Wong, M., Lee, E.J.D. and Chowbay, B. Impact of CYP2D6, CYP3A5, CYP2C9 and CYP2C19 polymorphisms on tamoxifen pharmacokinetics in Asian breast cancer patients. *British Journal of Clinical Pharmacology*. 2011; 71(5): 737-750.

78 Okishiro, M., Taguchi, T., Jin Kim, S., Shimazu, K., Tamaki, Y. and Noguchi, S. Genetic polymorphisms of CYP2D6*10 and CYP2C19*2,*3 are not associated with prognosis, endometrial thickness, or bone mineral density in Japanese breast cancer patients treated with adjuvant tamoxifen. *Cancer*. 2009; 115(5): 952-961.

79 Wegman, P., Elingarami, S., Carstensen, J., Stal, O., Nordenskjold, B. and Wingren, S. Genetic variants of CYP3A5, CYP2D6, SULT1A1, UGT2B15 and tamoxifen response in postmenopausal patients with breast cancer. *Breast Cancer Res*. 2007; 9(1): R7.

80 Toyama, T., Yamashita, H., Sugiura, H., Kondo, N., Iwase, H. and Fujii, Y. No association between CYP2D6*10 genotype and survival of node-negative Japanese breast cancer patients receiving adjuvant tamoxifen treatment. *Jpn J Clin Oncol*. 2009; 39(10): 651-656.

81 Criscitiello, C., Fumagalli, D., Saini, K.S. and Loi, S. Tamoxifen in early-stage estrogen receptor-positive breast cancer: overview of clinical use and

molecular biomarkers for patient selection. *Onco Targets Ther*. 2011; 41-11.

82 Yu, K.D., Huang, A.J. and Shao, Z.M. Tailoring adjuvant endocrine therapy for postmenopausal breast cancer: a CYP2D6 multiple-genotype-based modeling analysis and validation. *PLoS One*. 2010; 5(12): e15649.

83 Fox, E.M., Davis, R.J. and Shupnik, M.A. ERβ in breast cancer—Onlooker, passive player, or active protector?. *Steroids*. 2008; 73(11): 1039-1051.

84 McGuire, W.L. Endocrine therapy of breast cancer. *Annu Rev Med*. 1975; 26353-363.

85 Colditz, G.A. Relationship between estrogen levels, use of hormone replacement therapy, and breast cancer. *J Natl Cancer Inst*. 1998; 90(11): 814-823.

86 Tilghman, S.L., Nierth-Simpson, E.N., Wallace, R., Burow, M.E. and McLachlan, J.A. Environmental hormones: Multiple pathways for response may lead to multiple disease outcomes. *Steroids*. 2010; 75(8-9): 520-523.

87 Nilsson, S., Makela, S., Treuter, E., Tujague, M., Thomsen, J., Andersson, G., Enmark, E., Pettersson, K., Warner, M. and Gustafsson, J.A. Mechanisms of estrogen action. *Physiol Rev*. 2001; 81(4): 1535-1565.

88 Osborne, C.K. Tamoxifen in the treatment of breast cancer. *N Engl J Med*. 1998; 339(22): 1609-1618.

89 Herynk, M.H. and Fuqua, S.A. Estrogen receptor mutations in human disease. *Endocr Rev*. 2004; 25(6): 869-898.

90 Clarke, R., Liu, M.C., Bouker, K.B., Gu, Z., Lee, R.Y., Zhu, Y., Skaar, T.C., Gomez, B., O'Brien, K., Wang, Y. et al. Antiestrogen resistance in breast cancer and the role of estrogen receptor signaling. *Oncogene*. 2003; 22(47): 7316-7339.

91 Arpino, G., Weiss, H., Lee, A.V., Schiff, R., De Placido, S., Osborne, C.K. and Elledge, R.M. Estrogen receptor-positive, progesterone receptor-negative breast cancer: association with growth factor receptor expression and tamoxifen resistance. *J Natl Cancer Inst*. 2005; 97(17): 1254-1261.

