CASSIO CARDOSO FILHO

ASSOCIAÇÃO DOS POLIMORFISMOS *A4889G* E *T6235C* DO GENE *CYP1A1* COM CARACTERÍSTICAS CLÍNICAS E EPIDEMIOLÓGICAS DO CÂNCER DE MAMA

ASSOCIATION OF CYP1A1 GENE POLYMORPHISMS (A4889G AND T6235C) WITH CLINICAL AND EPIDEMIOLOGICAL FEATURES OF BREAST CANCER

CAMPINAS
2012

UNIVERSIDADE ESTADUAL DE CAMPINAS
Faculdade de Ciências Médicas

CASSIO CARDOSO FILHO

ASSOCIAÇÃO DOS POLIMORFISMOS *A4889G* E *T6235C* DO GENE *CYP1A1* COM CARACTERÍSTICAS CLÍNICAS E EPIDEMIOLÓGICAS DO CÂNCER DE MAMA

ORIENTADOR: Prof. Dr. LUIS OTAVIO ZANATTA SARIAN
COORIENTADORAS: Profª. Drª. MARIA SALETE COSTA GURGEL
Profª. Drª. CARMEN SILVIA PASSOS LIMA

ASSOCIATION OF CYP1A1 GENE POLYMORPHISMS (A4889G AND T6235C) WITH CLINICAL AND EPIDEMIOLOGICAL FEATURES OF BREAST CANCER

Tese de Doutorado apresentada ao Programa de Pós-Graduação da Faculdade de Ciências Médicas da Universidade Estadual de Campinas para obtenção do Título de Doutor em Ciências Médicas.

Doctorate Thesis presented to the Medical Sciences Postgraduation Programme of the School of Medical Sciences of the University of Campinas to obtain the Ph.D grade in Medical Sciences.

ESTE EXEMPLAR CORRESPONDE À VERSÃO FINAL DA TESE DEFENDIDA PELO ALUNO CASSIO CARDOSO FILHO E ORIENTADA PELO Prof. Dr. LUIS OTAVIO ZANATTA SARIAN

Assinatura do Orientador

_____

Campinas, 2012

FICHA CATALOGRÁFICA ELABORADA POR
MARISTELLA SOARES DOS SANTOS – CRB8/8402
BIBLIOTECA DA FACULDADE DE CIÊNCIAS MÉDICAS
UNICAMP

C179a

Cardoso Filho, Cassio, 1974-
Associação dos polimorfismos A4889G e T6235C do gene CYP1A1 com características clínicas e epidemiológicas do câncer de mama / Cassio Cardoso Filho. – Campinas, SP : [s.n.], 2012.

Orientador : Luis Otavio Zanatta Sarian.
Coorientador : Maria Salete Costa Gurgel.
Coorientador : Carmem Silvia Passos Lima.
Tese (Doutorado) - Universidade Estadual de Campinas, Faculdade de Ciências Médicas.

1. Neoplasias da mama. 2. Polimorfismo. 3. Citocromo P-450 CYP1A1. 4. Sobrevida. 5. Tamoxifeno. I. Sarian, Luis Otávio Zanatta, 1974-. II. Costa-Gurgel, Maria Salete, 1956-. III. Lima, Carmem Silvia Passos. IV. Universidade Estadual de Campinas. Faculdade de Ciências Médicas. V. Título.

Informações para Biblioteca Digital

Título em inglês: Association of CYP1A1 gene polymorphisms (A4889G and T6235C) with clinical and epidemiological features of breast cancer.

Palavras-chave em inglês:
Breast neoplasms
Polymorphism
Cytochrome P-450 CYP1A1
Survival
Tamoxifen

Área de concentração: Oncologia Ginecológica e Mamária

Titulação: Doutor em Ciências Médicas

Banca examinadora:
Luis Otavio Zanatta Sarian [Orientador]
Aarão Mendes Pinto Neto
Luiz Carlos Zeferino
Afonso Celso Pinto Nazário
Gil Facina

Data da defesa: 29-11-2012

Programa de Pós-Graduação: Tocoginecologia

Diagramação e arte-final: Assessoria Técnica do CAISM (ASTEC)

BANCA EXAMINADORA DA TESE DE DOUTORADO

Aluno: CASSIO CARDOSO FILHO

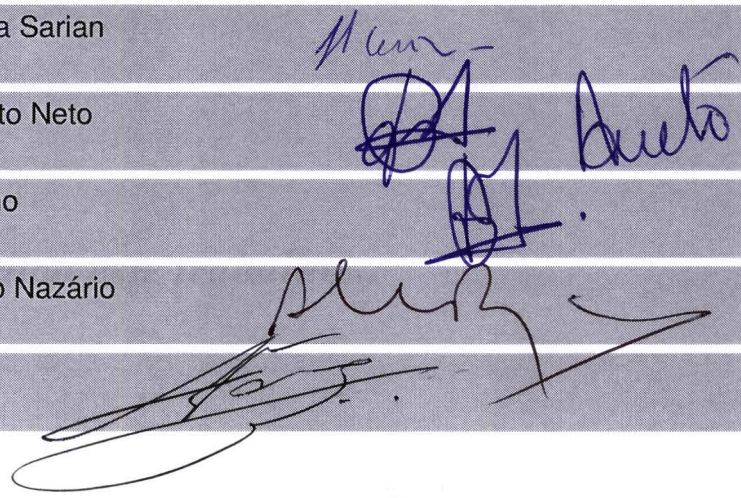
Orientador: Prof. Dr. LUIS OTAVIO ZANATTA SARIAN

Coorientadoras: Profª. Drª. MARIA SALETE COSTA GURGEL

Profª. Drª. CARMEN SILVIA PASSOS LIMA

Membros:

1. Luis Otavio Zanatta Sarian
2. Aarão Mendes Pinto Neto
3. Luiz Carlos Zeferino
4. Afonso Celso Pinto Nazário
5. Gil Facina



Curso de Pós-Graduação em Tocoginecologia da Faculdade de Ciências Médicas da Universidade Estadual de Campinas

Data: 29/11/2012

Dedico este trabalho...

A todas as mulheres que sofrem as mazelas do câncer mamário,
e a todos aqueles que buscam minorar as
dores e consequências causadas por esta doença.

Agradecimentos

A Deus, Uno e Trino, pelo AMOR incondicional por todos nós.

A minha amada esposa Adriana, parte de uma insondável tríplice aliança com Deus.

Aos meus pais, Cassio e Hilda, pelo dom da vida; aos meus irmãos, Rodrigo e Leandro, e demais parentes e amigos, pela companhia nesta estrada.

Aos professores e amigos de todas as minhas etapas de formação pelo exemplo de ensinar e aprender, em especial à Profª Drª Maria Salete Costa Gurgel e ao Prof. Dr. Luís Otávio Zanatta Sarian, pela atenção e paciência dispensadas em cada preciosa orientação. Estendo este agradecimento ao Prof. Dr. Luiz Carlos Zeferino e à Profª Drª Cassia Raquel Teatin Juliato, pelas valiosas sugestões por ocasião do exame de qualificação.

A todos os colegas de trabalho e de pesquisa do Laboratório de Genética do Câncer (LAGECA): Prof. Dr. Gustavo Jacob Lourenço, Dr. Leonardo Bossi, à bióloga Camila Borges Martins de Oliveira e, em especial, à também coorientadora Profª Drª Carmen Silvia Passos Lima, coletores de frutos de esperança.

A toda a equipe multiprofissional do CAISM – UNICAMP, pelo carinho na assistência às nossas pacientes, pela disciplina nas pesquisas e pela persistência no ensino de tantos outros profissionais que, assim como eu, têm escrito parte de sua história nesta instituição - e que carregarão, para sempre, marcas desta passagem.

A todos, agradeço imensamente pela compreensão comigo nos momentos em que me dediquei com a devida intensidade a cada etapa deste longo processo que, agora palpável, traduz a busca pelos meus rumos profissionais, a que chamo de "vocação".

Este estudo foi financiado:

Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), financiamento parcial através do auxílio-pesquisa – FAPESP 2004/06319-0: “Influência dos polimorfismos gênicos na susceptibilidade a tumores sólidos”.

Fundação de Apoio ao Ensino, Pesquisa e Extensão (FAEPEX) da Universidade Estadual de Campinas, financiamento parcial através do auxílio-pesquisa 101/05.

"Bendize a Deus em todo o tempo,
e pede-Lhe que dirija os teus passos,
de modo que os teus planos
estejam sempre de acordo
com a Sua vontade!"

Livro de Tobias, Capítulo 4 - versículo 20

Sumário

Símbolos, Siglas e Abreviaturas

A4889G – Polimorfismo M2 do gene *CYP1A1*

bp/pb – Pares de bases nitrogenadas

CYP – Sistema do citocromo P-450

DNA – Ácido desoxirribonucleico (*Deoxyribonucleic acid)*

et al. – E outro(s); e outra(s)

IC/CI – Intervalo de Confiança (*Confidence interval)*

LAGECA – Laboratório de Genética do Câncer

NA – Não se aplica

OR – *Odds ratio*

PCR – Reação em Cadeia da Polimerase (*Polymerase Chain Reaction)*

SNP – *Single nucleotide polymorphisms*

T6235C – Polimorfismo M1 do gene *CYP1A1*

UNICAMP – Universidade Estadual de Campinas

Resumo

Introdução: A terapêutica sistêmica para o câncer de mama envolve o uso do agente antiestrogênico tamoxifeno, fármaco metabolizado pelo fígado no sistema do citocromo P-450 (CYP). Este, por sua vez, é parcialmente codificado pelo gene *CYP1A1*, e alguns polimorfismos deste gene têm sido associados com interferências na sua eficácia metabólica. Além disso, diferenças interindividuais no CYP explicam parte das variações na resistência ao tamoxifeno e metabolismo dos estrogênios. Dentre esses polimorfismos, o *A4889G* (M2) *e* o *T6235C* (M1) são conhecidos por afetar a ativação da estrona e do estradiol, e por provocar a redução da concentração de metabólitos altamente ativos do tamoxifeno, reduzindo teoricamente o efeito antiestrogênico desta modalidade de hormonioterapia no tecido mamário. Embora plausíveis do ponto de vista biológico, as implicações clínicas dos polimorfismos do *CYP1A1*, ou seja, as características patológicas dos tumores e um pior prognóstico decorrente do aumento dos estrógenos circulantes e redução dos metabólitos ativos do tamoxifeno, não foram ainda avaliadas. **Objetivo:** Avaliar a associação entre os polimorfismos M1 e M2 do gene *CYP1A1* e as características patológicas e clínicas de mulheres com câncer de mama esporádico, em duas abordagens: 1) determinar as associações entre

estes polimorfismos e as características patológicas, clínicas e o padrão de sobrevida global em mulheres com câncer de mama esporádico e 2) determinar as associações entre estes polimorfismos e as caracteríasticas patológicas e o comportamento clínico de tumores de mama com receptores hormonais positivos na vigência do uso de tamoxifeno. **Métodos:** foram incluídas 741 mulheres com câncer de mama esporádico, 405 das quais com tumores positivos para receptores esteroides e que usaram tamoxifeno como terapia antiestrogênica primária, para as quais os dados referentes a cinco anos de seguimento estavam disponíveis. Foram avaliadas as associações de informações-chave patológicas e clínicas, incluindo a sobrevida geral em cinco anos, com as diferentes combinações de polimorfismos do gene *CYP1A1*. **Resultados:** Em mulheres portadoras de ambos os polimorfismos M1 e M2 do *CYP1A1*, a proporção de tumores grau histológico III (80,3%) foi significativamente menor que nas não-portadoras (89,6%); p ajustado <0,01. O mesmo ocorreu na análise restrita às mulheres com tumores RE+ usando tamoxifeno (76,1% vs. 85,9%; p ajustado= 0,02). Após 60 meses de seguimento, cerca de 75% das mulheres estavam vivas. Não houve diferença significativa na sobrevivência relacionada com o estado do gene *CYP1A1*. **Conclusões:** embora associados a tumores de menor grau histológico, não há nenhuma evidência da associação dos polimorfismos do *CYP1A1* com prognóstico do câncer da mama.

Palavras-chave: câncer, mama, polimorfismo, *CYP1A1*, sobrevida, tamoxifeno.

Summary

Introduction: systemic therapy for breast cancer involves the use of the anti-estrogen agent tamoxifen, which is metabolized by the liver cytochrome P-450 (CYP). This, in turn, is partially encoded by *CYP1A1*, and some polymorphisms of this gene have been associated with metabolic disturbance at their effectiveness. Moreover, interindividual differences in efficiency of CYP explain part of the variations in resistance to tamoxifen and estrogen metabolism. Among these polymorphisms, the *A4889G* (M2) and *T6235C* (M1) are known to affect the activation of estrone and estradiol, and cause the reduction of the concentration of highly active metabolites of tamoxifen, theoretically reducing the anti-estrogenic effect of this form of endocrine therapy in breast tissue. Although plausible from the biological point of view, the clinical implications of polymorphisms of *CYP1A1*, ie, the pathologic features of tumors and a worse prognosis due to increased circulating estrogens and reduction of active metabolites of tamoxifen have not yet been evaluated. **Objectives:** To evaluate the association between *CYP1A1 A4889G and T6235C* gene polymorphisms and clinical and pathological characteristics of women with sporadic breast cancer in two approaches: 1) determine the associations between *CYP1A1 A4889G and T6235C* gene polymorphisms and

the pathological characteristics of the tumors, and the clinical features, including overall survival, of women with sporadic breast cancer and 2) determine the associations between *CYP1A1 A4889G and T6235C* gene polymorphisms and the pathological characteristics and clinical behavior of estrogen receptor-positive breast tumors in patients using tamoxifen. **Methods:** We included 741 women with sporadic breast cancer, 405 of whom had tumors positive for steroid receptors and using tamoxifen as primary antiestrogen therapy, for which data on five years of follow-up were available. We evaluated the associations of key pathological and clinical features, including overall survival at five years, with different combinations of the *CYP1A1* gene polymorphisms. **Results:** In women with both polymorphisms of the *CYP1A1* gene, the proportion of grade III tumors (80.3%) was significantly lower than in non-carriers (89.6%), adjusted $p < 0.01$. The same was true for women with ER + tumors using tamoxifen (76.1% vs. 85.9%; adjusted $p = 0.02$). After 60 months of follow up, 75% of the women were alive. There was no significant difference in survival related to the state of the *CYP1A1* gene. **Conclusions:** Although associated with tumors of lower grade, there is no evidence of an association of *CYP1A1* polymorphisms with breast cancer prognosis.

Keywords: cancer, breast, polymorphism, *CYP1A1*, survival, tamoxifen.

1. Introdução

Dentre todas as neoplasias que acometem as mulheres, o câncer de mama tem-se apresentado mundialmente como o segundo mais frequente, com elevação de suas taxas de incidência (variação relativa de 80% nos últimos 30 anos), e é a principal causa de morte por neoplasia entre as mulheres brasileiras. Nos Estados Unidos da América são esperados, para 2012, 226.870 casos novos de câncer de mama, com 39.510 óbitos decorrentes desta neoplasia (1). O número de casos novos de câncer de mama esperados para o Brasil em 2012 será de 52.680 (52 para cada grupo de 100.000 mulheres), uma mortalidade estimada de 12.852, com aproximadamente 60% da casuística dos registros hospitalares composta por casos diagnosticados em estágios avançados (2).

Alguns aspectos genéticos, epidemiológicos e ambientais têm sido aventados para justificar o aumento gradual, porém constante, na incidência do câncer de mama nos últimos 50 anos. Destes, ressaltam-se o envelhecimento da população, decorrente de ações sociais de saúde que evitam mortes prematuras (3), e o estabelecimento de novos padrões reprodutivos e hormonais nas

últimas décadas, com aumento do número de ciclos ovulatórios vivenciados pelas mulheres durante a sua vida (3,4,5,6).

Nesta linha, tornam-se explicáveis as associações epidemiológicas observadas para o aumento do risco da neoplasia em relação à: menarca precoce (antes dos 12 anos), menopausa tardia (após os 54 anos), idade tardia da primeira gravidez a termo (após os 30 anos de idade) e não-lactação (7). Além destes, está estabelecido o aumento de risco com o uso de terapia hormonal (TH) por mais de cinco anos consecutivos, mormente com os esquemas que utilizam estrogênios conjugados associados a progestagênios sintéticos de uso contínuo (8,9).

Dieta rica em gordura, ganho de peso na pós-menopausa e obesidade também estão elencados como fatores de risco para o câncer de mama (7). O consumo de álcool acima de duas doses ao dia parece ampliar o risco, possivelmente pelo aumento de precursores de carcinógenos por vias ainda não completamente determinadas (10,11).

Quanto ao hábito de fumar, os dados são controversos, uma vez que não existem mecanismos biológicos que expliquem possíveis associações, além da disparidade dos estudos, quanto à quantificação do número de cigarros consumidos, população, tempo de uso, fumo ativo ou passivo, ou outros fatores de risco associados (12, 13,14).

Em relação ao componente racial, parece haver uma associação entre o câncer de mama em mulheres não-brancas na população estadunidense

ajustada por idade, com risco relativo de 1,3 para as não-brancas (15); mas as dificuldades e os desafios na obtenção de informações precisas sobre dados étnicos e possível miscigenação permanecem na literatura (16), bem como outros fatores confundidores associados à raça (17).

Dentre os aspectos genéticos, 10% a 15% dos casos de câncer de mama têm história familiar positiva para a doença, sendo que apenas 5% podem ser explicados pelas raras (mas de grande penetrância genética) mutações dos genes BRCA1 e BRCA2 (18,19,20). Isso leva a crer que há outras variações genéticas comuns, de baixa penetrância, que influenciam a predisposição ao câncer de mama.

Recentes estudos na literatura têm aumentado as evidências de que polimorfismos genéticos de baixa penetrância podem aumentar o risco do câncer de mama, apesar do impacto em termos de prevenção ainda ser desconhecido (21). Outros estudos ressaltam, ainda, a importância do estabelecimento das "assinaturas gênicas" dos tumores de mama na particularização do prognóstico e dos tratamentos dispensados a cada subgrupo de pacientes, por exemplo, usando kits comerciais, como o Oncotype-Dx® (Genomic Health, Redwood City, CA, USA), que analisa 21 expressões gênicas (22, 23,24,25).

Há dois grupos principais de genes potenciais candidatos aos papéis relatados acima (26): aqueles que codificam proteínas envolvidas no metabolismo dos hormônios esteroides (*CYP17, CYP19*) e outros relacionados à expressão de enzimas envolvidas no processo de carcinogênese (*CYP1A1, CYP2D6, CYP2E1, GSTM1, GSTT1, NAT1, NAT2*). Há inclusive evidências na literatura relacionando dois ou mais grupos de genes, com as potenciais interações entre suas ações (27).

Cumpre ressaltar a possível interação entre fatores ambientais epidemiológicos e genéticos, potencializando o risco do desenvolvimento do câncer de mama: fatores de risco relacionados à maior exposição estrogênica ou tabagismo, e os polimorfismos de baixa penetrância (28).

1.1. Fatores Prognósticos

A fim de padronizar o acompanhamento dos casos de câncer de mama, no que tange ao diagnóstico, aplicabilidade de técnicas terapêuticas e prognóstico, utiliza-se o sistema de estadiamento do *American Joint Committee on Cancer* (AJCC) (29). Este sistema considera a extensão do acometimento do tumor na mama e em tecidos adjacentes, como a pele e a parede torácica (T), a identificação da presença de metástases para linfonodos regionais como cadeias axilares, mamária interna, infraclavicular e fossa supraclavicular (N) e a identificação da presença de metástases a distância (M). Nove faixas denominadas estádios podem ser determinadas (estádios 0, Ia, Ib, IIa, IIb, IIIa, IIIb, IIIc e IV), com as probabilidades de sobrevida em cinco anos decrescentes de 92% a menos de 13%. Com base em registros hospitalares brasileiros, aproximadamente 60% das mulheres têm o câncer de mama diagnosticado em fase avançada – estádios III e IV (2) – onde a sobrevida em cinco anos varia de 13% a 40%, a despeito dos avanços terapêuticos.

A progressão do câncer de mama primário varia independentemente do poder preditivo do estadiamento. Parte dessas variações é explicada por fatores prognósticos, que conferem diferenças nas taxas de crescimento do tumor,

poder de invasão, potencial metastático e outros mecanismos ainda não completamente conhecidos (30).

Podem ser divididos em fatores anatomopatológicos e biológicos. Dentre os anatomopatológicos há o tipo histológico (31), grau de diferenciação tumoral (32;33,34), estado linfonodal axilar (35;36), tamanho tumoral (37) e angiogênese peritumoral, relacionada diretamente com pior prognóstico nas mulheres com câncer de mama (38). Quanto aos fatores prognósticos biológicos, mais estudados nos últimos 20 anos, estes adquirem maior expressão nas mulheres sem acometimento linfonodal axilar, onde exercem papel selecionador de mulheres de maior risco para recidiva (39). São eles: expressão de receptores hormonais de estrogênio e progesterona, índice de atividade proliferativa (índice de marcação, citofluorometria na fase S, Ki 67), ploidia ou índice de DNA, receptores para fatores de crescimento (EGF-R, IGF-IR, SS-R receptor de somatostatina, TGF), oncogenes (*Her-2-Neu, p53, BCL2*), catepsina D, e outros (39, 40; 41, 42;43;44). É necessário maior conhecimento sobre a biologia molecular do tumor para tentar identificar quais os fatores isolados, ou em associação com os já bem estabelecidos, que poderiam predizer a evolução da doença.