92 Shupnik, M.A. Crosstalk between steroid receptors and the c-Src-receptor tyrosine kinase pathways: implications for cell proliferation. *Oncogene*. 2004; 23(48): 7979-7989.

93 Osborne, C.K., Shou, J., Massarweh, S. and Schiff, R. Crosstalk between estrogen receptor and growth factor receptor pathways as a cause for endocrine therapy resistance in breast cancer. *Clin Cancer Res*. 2005; 11(2 Pt 2): 865s-870s.

94 Bogush, T.A., Dudko, E.A., Beme, A.A., Bogush, E.A., Polotskii, B.E., Tiuliandin, S.A. and Davydov, M.I. [Estrogen receptor expression in tumors different from breast cancer]. *Antibiot Khimioter*. 2009; 54(7-8): 41-49.

95 Gruvberger-Saal, S.K., Bendahl, P.O., Saal, L.H., Laakso, M., Hegardt, C., Eden, P., Peterson, C., Malmstrom, P., Isola, J., Borg, A. et al. Estrogen receptor beta expression is associated with tamoxifen response in ERalpha-negative breast carcinoma. *Clin Cancer Res*. 2007; 13(7): 1987-1994.

96 Wu, L., Williams, P.M. and Koch, W.H. Clinical applications of microarray-based diagnostic tests. *Biotechniques*. 2005; 39(4): 577-582.

97 Li, X., Quigg, R.J., Zhou, J., Gu, W., Nagesh Rao, P. and Reed, E.F. Clinical utility of microarrays: current status, existing challenges and future outlook. *Curr Genomics*. 2008; 9(7): 466-474.

98 Patel, A.C. Basic science for the practicing physician: gene expression microarrays. *Ann Allergy Asthma Immunol*. 2008; 101(3): 325-332.

99 Wu, L., Williams, P.M. and Koch, W. Clinical applications of microarray-based diagnostic tests. Biotechniques. 2005; 39(10 Suppl): S577-582.

100 de Leon, J., Susce, M.T. and Murray-Carmichael, E. The AmpliChip CYP450 genotyping test: Integrating a new clinical tool. *Mol Diagn Ther*. 2006; 10(3): 135-151.

101 Wedlund, P.J. The CYP2C19 enzyme polymorphism. *Pharmacology*. 2000; 61(3): 174-183.

102 Phillips, K.A. and Van Bebber, S.L. Measuring the value of pharmacogenomics. *Nat Rev Drug Discov*. 2005; 4(6): 500-509.

103 Stoughton, R.B. Applications of DNA microarrays in biology. *Annu Rev Biochem*. 2005; 7453-82.

104 Schena, M., Heller, R.A., Theriault, T.P., Konrad, K., Lachenmeier, E. and Davis, R.W. Microarrays: biotechnology's discovery platform for functional genomics. *Trends Biotechnol*. 1998; 16(7): 301-306.

105 Menoyo, A., del Rio, E. and Baiget, M. Characterization of variant alleles of cytochrome CYP2D6 in a Spanish population. *Cell Biochem Funct*. 2006; 24(5): 381-385.

106 Wang, S.L., Huang, J.D., Lai, M.D., Liu, B.H. and Lai, M.L. Molecular basis of genetic variation in debrisoquin hydroxylation in Chinese subjects: polymorphism in RFLP and DNA sequence of CYP2D6. *Clin Pharmacol Ther*. 1993; 53(4): 410-418.

107 Ikenaga, Y., Fukuda, T., Fukuda, K., Nishida, Y., Naohara, M., Maune, H. and Azuma, J. The frequency of candidate alleles for CYP2D6 genotyping in the Japanese population with an additional respect to the -1584C to G substitution. *Drug Metab Pharmacokinet*. 2005; 20(2): 113-116.

108 Bradford, L.D. CYP2D6 allele frequency in European Caucasians, Asians, Africans and their descendants. *Pharmacogenomics*. 2002; 3(2): 229-243.