1.2. Polimorfismos A4889G e T6235C do gene CYP1A1

O gene *CYP1A1* está localizado no braço longo do cromossomo 15. O polimorfismo A4889G do gene *CYP1A1* determina a troca de uma adenina por uma guanina na posição 4889 do éxon 7 do gene e a substituição de uma isoleucina por uma valina na proteína resultantemente codificada. O alelo

variante codifica uma proteína com maior atividade na ativação de estrona e estradiol do que o alelo selvagem (45).

O genótipo variante em homozigose, GG, foi identificado em cerca de 4% a 40% dos indivíduos de diferentes populações étnicas (46;47; 48;49, 50;51; 52; 53; 54;55, 56,57). É incerto seu papel no risco de ocorrência do câncer de mama em mulheres (49; 52; 55; 56, 57).

Um outro polimorfismo, o *T6235C*, determina a troca de uma base nitrogenada timina por uma citosina na região 6235 da cadeia de DNA do gene CYP1A1, em região não codificadora do gene. Assim como o polimorfismo *A4889G*, o alelo variante também codifica uma proteína mais eficaz na ativação de estrona e estradiol do que o alelo selvagem (58).

O genótipo variante em homozigose do polimorfismo *T6235C*, CC, foi identificado em cerca de 5% a 20% dos indivíduos de diferentes populações étnicas (46, 47,48, 49, 50, 51, 52, 53, 54, 56, 59; 60, 62; 63; 64, 65). Entretanto, seu papel no risco de ocorrência do câncer de mama é incerto (49, 52,54, 55, 56, 59, 60,61,62, 63, 64).

Vale ainda comentar que o quimioterápico ciclofosfamida (66, 67, 68) e o antiestrogênio tamoxifeno (69, 70, 71), utilizados geralmente no tratamento de pacientes com câncer de mama, são metabolizados no fígado por enzimas do CYP, incluindo as *CYP1A1*. Até onde atinge o conhecimento deste pesquisador, são também desconhecidos os papéis deste polimorfismos gênicos na sobrevida de pacientes com câncer de mama tratadas de forma convencional.

1.3. Justificativa

O câncer é a segunda causa de óbito na região Sudeste do Brasil e o câncer de mama foi descrito como um problema importante de saúde nessa região do país (2). Ainda, a população brasileira é altamente heterogênea, constituída por indígenas e imigrantes da Europa, África e Ásia, o que promoveu a miscigenação racial, uma das características desta população. (72, 73).

Frente ao exposto, pareceu interessante identificar os papéis dos polimorfismos *A4889G* e T6235C do gene *CYP1A1* no risco e sobrevida de portadoras de câncer de mama em uma população heterogênea de mulheres com alta incidência da doença.

2. Objetivos

2.1. Objetivo Geral

Avaliar a associação entre os polimorfismos *A4889G* e *T6235C* do gene *CYP1A1* e as características epidemiológicas e clínicas de mulheres com câncer de mama esporádico.

2.2. Objetivos Específicos

- **Artigo1:** Determinar as associações entre os polimorfismos *A4889G* e *T6235C* do gene *CYP1A1* e as características epidemiológicas, clínicas e patológicas do tumor, e o padrão de sobrevida global em mulheres com câncer de mama esporádico.

- **Artigo 2:** Determinar as associações entre os polimorfismos *A4889G* e *T6235C* do gene *CYP1A1* e o comportamento clínico de tumores de mama com receptores hormonais positivos na vigência do uso de tamoxifeno.

3. Publicações

Artigo 1 – **The *CYP1A1* gene polymorphisms *A4889G* and *T6235C* are associated with low-grade ductal carcinomas of the breast but not with prognosis**

Artigo 2 – **The *CYP1A1 A4889G and T6235C* gene polymorphisms are not related to the clinical outcomes of ER/PR positive breast cancer in women using tamoxifen**

3.1. Artigo 1

from: Breast Cancer Research and Treatment (BREA) naresh.santhanam@springer.com via editorialmanager.com
to: CASSIO CARDOSO-FILHO <cardosofilho@gmail.com>
date: Wed, Oct 31, 2012 at 1:31 AM
subject: BREA - Acknowledgement of Receipt
mailed-by: editorialmanager.com

Dear CASSIO CARDOSO-FILHO,

Thank you for submitting your manuscript, The CYP1A1 gene polymorphisms A4889G and T6235C are associated with low-grade ductal carcinomas of the breast but not with prognosis., to Breast Cancer Research and Treatment.

During the review process, you can keep track of the status of your manuscript by accessing the following web site:

http://brea.edmgr.com/

Should you require any further assistance please feel free to contact the Editorial Office by clicking on the "contact us" in the menu bar to send an email to us.

Alternatively, please call us at +91 44 42197752 anytime between 9.00 - 17.00 hrs IST/5.00 - 13.00 hrs CET.

With kind regards,
Springer Journals Editorial Office
Breast Cancer Research and Treatment

The *CYP1A1* gene polymorphisms *A4889G* and *T6235C* are associated with low-grade ductal carcinomas of the breast but not with prognosis

Authors: Cassio Cardoso Filho, M.D. [1]

Luis Otavio Sarian, M.D., PhD [1]

Camila Borges Martins de Oliveira [2]

Maria Salete Costa Gurgel, M.D., PhD [1]

Carmen Silvia Passos Lima, M.D., PhD [2]

Leonardo da Silveira Bossi, M.D. [1]

Gustavo Jacob Lourenço, PhD [2]

1. Campinas State University, Department of Obstetrics and Gynecology, Campinas, Sao Paulo, Brazil
2. Campinas State University, Department of Internal Medicine, Campinas, Sao Paulo, Brazil

Correspondence to:

Prof. Luis Otávio Sarian

Campinas State University

Department of Obstetrics and Gynecology

Campinas, Sao Paulo, Brazil

e-mail: sarian@unicamp.br

Abstract

Background: Systemic therapy for breast cancer generally involves the use of the chemotherapy agent cyclophosphamide and the antiestrogen drug tamoxifen, which are drugs metabolized by the liver cytochrome P-450 (CYP). This later is encoded by the *CYP1A1* gene, and a few polymorphisms of this gene have been demonstrated to interfere with the metabolic efficiency of CYP. **Objective:** Evaluate whether the *T6235C* and *A4889G* gene polymorphisms are related to the pathological presentation and clinical outcomes of breast cancer. **Methods:** We included 741 women with breast cancer for which 5 year follow-up data were available. We evaluated the associations of the key pathological and clinical information, including overall 5-year survival, with the *CYP1A1* gene statuses. **Results:** White ethnicity was positively associated with the AG/GG genotypes in both uni- and multivariate analyses (p=0.04 and 0.03, respectively). Women harboring the AG/GG genotypes had a slightly lower prevalence of high-grade (grade III tumors) compared to those with the AA genotype (p=0.01 and 0.02). The same was true for women with the TC/CC genotypes (p=0.03 for both uni- and multivariate analyses). Women with any genotype other than the AA/TT were slightly more prone at developing low-grade invasive carcinomas (adjusted p<0.01; OR (for grade III carcinomas) = 0.4; 95%CI 0.4 to 0.68). After 60 months of follow-up, roughly 75% of the women were alive. There was no significant difference in survival related to the *CYP1A1* status. **Conclusions:** The *CYP1A1* gene polymorphisms were associated with less aggressive tumors, but this association did not translate clinically into a better prognosis.

Introduction

The *CYP1A1* gene is located on the long arm of chromosome 15. The polymorphism *T6235C* determines the change of a nitrogenous base thymine to cytosine in the region 6235 of the DNA chain of the *CYP1A1* gene in non-coding region of the gene. The variant allele encodes a protein more effective in activating estrone and estradiol than the wild-type allele [1]. The variant genotype in homozygous polymorphism *T6235C*, CC, was identified in approximately 5% to 20% of the individuals of different ethnic populations [2-17]. Another polymorphism of the *CYP1A1* gene, the *A4889G*, determines the exchange of adenine for guanine at position 4889 in exon 7 of the gene and the substitution of a valine by an isoleucine in the encoded protein. The variant allele encodes a protein with increased activity in the activation of estrone and estradiol than the wild-type allele [18].

The chemotherapy drug cyclophosphamide [19-21] and the antiestrogen tamoxifen [22-24] used commonly in the treatment of patients with breast cancer, are metabolized by the liver cytochrome CYP, which is encoded by the *CYP1A1* gene. Based on these, it seemed of interest to identify the roles of the polymorphisms *T6235C* and *A4889G CYP1A1* on risk and survival of Brazilian women with breast cancer.

Subjects and methods

Selection of subjects

In this study, we included 741 women breast carcinomas, for which minimal 5-year follow-up were available. The cohort of this study originally integrated a prospective study addressing the glutathione S-transferase system (GST) polymorphisms and sporadic breast cancer, as described elsewhere [25-26]. Women aged 25 years or older and with no previous personal history or family history of breast cancer (BC) in a first-degree relative

were included, associated with the histopathologic diagnosis of invasive breast carcinoma. Women who had no knowledge of their family history (adoption, in vitro fertilization), or those incapable of informing their clinical data, were excluded from the analysis.

These women were invited to participate in the study at the time of hospital admission for their surgical treatment. Peripheral blood was drawn for DNA analysis and study of the polymorphisms.

This study was approved by the Ethics in Research Committee of the School of Medicine of UNICAMP and by the National Commission of Ethics in Research (CONEP), following the precepts of the Declaration of Helsinke (2004) and Resolution 196/96 of the National Council of Health (Brazil, 1996). All subjects signed an informed consent term, and no one refused to participate in the study.

Obtainment of clinical and biological characteristics

From review of the medical charts and interview to present the informed consent term, the following information was obtained: age, ethnic group, age at menarche, menopausal status, clinical tumor staging, histologic type, histologic and nuclear grades, in addition to the expression of hormone receptors.

Extraction of DNA from leucocytes

Genomic DNA was obtained from peripheral blood samples of patients with BC, using the technique of extraction by lithium chloride and proteinase K [27].

Identification of polymorphisms CYP1A1 A4889G and T6235C

The exon 7 of the *CYP1A1* gene is amplified by polymerase chain reaction (PCR) for the genotyping of polymorphisms *A4889G* and *T6235C*. After the reaction, a

fragment of 340 base pairs (bp) was obtained using the primers described by Canalle et al [5]. The genotype of the polymorphism *T6235C* was identified by enzymatic digestion of the amplified fragments with the use of the enzyme MspI. The reaction products were evaluated in electrophoresis on agarose gels of 3%. A single fragment of 340bp corresponded to the wild-type allele. Fragments of 200bp and 140bp correspond to the variant allele. Positive and negative controls were used in all reactions [4].

The genotype of the polymorphism *A4889G* was identified by enzymatic digestion of the amplified fragments using the enzyme NcoI. The reaction products were evaluated in electrophoresis on agarose gels of 3%. A single fragment of 263bp corresponded to the wild type allele, while fragments of 232bp and 31bp corresponded to the variant allele [6]. Genotyping was performed in the "Laboratory of Cancer Genetics", Faculty of Medical Sciences, State University of Campinas (http://www.fcm.unicamp.br/grupos/lageca).

Statistical analysis

All statistical analyses were performed with the R Environment for Statistical Computing (R Project). Significance was set at 95% (p=0.05) and 95% confidence intervals were used (95%CI). Chi-squares and Fisher's exact test were used to assess the relationships between the clinical and epidemiological variables and the gene statuses. Next, we used a multivariate regression model to adjust the p-values. We then formed two groups using the *A4889G* and *T6235C* statuses: one group consisting of women with both AA and TT genotypes (wild alleles) and the other consisting of women with any other combination of the genotypes (AA/TC; AA/CC; AG/TT; AG/TC; AG/CC; GG/TT; GG/TC or GG/CC). The relationship of the clinical and epidemiological features with these combined genotype statuses was assessed using uni- and multivariate analyses as

described above for the analyses of the individual gene statuses. Odds ratios were obtained with the exponentiation of the logistic regression coefficients. Finally, we produced univariate Kaplan-Meyer survival curves comparing the 5-year overall and disease-free survival of the women, grouped according to the individual and combined gene statuses. Differences in survival were assessed with the Log-Rank test.

Results

The *CYP1A1* genotypes were distributed as follows: For the *A4889G* polymorphism: AA= 527 (71.1%) cases, AG= 194 (26.2%) cases and GG= 20 (2.7%) cases, resulting in a G allele frequency of 31.7%; for the *T6235C* polymorphism: TT= 464 (62.6%) cases, TC= 235 (31.7%) cases, CC= 42 (5.7%) cases, resulting in a C allele frequency of 43.2% (data not shown in Tables).

Table 1 lists the main clinical and epidemiological features of the women as related to the genotypes. White ethnicity was positively associated with the AG/GG genotypes in both uni- and multivariate analyses (p=0.04 and 0.03, respectively). Women harboring the AG/GG genotypes had a slightly lower prevalence of high-grade (grade III tumors) compared to those with the AA genotype (p=0.01 and 0.02). The same was true for women with the TC/CC genotypes (p=0.03 for both uni- and multivariate analyses).

Table 2 shows the relationship of the combined genotypes with patients' characteristics. Women with any genotype other than the AA/TT were slightly more prone at developing low-grade invasive carcinomas (adjusted p<0.01; OR (for grade III carcinomas) = 0.4; 95%CI 0.4 to 0.68).

Figures 1 and 2 convey the Kaplan-Meyer representation of overall survival as related to the different approaches to the *CYP1A1* polymorphisms. Mean follow-up time

was 57.2 months (interquartile range= 36.5 months; data not shown in figure). After 60 months of follow-up, roughly 75% of the women were alive. There was no significant difference in survival related to the *CYP1A1* status.

Discussion

In our study, the *CYP1A1* gene polymorphisms were associated with low-grade breast carcinomas, but did not correlate with overall survival of the women. The polymorphisms were also more prevalent in Caucasian women, who form the majority of our cohort. It is important to emphasize, as far as our knowledge goes, most studies about the *CYP1A1* gene polymorphisms focus on the association of these single nucleotide polymorphisms (SNP) with risk for breast cancer development. We, by contrast, evaluated the clinical and pathological implications of the *CYP1A1* gene polymorphisms in women who already developed the disease.

We studied a population of southeastern Brazilian women, with large numbers of descendants of Europeans [28-29]. The *CYP1A1* gene is highly polymorphic in human populations and ethnic differences in the distribution of these polymorphisms have been reported [30]. As we have mentioned above, most studies about *CYP1A1* polymorphisms address the association of the SNP with risk of developing breast cancer. The results reported in most studies are controversial, with the risk estimates for the SNP carriers varying between studies [31]. In Caucasian populations similar to ours, and in studies evaluating the same polymorphisms, no relationship between the *A4889G* and *T6235C* SNP and breast cancer risk has been found.

We detected a higher prevalence of both *A4889G* and *T6235C* SNP in Caucasians. Okobia and cols [10], studying Nigerian women, found only one *A4889G* heterozygous

carrier among 250 cancer subjects. This same polymorphism has been reported to have allele frequencies of 0.69 and 0.31 among Japanese women [32], and studies in Asian women have indicated that more than 10% of the population possesses variant genotypes [33]. However, in Caucasians, the allele frequencies of the *A4889G* and *T6235C* SNPs have been reported to be low [34]. Not unexpectedly, the only study where the authors reported an increased risk of developing breast cancer in *CYP1A1* polymorphism was based on a population of African women, in which the prevalence of the SNP is known to be higher than that in Caucasian and Asian women [35].

There is only one study where the authors investigated the association between polymorphisms of the *CYP1A1* gene and the morphological characteristics of breast tumors [36]. In that study, the *A4889G* polymorphism was associated with high-grade tumors, a finding dissimilar to ours. However, contrary to what we found, the *T6235C* polymorphism was not associated with tumor characteristics in that study.

The *CYP1A1* gene plays a pivotal role in the 2-hydroxylation of estradiol and estrone to 2-hydroxy catechol metabolites for subsequent O-methylation to 2-methoxy intermediates. The pathway of 16a-hydroxylation leads to metabolites with strong estrogenic properties and have been linked to estrogen-induced carcinogenesis in both laboratory animals and humans [37-39]. The estrogen receptor positive models of carcinogenesis encompasses lesions that progress from low to high-grade due to the acquisition of genetic instability and the accumulation of stochastic genetic events due to the presence of estrogen and its metabolites [40]. It is not known, however, whether the carcinogenic estrogen metabolites derived from polymorphic CYP may induce the formation of a higher proportion of high-grade tumors in *CYP1A1* gene polymorphism carriers.

Even though the proportion of high-grade breast tumors in *CYP1A1* carriers was lower, we detected no difference in overall survival as related to the presence of any of the two SNPs. Tumor grade is a well-established risk factor for disease recurrence and shorter survival [41]. It is possible that our cohort was not sufficiently powered to allow the detection of slight differences in survival related to the presence of the SNPs, given the low prevalence of the polymorphisms in our preponderantly Caucasian population. It is also worth noting that we had a large proportion of high-grade tumors in carriers and non-carriers, surpassing 80% in both cases.

In conclusion, we have not detected any difference in clinical behavior of breast cancer in *CYP1A1* gene polymorphism carriers compared to non-carriers. It is worth noting, however, that the SNPs were associated with low-grade tumors, which are known to bear a better prognosis. Whether the lack of association between SNP carrier status and disease prognosis in our study is a consequence of the low prevalence of the SNP in the studied population or another unknown methodological artifact, we have no clues.

Conflict of interest

The authors have no conflicts of interest to disclose.

References

1. Parl FF; Dawling S, Roodi N, Crooke PS. Estrogen metabolism and breast cancer: a risk model. Ann NY Acad Sci. 2009;1155:68-75.

2. Aynacioglu AS, Cascorbi I, Mrozikiewicz PM, Roots I. High frequency of CYP1A1 mutations in a Turkish population. Arch Toxicol. 1998;72:215-218.

3. da Fonte de Amorim L, Rossini A, Mendonça G, Lotsch P, de Almeida Simão T, de Moura Gallo C et al. CYP1A1, GSTM1, and GSTT1 polymorphisms and breast cancer risk in Brazilian women. Cancer Lett. 2002;181(2):179-186.

4. Wang J, Deng Y, Li L, Kuriki K, Ding J, Pan X et al. Association of GSTM1, CYP1A1 and CYP2E1 genetic polymorphisms with susceptibility to lung adenocarcinoma: a case-control study in Chinese population. Cancer Sci. 2003;94:448-452.

5. Canalle R, Burim RV, Tone LG, Takahashi CS. Genetic polymorphisms and susceptibility to childhood acute lymphoblastic leukemia. Environ Mol Mutagen. 2004;43(2):100-109.

6. Joseph T, Kusumakumary P, Chacko P, Abraham A, Radhakrishna Pillai M. Genetic polymorphisms of CYP1A1, CYP2D6, GSTM1, GSTT1 and susceptibility to acute lymphoblastic leukaemia in Indian children. Pediatr Blood Cancer. 2004;43:560-567.

7. Li Y, Millikan RC, Bell DA, Cui L, Tse CK, Newman B et al. Cigarette smoking, cytochrome P4501A1 polymorphisms, and breast cancer among African-American and white women. Breast Cancer Res. 2004;6:R460-R473.

8. Hefler LA, Tempfer CB, Grimm C, Lebrecht A, Ulbrich E, Heinze G et al. Estrogen-metabolizing gene polymorphisms in the assessment of breast carcinoma risk and fibroadenoma risk in Caucasian women. Cancer. 2004;101(2):264-269.

9. Li Y, Millikan RC, Bell DA, Cui L, Tse CK, Newman B et al. Polychlorinated biphenyls, cytochrome P450 1A1 (CYP1A1) polymorphisms, and breast cancer risk

among African American women and white women in North Carolina: a population-based case-control study. Breast Cancer Res. 2005;7(1):R12-8.

10. Okobia M, Bunker C, Zmuda J, Kammerer C, Vogel V, Uche Eet al. Cytochrome P4501A1 genetic polymorphisms and breast cancer risk in Nigerian women. Breast Cancer Res Treat. 2005;94(3):285-93.

11. Seremak-Mrozikiewicz A, Drews K, Semczuk A, Jakowicki JA, Mrozikiewicz PM. CYP1A1 alleles in female genital cancers in the Polish population. Eur J Obstet Gynecol Reprod Biol. 2005;118(2):246-50.

12. Zhu K, Hunter S, Payne-Wilks K, Sutcliffe C, Bentley C, Roland CL et al. Potential differences in breast cancer risk factors based on CYP1A1 MspI and African-American-specific genotypes. Ethn Dis. 2006;16(1):207-15.

13. Shin A, Kang D, Choi JY, Lee KM, Park SK, Noh DY et al. Cytochrome P450 1A1 (CYP1A1) polymorphisms and breast cancer risk in Korean women. Exp Mol Med. 2007;39(3):361-6.