109 Luo, H.R., Poland, R.E., Lin, K.M. and Wan, Y.J. Genetic polymorphism of cytochrome P450 2C19 in Mexican Americans: a cross-ethnic comparative study. *Clin Pharmacol Ther*. 2006; 80(1): 33-40.

110 Goetz, M.P., Knox, S.K., Suman, V.J., Rae, J.M., Safgren, S.L., Ames, M.M., Visscher, D.W., Reynolds, C., Couch, F.J., Lingle, W.L. et al. The impact of cytochrome P450 2D6 metabolism in women receiving adjuvant tamoxifen. *Breast Cancer Res Treat*. 2007; 101(1): 113-121.

111 Teh, L., Mohamed, N., Salleh, M., Rohaizak, M., Shahrun, N., Saladina, J., Shia, J., Roslan, H., Sood, S., Rajoo, T. et al. The Risk of Recurrence in Breast Cancer Patients Treated with Tamoxifen: polymorphisms of CYP2D6 and ABCB1. *AAPS J*. 2012 ;14(1):52-9.

112 Lim, H.S., Ju Lee, H., Seok Lee, K., Sook Lee, E., Jang, I.J. and Ro, J. Clinical implications of CYP2D6 genotypes predictive of tamoxifen pharmacokinetics in metastatic breast cancer. *J Clin Oncol*. 2007; 25(25): 3837-3845.

# APPENDIX

**Suppliers**

- MicroAmp (0.2 mL) Reaction Tubes, Caps, Tray/Retainers or MicroAmp Reaction Tubes/Tray/Retainer Assembly and Base
- Tough-Spots labels, small (USA Scientific, Inc., Ocala, FL)
- 2.0 mL screw cap tubes: Sarstedt
- 1.5 mL microfuge tubes: VWR
- Sterile polypropylene conical tubes; 15 mL
- 500 mL square media bottles: Nalgene
- Adjustable Pipettors
- Dry Heat Block set at 95 $^{o}$C
- Plastic resealable bag
- Sterile disposable serological pipettes (5 and 10 mL)
- Graduated vessels
- Vortex mixer
- Tube racks
- Disposable gloves, powderless
- Ice water bath
- Aluminum foil

Solution for Electrophoresis and ethidium bromide staining

1. 10X TBE (stock solution)

Makes 1 L. Store at room temperature indefinitely.

- 1 g of NaOH
- 108 g of Tris base (m.w. 121.10)
- 55 g of boric acid (m.w. 61.83)
- 9.5 g of ethylene diamine tetraacetic acid (EDTA, disodium salt, m.w. 372.24)

Add all dry ingredients to 700 mL of deionized or distilled water in a 2 L flask. Sterile to dissolve preferably is using a magnetic stirrer until the dry was dissolved and added distilled water to bring the total solution to 1L.

Working solution (1X TBE) was prepared by adding 100 ml of 10X TBE with 900 ml of DW.

2. 50 mg/ml Ethidium bromide 100 ml

1. Ethidium bromide 0.5 g

2. Distilled water 100 ml

Dissolved ethidium bromide with distill water 100 ml (5 mg/ml) and sterile on magnetic sterer until the dry was dissolved. Store in the dark bottle and keep at room temperature.

# BIOGRAPHY


| | |
|---|---|
| **NAME** | Mr.Montri Chamnanphon |
| **DATE OF BIRTH** | 19 Sep 1979 |
| **PLACE OF BIRTH** | Bangkok, Thailand |
| **INSTITUTIONS ATTENDED** | Khonkaen University, 1999–2002:<br>Bachelor of Science<br>(Medical Technology)<br>Mahidol University, 2010–2012:<br>Master of Science<br>(Clinical Pathology) |
| **RESEARCH GRANT** | Supported in part by the Thesis Grant,<br>Thailand Center of Excellent Life Science<br>(TCELS). |
| **HOME ADDRESS** | 112/11 Nongpai District,<br>Amphur Kaengkhro, Chaiyaphum Province<br>Thailand 36150<br>E-mail: montyne_ch@hotmail.com |