14. Diergaarde B, Potter JD, Jupe ER, Manjeshwar S, Shimasaki CD, Pugh TW et al. Polymorphisms in genes involved in sex hormone metabolism, estrogen plus progestin hormone therapy use, and risk of postmenopausal breast cancer. Cancer Epidemiol Biomarkers Prev. 2008;17(7):1751-9.

15. Gulyaeva LF, Mikhailova ON, PustyInyak VO, Kim IV 4th, Gerasimov AV, Krasilnikov SE et al. Comparative analysis of SNP in estrogen-metabolizing enzymes for ovarian, endometrial, and breast cancers in Novosibirsk, Russia. Adv Exp Med Biol. 2008;617:359-66.

16. Shimada N, Iwasaki M, Kasuga Y, Yokoyama S, Onuma H, Nishimura H et al. Genetic polymorphisms in estrogen metabolism and breast cancer risk in case-control studies in Japanese, Japanese Brazilians and non-Japanese Brazilians. J Hum Genet. 2009;54(4):209-15.

17. Yamaguti GG, Lourenço GJ, Costa FF, Lima CS. High risk of 'de novo' acute myeloid leukaemia in individuals with cytochrome P450 A1 (CYP1A1) and NAD(P)H: quinone oxidoreductase 1 (NQO1) gene defects. Eur J Haematol. 2009;83(3):270-2.

18. Tsuchiya Y, Nakajima M, Yokoi T. Cytochrome P450-mediated metabolism of estrogens and its regulation in human. Cancer Lett. 2005;227(2):115-24.

19. Sreerama L, Sladek NE. Cellular levels of class 1 and class 3 aldehyde dehydrogenases and certain other drug-metabolizing enzymes in human breast malignancies. Clin Cancer Res. 1997;3(11):1901-14.

20. Montero R, Serrano L, Dávila VM, Ito A, Plancarte A. Infection of rats with Taenia taeniformis metacestodes increases hepatic CYP450, induces the activity of CYP1A1, CYP2B1 and COH isoforms and increases the genotoxicity of the procarcinogens benzo[a]pyrene, cyclophosphamide and aflatoxin B(1). Mutagenesis. 2003;18(2):211-6.

21. Oesch-Bartlmowicz B, Oesch F. Modulation of mutagenicity by phosphorylation of mutagen-metabolizing enzymes. Arch Biochem Biophys. 2004;423(1):31-6.

22. Sharma M, Shubert DE, Sharma M, Lewis J, McGarrigle BP, Bofinger DP et al. Biotransformation of tamoxifen in a human endometrial explant culture model. Chem Biol Interact. 2003;146(3):237-49.

23. Parte P, Kupfer D. Oxidation of tamoxifen by human flavin-containing monooxygenase (FMO) 1 and FMO3 to tamoxifen-N-oxide and its novel reduction back to tamoxifen by human cytochromes P450 and hemoglobin. Drug Metab Dispos. 2005;33(10):1446-52.

24. van Schaik RH. CYP450 pharmacogenetics for personalizing cancer therapy. Drug Resist Updat. 2008;11(3):77-98.

25. Oliveira C, Lourenço GJ, Silva PM, Cardoso-Filho C, Favarelli MH, Gonçales NS et al. Polymorphisms in the 5'- and 3'-untranslated region of the VEGF gene and sporadic breast cancer risk and clinicopathologic characteristics. Tumour Biol. 2011;32(2):295-300.

26. Cardoso-Filho C, Lourenço GJ, Shinzato JY, Zeferino LC, Costa FF. Clinical and pathological implications of GSTM1 and GSTT1 gene deletions in sporadic breast cancer. Oncol Rev. 2008;2(1):36-43.

27. Woodhead JL, Fallon R, Figuered H, Longdale J, Malcom AD – Alternative methodology of gene diagnosis. In: Human Genetic Diaseases: A Practical Approach. K.E. Davies, ed. Oxford: IRL Press. p.51-64.

28. Alves-Silva J, da Silva Santos M, Guimarães PE, Ferreira AC, Bandelt HJ, Pena SD et al. The ancestry of Brazilian mtDNA lineages. Am J Hum Genet. 2000;67(2):444-61.

29. Carvalho-Silva DR, Santos FR, Rocha J, Pena SD. The phylogeography of Brazilian Y-chromosome lineages. Am J Hum Genet. 2001;68(1):281-6.

30. Le Marchand L, Donlon T, Kolonel LN, Henderson BE, Wilkens LR. Estrogen metabolism-related genes and breast cancer risk: the multiethnic cohort study. Cancer Epidemiol Biomarkers Prev. 2005;14(8):1998-2003.

31. Taioli E, Trachman J, Chen X, Toniolo P, Garte SJ. A CYP1A1 restriction fragment length polymorphism is associated with breast cancer in African-American women. Cancer Res. 1995;55:3757–3758.

32. Klauber N, Parangi S, Flymn E, Hamel E, D'Amato RJ: Inhibition of angiogenesis and breast cancer in mice by the microtubulin inhibitors 2-methoxyestradiol and taxol. Cancer Res. 1997;57:81–86.

33. Huang CS, Shen CY, Chang KJ, Hsu SM, Chern HD: Cytochrome P4501A1 polymorphism as a susceptibility factor for breast cancer in postmenopausal Chinese women in Taiwan. Br J Cancer. 1999;80(11): 1838–1843.

34. Rebbeck TR, Rosvold EA, Duggan DJ, Zhang J, Buetow KH: Genetics of CYP1A1: Coamplification of specific alleles by Polymerase Chain Reaction and association with breast cancer. Cancer Epidemiol Biomarkers Prev. 1994;3:511–514.

35. Kato I, Cichon M, Yee CL, Land S, Korczak JF. African American-preponderant single nucleotide polymorphisms (SNPs) and risk of breast cancer. Cancer Epidemiol. 2009;33(1):24-30.

36. Singh N, Mitra AK, Garg VK, Agarwal A, Sharma M, Chaturvedi R et al. Association of CYP1A1 polymorphisms with breast cancer in North Indian women. Oncol Res. 2007;16(12):587-97.

37. Schneider J, Huh MM, Bradlow LH, Fishman J: Antiestrogen action of 2-hydrxyestrone on MCF-7 human breast cancer cells. J Biol Chem. 1984;259:4840–4845.

38. Fotsis T, Zhang Y, Pepper MS, Aldercreutz H, Montesano R, Nawroth PP, Schwelgerer L: The endogenous estrogen metabolite 2-methoxyestradiol inhibits angiogenesis and suppresses tumor growth. Nature. 1994;368: 237–239.

39. Cushman M, He HM, Katzenellenbogan JA, Lin CM, Hamel E: Synthesis, antitubulin and antimitotic activity, and cytotoxicity of analogs of 2-methoxyestradiol, an endogenous mammalian metabolite of estradiol that inhibits tubulin polymerization by binding to the colchicines binding site. J Med Chem. 1995;38:2041–2049.

40. Lopez-Garcia MA, Geyer FC, Lacroix-Triki M, Marchió C, Reis-Filho JS.Breast cancer precursors revisited: molecular features and progression pathways. Histopathology. 2010;57(2):171-92.

41. Elston CW, Ellis IO: Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology. 1991;19:403–410.

Table 1 – *A4889G* and *T6235C* genotypes as related to key epidemiological features

	***A4889G* genotype**						***T6235C* genotype**					
	AA		**AG/GG**				**TT**		**TC/CC**			
	n	**(%)**	**n**	**(%)**	**p**	**p adj**	**n**	**(%)**	**n**	**(%)**	**p**	**p adj**
Age					1	0.72					0.4	0.77
≥35 years	494	(93.9)	202	(94.0)			438	(94.6)	258	(92.8)		
<35 years	32	(6.1)	13	(6.0)			25	(5.4)	20	(7.2)		
Unknown	1		0				1		0			
Menarche					0.14	0.09					0.34	0.15
≥12 years	289	(58.9)	106	(52.5)			255	(58.5)	140	(54.5)		
<12 years	202	(41.1)	96	(47.5)			181	(41.5)	117	(45.5)		
Unknown	36		13				28		21			
BMI					0.38	0.57					1	0.81
≤25	186	(37.1)	69	(33.3)			161	(36.1)	94	(35.9)		
>25	315	(62.9)	138	(66.7)			285	(63.9)	168	(64.1)		
Unknown	26		8				18		16			
Ethnicity					**0.04**	**0.03**					0.44	0.81
White	444	(84.3)	194	(90.2)			403	(86.9)	235	(84.5)		
Non-white	83	(15.7)	21	(9.8)			61	(13.1)	43	(15.5)		
Unknown	0		0				0		0			
Menopause					0.9	0.97					0.36	0.6
Post	231	(45.4)	98	(46.2)			250	(55.8)	142	(52.0)		
Pre	278	(54.6)	114	(53.8)			198	(44.2)	131	(48.0)		
Unknown	18		3				16		5			
Disease stage					0.23	0.22					0.58	0.51
0-II	307	(58.3)	136	(63.3)			273	(58.8)	170	(61.2)		
III-IV	220	(41.7)	79	(36.7)			191	(41.2)	108	(38.8)		
Unknown	0		0				0		0			
Histology					0.14	0.07					0.057	0.07
Ductal invasive	357	(67.7)	158	(73.5)			310	(66.8)	205	(73.7)		
Other	170	(32.3)	57	(26.5)			154	(33.2)	73	(26.3)		
Unknown	0		0				0		0			
Grade					**0.01**	**0.02**					**0.03**	**0.03**
I-II	56	(12.3)	37	(20.0)			48	(12.0)	45	(18.6)		
III	400	(87.7)	148	(80.0)			351	(88.0)	197	(81.4)		
Unknown/NA	71		30				65		36			
Nuclear grade					0.85	0.365					0.66	0.88
1-2	169	(34.8)	66	(33.7)			144	(33.7)	91	(35.7)		
3	317	(65.2)	130	(66.3)			283	(66.3)	164	(64.3)		
Unknown/NA	41		19				37		22			
Hormonal receptor					1	0.99					0.21	0.11
One positive	335	(69.9)	142	(70.0)			307	(71.7)	170	(66.9)		
Both negative	144	(30.1)	61	(30.0)			121	(28.3)	84	(33.1)		
Unknown	48		12				36		24			

Table 2 – Combined analysis of *A4889G* and *T6235C* genotypes as related to clinical and epidemiological features

	Combined genotypes							
	AA and TT		Other					
	n	**(%)**	**n**	**(%)**	**p**	**p adj**	**OR**	**(IC95%)**
Age								
≥35 years	389	(94.4)	307	(93.3)				
<35 years	23	(5.6)	22	(6.7)	0.63	0.95	1.02	(0.49 to 2.12)
Unknown	1		0					
Menarche								
≥12 years	226	(58.5)	169	(55.0)				
<12 years	160	(41.5)	138	(45.0)	0.39	0.19	1.26	(0.89 to 1.8)
Unknown	27		22					
BMI								
≤25	144	(36.5)	111	(35.5)				
>25	251	(63.5)	202	(64.5)	0.84	0.98	0.99	(0.68 to 1.44)
Unknown	18		16					
Ethnicity								
White	357	(86.4)	281	(85.4)				
Non-white	56	(13.6)	48	(14.6)	0.76	0.69	1.1	(0.67 to 1.83)
Unknown	0		0					
Menopause								
Post	219	(55.2)	173	(53.4)				
Pre	178	(44.8)	151	(46.6)	0.68	0.72	0.94	(0.65 to 1.35)
Unknown	16		5					
Disease stage								
0-II	242	(58.6)	197	(59.9)				
III-IV	171	(41.4)	132	(40.1)	0.78	0.99	1.00	(0.69 to 1.43)
Unknown	0		0					
Histology								
Ductal invasive	278	(67.3)	237	(72.0)				
Other	135	(32.7)	92	(28.0)	0.19	0.06	1.5	(0.97 to 2.32)
Unknown	0		0					
Grade								
I-II	37	(10.4)	56	(19.7)				
III	320	(89.6)	228	(80.3)	**<0.01**	**<0.01**	**0.4**	**(0.24 to 0.68)**
Unknown / NA	56		45					
Nuclear grade								
1-2	132	(34.6)	103	(34.3)				
3	250	(65.4)	197	(65.7)	1.00	0.38	1.19	(0.80 to 1.76)
Unknown / NA	31		29					
Hormonal receptor								
One positive	269	(71.2)	208	(68.4)				
Both negative	109	(28.8)	96	(31.6)	0.48	0.30	0.81	(0.54 to 1.21)
Unknown	35		25					

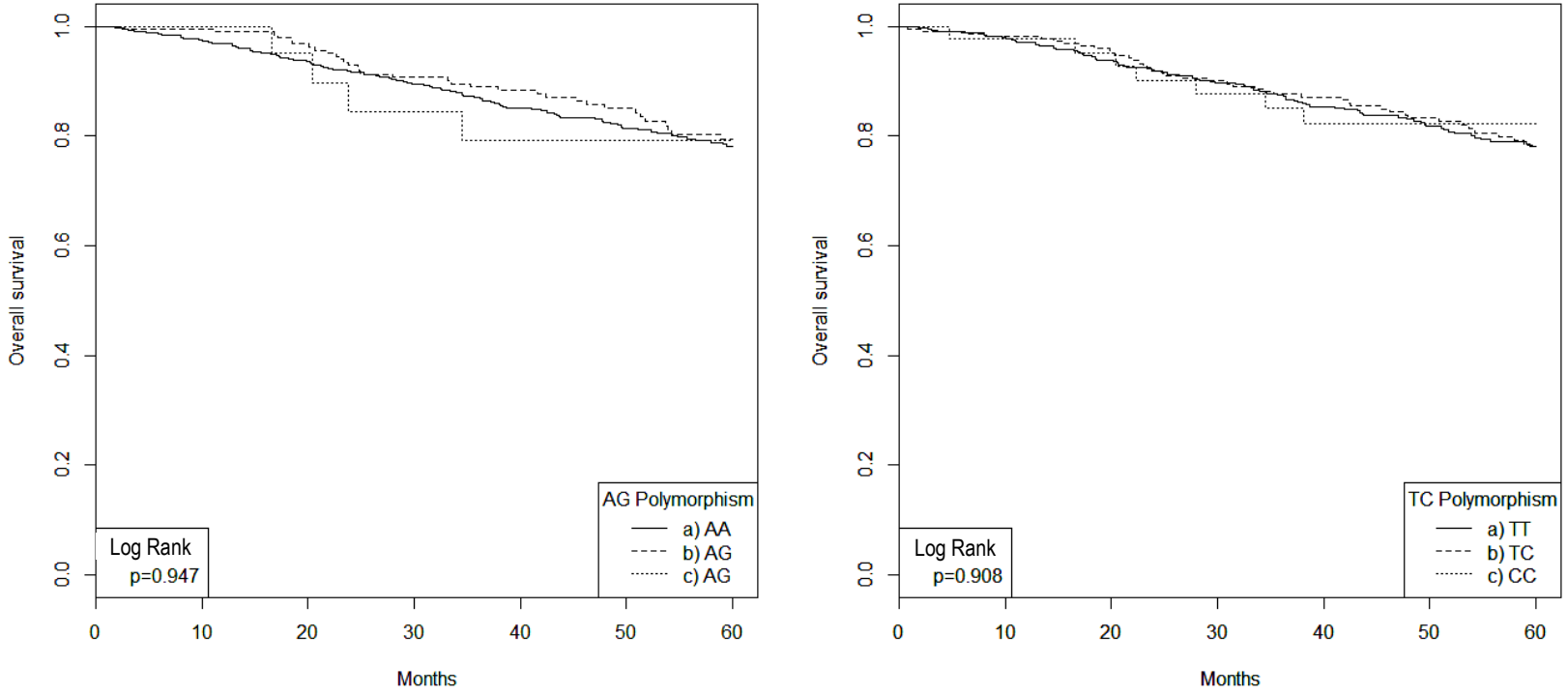


Figure 1. Kaplan-Meyer depiction of the overall survival as related to the *A4889G* (left) and *T6235C* (right) genotypes.

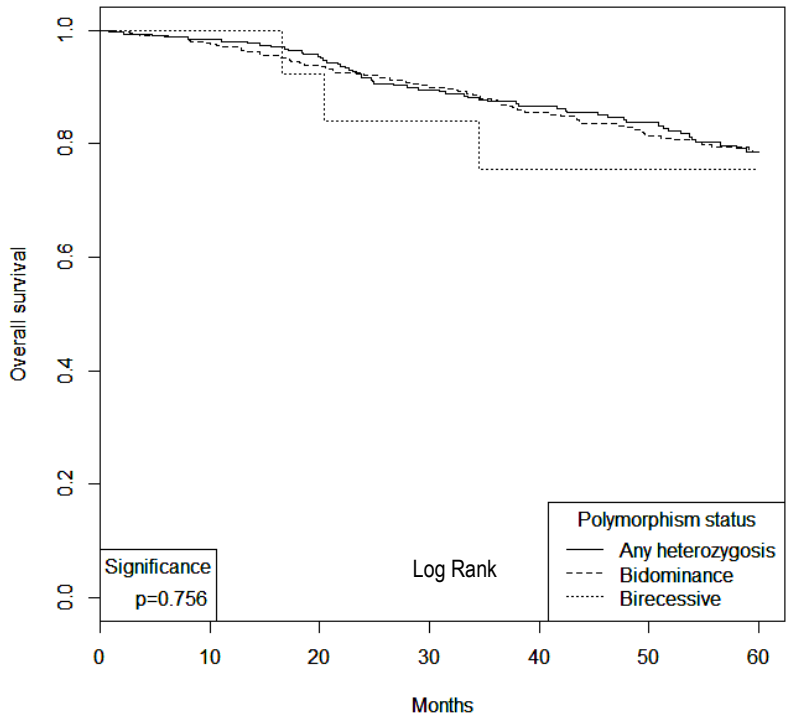


Figure 2. Kaplan-Meyer depiction of the overall survival as related to the combined *A4889G* and *T6235C* genotypes

3.2. Artigo 2

from: Breast Cancer Research and Treatment (BREA) naresh.santhanam@springer.com via editorialmanager.com
to: CASSIO CARDOSO-FILHO <cardosofilho@gmail.com>
date: Tue, Nov 6, 2012 at 10:18 PM
subject: BREA - Acknowledgement of Receipt
mailed-by: editorialmanager.com

Dear CASSIO CARDOSO-FILHO,

Thank you for submitting your manuscript, The CYP1A1 A4889G and T6235C gene polymorphisms are not related to the clinical outcomes of ER/PR positive breast cancer in women using tamoxifen., to Breast Cancer Research and Treatment.

During the review process, you can keep track of the status of your manuscript by accessing the following web site:

http://brea.edmgr.com/

Should you require any further assistance please feel free to contact the Editorial Office by clicking on the "contact us" in the menu bar to send an email to us.

Alternatively, please call us at +91 44 42197752 anytime between 9.00 - 17.00 hrs IST/5.00 - 13.00 hrs CET.

With kind regards,
Springer Journals Editorial Office

The *CYP1A1 A4889G and T6235C* gene polymorphisms are not related to the clinical outcomes of ER/PR positive breast cancer in women using tamoxifen

Authors: Cassio Cardoso Filho, M.D. [1]

Luis Otavio Sarian, M.D., PhD [1]

Camila Borges Martins de Oliveira [2]

Maria Salete Costa Gurgel, M.D., PhD [1]

Carmen Silvia Passos Lima, M.D., PhD [2]

Leonardo da Silveira Bossi, M.D. [1]

Gustavo Jacob Lourenço, PhD [2]

1. Campinas State University, Department of Obstetrics and Gynecology, Campinas, Sao Paulo, Brazil
2. Campinas State University, Department of Internal Medicine, Campinas, Sao Paulo, Brazil

Correspondence to:

Prof. Luis Otávio Sarian

Campinas State University

Department of Obstetrics and Gynecology

Campinas, Sao Paulo, Brazil

e-mail: sarian@unicamp.br

Abstract

Background: Inter-individual differences in cytochrome P-450 (CYP) efficiency explain part of the variations in resistance to tamoxifen and oestrogen metabolism. Two polymorphisms of the *CYP1A1* gene (*A4889G* and *T6235C*) are known to affect the activation of estrone and estradiol and to deregulate the concentration of highly active tamoxifen metabolites. However, the clinical translation of these laboratorial findings has not yet been evaluated. **Objective:** Evaluate whether the *T6235C* and *A4889G* gene polymorphisms are related to the pathological presentation and clinical outcomes of ER/PR breast cancer in women using tamoxifen. **Methods:** We included 405 women with ER/PR positive tumors, who used tamoxifen as the primary antiestrogen therapy, and for which 5 year follow-up data were available. We evaluated the associations of the key pathological and clinical information, including overall 5-year survival, with the *CYP1A1* gene statuses. **Results:** In univariate analysis, a slightly higher proportion of whites was found in women with the AG/GG genotypes (p=0.05). Also in the univariate analysis, a significant association between premenopausal status (p=0.01) and TC/CC genotypes was found. However, after multivariate adjustment, no significant association remained. Women with *CYP1A1* genotypes other than the AA and TT were slightly more prone at developing low-grade tumors (85.9% of the tumors in the AA and TT genotypes group were grade III, contrasted to only 76.1% in carriers of the polymorphisms; adjusted p= 0.02; OR (for grade III disease)= 0.51; CI95% 0.28 to 0.93). After 60 months of follow-up, roughly 75% of the women were alive. There was no significant difference in survival related to the *CYP1A1* gene status. **Conclusions:** Although highly plausible from the biological standpoint, there is no evidence of a worse prognosis of breast cancer in patients carrying *CYP1A1* gene polymorphisms.

Introduction

In a proportion of steroid receptor positive breast cancer (BC) patients, tumours are resistant to tamoxifen, or become so, with a subsequent relapse of the disease. The mechanisms underlying the resistance are not fully understood. Because there is convincing evidence that tamoxifen is converted to anti-oestrogenic metabolites that are more potent than the mother substance, one hypothesis is that altered metabolism might contribute to inter-individual variability in serum concentrations, which in turn may influence the action on ER and the response to treatment [1-3]. Some studies have demonstrated considerable inter-individual variation in concentrations of tamoxifen metabolites both in plasma and locally in the breast [4-5].

The interindividual variability in tamoxifen serum concentrations can be, at least in part, explained by the individuals' variations in liver CYP efficiency [6-8]. Tamoxifen is converted in vivo into potent anti-oestrogenic metabolites by several CYP enzymes [9]. The active metabolites of tamoxifen are metabolised further into inactive compounds by conjugation via phase 2 liver enzymes [10-12].

Several gene polymorphisms have been suggested as potential candidates to explain the inter-individual differences in CYP efficiency. The polymorphism *T6235C* of the *CYP1A1* gene encodes a protein more effective in activating estrone and estradiol than the wild-type allele [13]. Another polymorphism of the *CYP1A1* gene, the *A4889G*, encodes another protein with increased activity in the activation of estrone and estradiol than the wild-type allele [14].

In this clinical study, we evaluated the clinical and pathological implications of the *CYP1A1* gene polymorphisms, taking advantage of a large cohort of breast cancer patients with steroid receptor positive tumors using tamoxifen with complete 5-year follow-up data.

Subjects and methods

Selection of subjects

In this study, we included 405 women, sequentially examined at Center for Women's Integrated Healthcare (University of Campinas – UNICAMP) from December 2002 to October 2004, with ER/PR positive breast carcinomas using tamoxifen. Minimal follow-up time for inclusion was arbitrarily set at five years. The cohort of this study, however, originally integrated a prospective study addressing the glutathione S-transferase system (GST) polymorphisms and sporadic brast cancer, as described elsewhere [15-16]. As another inclusion criteria, women aged 25 years or older and with no previous personal history or family history of BC in a first-degree relative were included, associated with the histopathological diagnosis of hormonal receptor-positive invasive breast carcinoma using Tamoxifen. Women who had no knowledge of their family history (adoption, in vitro fertilization), or those incapable of informing their clinical data, were excluded from the analysis.

These women were invited to participate in the study at the time of hospital admission for their surgical treatment. Peripheral blood was drawn for DNA analysis and study of the polymorphisms.

This study was approved by the Ethics in Research Committee of the School of Medicine of UNICAMP and by the National Commission of Ethics in Research (CONEP), following the precepts of the Declaration of Helsinke (2004) and Resolution 196/96 of the National Council of Health (Brazil, 1996). All subjects signed an informed consent term, and no one refused to participate in the study.

Obtainment of clinical and biological characteristics

From review of the medical charts and interview to present the informed consent term, the following information was obtained: age, ethnic group, age at menarche, menopausal status, clinical tumor staging, histologic type, histologic and nuclear grades, in addition to the expression of hormone receptors. Compliance to tamoxifen prescription was ascertained by the hospital logs of delivery of drugs to patients.

Extraction of DNA from leucocytes

Genomic DNA was obtained from peripheral blood samples of patients with BC, using the technique of extraction by lithium chloride and proteinase K [17].

Identification of polymorphisms CYP1A1 A4889G and T6235C

The exon 7 of the *CYP1A1* gene is amplified by polymerase chain reaction (PCR) for the genotyping of polymorphisms *A4889G* and *T6235C*. After the reaction, a fragment of 340 base pairs (bp) was obtained using the primers described by Canalle et cols [18]. The genotype of the polymorphism *T6235C* was identified by enzymatic digestion of the amplified fragments with the use of the enzyme MspI. The reaction products were evaluated in electrophoresis on agarose gels of 3%. A single fragment of 340bp corresponded to the wild-type allele. Fragments of 200bp and 140bp correspond to the variant allele. Positive and negative controls were used in all reactions [19].

The genotype of the polymorphism *A4889G* was identified by enzymatic digestion of the amplified fragments using the enzyme NcoI. The reaction products were evaluated in electrophoresis on agarose gels of 3%. A single fragment of 263bp corresponded to the wild type allele, while fragments of 232bp and 31bp corresponded to the variant allele [20].

Genotyping was performed in the "Laboratory of Cancer Genetics", Faculty of Medical Sciences, State University of Campinas (http://www.fcm.unicamp.br/grupos/lageca/index.html).

Statistical analysis

All statistical analyses were performed with the R Environment for Statistical Computing (R Project). Significance was set at 95% (p=0.05) and 95% confidence intervals were used (95%CI). Chi-squares and Fisher's exact test were used to assess the relationships between the clinical and epidemiological variables and the gene statuses. Next, we used a multivariate regression model to adjust the p-values. We then formed two groups using the *A4889G* and *T6235C* statuses: one group consisting of women with both AA and TT genotypes (wild alleles) and the other consisting of women with any other combination of the genotypes (AA/TC; AA/CC; AG/TT; AG/TC; AG/CC; GG/TT; GG/TC or GG/CC). The relationship of the clinical and epidemiological features with these combined genotype statuses was assessed using uni- and multivariate analyses as described above for the analyses of the individual gene statuses. Odds ratios were obtained with the exponentiation of the logistic regression coefficients. Finally, we produced univariate Kaplan-Meyer survival curves comparing the 5-year overall and disease-free survival of the women, grouped according to the individual and combined gene statuses. Differences in survival were assessed with the Log-Rank test.

Results

The *CYP1A1* genotypes were distributed as follows: For the *A4889G* polymorphism: AA= 288 (71.0%) cases, AG= 105 (26.3%) cases and GG= 12 (2.7%) cases, resulting in a G allele frequency of 31.9%; for the *T6235C* polymorphism: TT= 259 (63.9%) cases,

TC= 127 (31.4%) cases, CC= 42 (4.7%) cases, resulting in a C allele frequency of 40.7% (data not shown in Tables).

Table 1 shows the relationships between the *CYP1A1* gene statuses and the main features of the BC patients using tamoxifen. In univariate analysis, a slightly higher proportion of caucasians was found in women with the AG/GG genotypes (p=0.05). Also in the univariate analysis, a significant association between premenopausal status (p=0.01) and TC/CC genotypes was found. However, after multivariate adjustment, no significant association remained.

Table 2 shows the associations between the combined *CYP1A1* gene statuses and the features of the women. Women with *CYP1A1* genotypes other than the AA and TT were slightly more prone at developing low-grade tumors (85.9% of the tumors in the AA and TT genotypes group were grade III, contrasted to only 76.1% in carriers of the polymorphisms; adjusted p= 0.02; OR (for grade III disease)= 0.51; CI95% 0.28 to 0.93).

Figures 1 and 2 convey the Kaplan-Meyer representation of overall survival as related to the different approaches to the *CYP1A1* polymorphisms. Mean follow-up time was 55.9 months (interquartile range= 31.2 months; data not shown in figure). After 60 months of follow-up, roughly 75% of the women were alive. There was no significant difference in survival related to the *CYP1A1* status.

Discussion

In the present study, the *CYP1A1* gene status was not associated with any of the studied clinical and pathological features of ER/PR positive breast cancer in a large sample of tamoxifen users. Also, the two SNPs were not correlated, in a stand alone or combined fashion, with the clinical outcome (overall survival) of breast cancer. Contrary

to our expectations, all the theoretically plausible implications of an increased exposure of ER/PR positive tumors to tamoxifen metabolites, such as a lower relapse/progression rate and increased survival, were not detected in our sample.

The theoretical basis of the present study relies on the known fact that the *CYP1A1* gene plays a pivotal role in estrogen-related metabolic pathways. The SNP carriers would be exposed to two important breast cancer promoting events: 1) reduced efficacy of tamoxifen and 2) increased serum concentrations of active proestrogenic molecules. The *CYP1A1* regulates both the 2-hydroxylation of estradiol and estrone to 2-hydroxy catechol metabolites for subsequent O-methylation to 2-methoxy intermediates and in tamoxifen conversion to its active metabolite. The pathway of 16a-hydroxylation leads to metabolites with strong estrogenic properties and have been linked to estrogen-induced carcinogenesis in both laboratory animals and humans [21-23]. The estrogen receptor positive models of carcinogenesis encompasses lesions that progress from low to high-grade due to the acquisition of genetic instability and the accumulation of stochastic genetic events due to the presence of estrogen and its metabolites [24].

One of the major weaknesses of our study relies on the fact that we have not an empirical confirmation of the increase in serum concentrations of tamoxifen metabolites in the *CYP1A1* gene polymorphism carriers. In the literature, there is no report of a study addressing concomitantly the serum levels of tamoxifen metabolites and estrogen and the clinical behavior of breast cancer as related to the *CYP1A1* gene status. Although some studies have demonstrated considerable inter-individual variation in concentrations of tamoxifen metabolites both in plasma and locally in the breast [4-5], and some others have suggested that the interindividual variability in tamoxifen serum concentrations could be related to less prevalent presentations of the genes that encode the CYP

proteins, such as the *CYP1A1* [6-8], none has integrated all this data into clinically significant information. We made an attempt at addressing the clinical question as to whether the already proven relationship of the *CYP1A1* polymorphisms with the steroid regulatory mechanisms translate into pathological and/or clinical singularities of breast cancer in SNP carriers.

In conclusion, although the laboratorial, ex-vivo and in vivo data have pointed towards a plausible relationship between *CYP1A1 A4889G* and *T6235C* genotypes and a worse prognosis of ER/PR positive breast cancer, we found no clinical evidence confirming this relationship.

Conflict of interest

The authors have no conflicts of interest to disclose.

References

1. Stearns V, Johnson MD, Rae JM, Morocho A, Novielli A, Bhargava P et al. Active tamoxifen metabolite plasma concentrations after coadministration of tamoxifen and the selective serotonin reuptake inhibitor paroxetine. J Natl Cancer Inst. 2003;95(23):1758-64.
2. Johnson MD, Zuo H, Lee KH, Trebley JP, Rae JM, Weatherman RV ET al. Pharmacological characterization of 4-hydroxy-N-desmethyl tamoxifen, a novel active metabolite of tamoxifen. Breast Cancer Res Treat. 2004;85(2):151-9.
3. Lim YC, Desta Z, Flockhart DA, Skaar TC. Endoxifen (4-hydroxy-N-desmethyl-tamoxifen) has anti-estrogenic effects in breast cancer cells with potency similar to 4-hydroxy-tamoxifen. Cancer Chemother Pharmacol. 2005;55(5):471-8.

4. MacCallum J, Cummings J, Dixon JM, Miller WR. Concentrations of tamoxifen and its major metabolites in hormone responsive and resistant breast tumours. Br J Cancer. 2000;82(10):1629-35.

5. Kisanga ER, Gjerde J, Guerrieri-Gonzaga A, Pigatto F, Pesci-Feltri A, Robertson C et al. Tamoxifen and metabolite concentrations in serum and breast cancer tissue during three dose regimens in a randomized preoperative trial. Clin Cancer Res. 2004;10(7):2336-43.

6. Sharma M, Shubert DE, Sharma M, Lewis J, McGarrigle BP, Bofinger DP et al. Biotransformation of tamoxifen in a human endometrial explant culture model. Chem Biol Interact. 2003;146(3):237-49.

7. Parte P, Kupfer D. Oxidation of tamoxifen by human flavin-containing monooxygenase (FMO) 1 and FMO3 to tamoxifen-N-oxide and its novel reduction back to tamoxifen by human cytochromes P450 and hemoglobin. Drug Metab Dispos. 2005;33(10):1446-52.

8. van Schaik RH. CYP450 pharmacogenetics for personalizing cancer therapy. Drug Resist Updat. 2008;11(3):77-98.

9. Desta Z, Ward BA, Soukhova NV, Flockhart DA. 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):1062-75.

10. Sun D, Sharma AK, Dellinger RW, Blevins-Primeau AS, Balliet RM, Chen G et al. Glucuronidation of active tamoxifen metabolites by the human UDP glucuronosyltransferases. Drug Metab Dispos. 2007;35(11):2006-14.

11. Nishiyama T, Ogura K, Nakano H, Ohnuma T, Kaku T, Hiratsuka A et al. Reverse geometrical selectivity in glucuronidation and sulfation of cis- and trans-4-hydroxytamoxifens by human liver UDP-glucuronosyltransferases and sulfotransferases. Biochem Pharmacol. 2002;63(10):1817-30.

12. Nowell S, Falany CN. Pharmacogenetics of human cytosolic sulfotransferases. Oncogene. 2006;25(11):1673-8.

13. Parl FF; Dawling S, Roodi N, Crooke PS. Estrogen metabolism and breast cancer: a risk model. Ann NY Acad Sci. 2009;1155:68-75.

14. Tsuchiya Y, Nakajima M, Yokoi T. Cytochrome P450-mediated metabolism of estrogens and its regulation in human. Cancer Lett. 2005;227(2):115-24.

15. Oliveira C, Lourenço GJ, Silva PM, Cardoso-Filho C, Favarelli MH, Gonçales NS et al. Polymorphisms in the 5'- and 3'-untranslated region of the VEGF gene and sporadic breast cancer risk and clinicopathologic characteristics. Tumour Biol. 2011;32(2):295-300.

16. Cardoso-Filho C, Lourenço GJ, Shinzato JY, Zeferino LC, Costa FF. Clinical and pathological implications of GSTM1 and GSTT1 gene deletions in sporadic breast cancer. Oncol Rev. 2008;2(1):36-43.

17. Woodhead JL, Fallon R, Figuered H, Longdale J, Malcom AD – Alternative methodology of gene diagnosis. In: Human Genetic Diaseases: A Practical Approach. K.E. Davies, ed. Oxford: IRL Press. p.51-64.

18. Canalle R, Burim RV, Tone LG, Takahashi CS. Genetic polymorphisms and susceptibility to childhood acute lymphoblastic leukemia. Environ Mol Mutagen. 2004;43(2):100-109.

19. Wang J, Deng Y, Li L, Kuriki K, Ding J, Pan X et al. Association of GSTM1, CYP1A1 and CYP2E1 genetic polymorphisms with susceptibility to lung adenocarcinoma: a case-control study in Chinese population. Cancer Sci. 2003;94:448-452.

20. Joseph T, Kusumakumary P, Chacko P, Abraham A, Radhakrishna Pillai M. Genetic polymorphisms of CYP1A1, CYP2D6, GSTM1, GSTT1 and susceptibility to acute lymphoblastic leukaemia in Indian children. Pediatr Blood Cancer. 2004;43:560-567.

21. Schneider J, Huh MM, Bradlow LH, Fishman J: Antiestrogen action of 2-hydrxyestrone on MCF-7 human breast cancer cells. J Biol Chem. 1984;259:4840–4845.

22. Fotsis T, Zhang Y, Pepper MS, Aldercreutz H, Montesano R, Nawroth PP, Schwelgerer L: The endogenous estrogen metabolite 2-methoxyestradiol inhibits angiogenesis and suppresses tumor growth. Nature. 1994;368: 237–239.

23. Cushman M, He HM, Katzenellenbogan JA, Lin CM, Hamel E: Synthesis, antitubulin and antimitotic activity, and cytotoxicity of analogs of 2-methoxyestradiol, an endogenous mammalian metabolite of estradiol that inhibits tubulin polymerization by binding to the colchicines binding site. J Med Chem. 1995;38: 2041–2049.

24. Lopez-Garcia MA, Geyer FC, Lacroix-Triki M, Marchió C, Reis-Filho JS.Breast cancer precursors revisited: molecular features and progression pathways. Histopathology. 2010;57(2):171-92.

Table 1 – *A4889G* and *T6235C* genotypes as related to key epidemiological features of women with positive steroid hormone receptors using tamoxifen

	***A4889G* genotype**						***T6235C* genotype**					
	AA		**AG/GG**				**TT**		**TC/CC**			
	n	**(%)**	**n**	**(%)**	**p**	**p adj**	**n**	**(%)**	**n**	**(%)**	**p**	**p adj**
Age					0.38	0.73					0.53	0.78
≥35 years	276	(95.8)	109	(93.2)			248	(95.8)	137	(93.8)		
<35 years	12	(4.2)	8	(6.8)			11	(4.2)	9	(6.2)		
Unknown	0		0				0		0			
Menarche					0.15	0.14					0.10	0.4
≥12 years	163	(59.9)	56	(51.4)			150	(60.7)	69	(51.5)		
<12 years	109	(40.1)	53	(48.6)			97	(39.3)	65	(48.5)		
Unknown	16		8				12		12			
BMI					0.86	0.81					0.99	0.82
≤25	94	(34.4)	41	(36.0)			87	(35.1)	48	(34.5)		
>25	179	(65.6)	73	(64.0)			161	(64.9)	91	(65.5)		
Unknown	15		3				11		5			
Ethnicity					**0.05**	0.06					0.62	0.70
White	244	(84.7)	108	(92.3)			223	(86.1)	129	(88.4)		
Non-white	44	(15.3)	9	(7.7)			36	(13.9)	17	(11.6)		
Unknown	0		0				0		0			
Menopause					0.37	0.82					**0.01**	0.10
Post	158	(57.2)	60	(51.7)			151	(60.6)	67	(46.9)		
Pre	118	(42.8)	56	(48.3)			98	(39.4)	76	(53.1)		
Unknown	12		1				10		3			
Disease stage					0.77	0.85					0.32	0.15
0-II	176	(61.1)	74	(63.2)			165	(63.7)	85	(58.2)		
III-IV	112	(38.9)	43	(36.8)			94	(36.3)	61	(41.8)		
Unknown	0		0				0		0			
Histology					0.32	0.27					0.26	0.42
Ductal invasive	206	(71.5)	90	(76.9)			184	(71.0)	112	(76.7)		
Other	82	(28.5)	27	(23.1)			75	(29.0)	34	(23.3)		
Unknown	0		0				0		0			
Grade					0.18	0.19					0.08	0.17
I-II	43	(16.5)	24	(23.1)			36	(15.5)	31	(23.3)		
III	218	(83.5)	80	(76.9)			196	(84.5)	102	(76.7)		
Unknown / NA	27		13				27		13			
Nuclear grade					0.73	0.51					0.15	0.13
1-2	120	(45.5)	45	(42.9)			98	(41.7)	67	(50.0)		
3	144	(54.5)	60	(57.1)			137	(58.3)	67	(50.0)		
Unknown / NA	24		12				24		12			

Table 2 – Combined analysis of *A4889G* and *T6235C* genotypes as related to clinical and epidemiological features of women with positive steroid hormone receptors using tamoxifen

	Combined genotypes					
	AA and TT	Other	p	p adjusted	OR	(IC95%)
	n (%)	n (%)				
Age			0.74	0.69	0.82	(0.29 to 2.27)
≥35 years	217 (95.6)	168 (94.4)				
<35 years	10 (4.4)	40 (5.6)				
Unknown	0	0				
Menarche			0.09	0.06	1.54	(0.97 to 2.43)
≥12 years	132 (61.4)	87 (52.4)				
<12 years	83 (38.6)	79 (47.6)				
Unknown	12	12				
BMI			1.00	0.88	0.96	(0.59 to 1.58)
≤25	75 (34.7)	60 (35.1)				
>25	141 (65.3)	111 (64.9)				
Unknown	11	7				
Ethnicity			0.59	0.50	1.25	(0.64 to 2.44)
White	195 (85.9)	157 (88.2)				
Non-white	32 (14.1)	21 (11.8)				
Unknown	0	0				
Menopause			0.07	0.32	0.78	(0.48 to 1.27)
Post	130 (59.9)	88 (50.3)				
Pre	87 (40.1)	87 (49.7)				
Unknown	10	3				
Disease stage			0.48	0.18	1.37	(0.86 to 2.19)
0-II	144 (63.4)	106 (59.6)				
III-IV	83 (36.6)	72 (40.4)				
Unknown	0	0				
Histology			0.58	0.37	1.30	(0.72 to 2.34)
Ductal invasive	163 (71.8)	133 (74.7)				
Other	64 (28.2)	45 (25.3)				
Unknown	0	0				
Grade			**0.02**	**0.02**	0.51	(0.28 to 0.93)
I-II	29 (14.1)	38 (23.9)				
III	177 (85.9)	121 (76.1)				
Unknown / NA	21	19				
Nuclear grade			0.75	0.87	0.96	(0.61 to 1.53)
1-2	91 (43.8)	74 (46.0)				
3	117 (56.2)	87 (54.0)				
Unknown / NA	19	17				

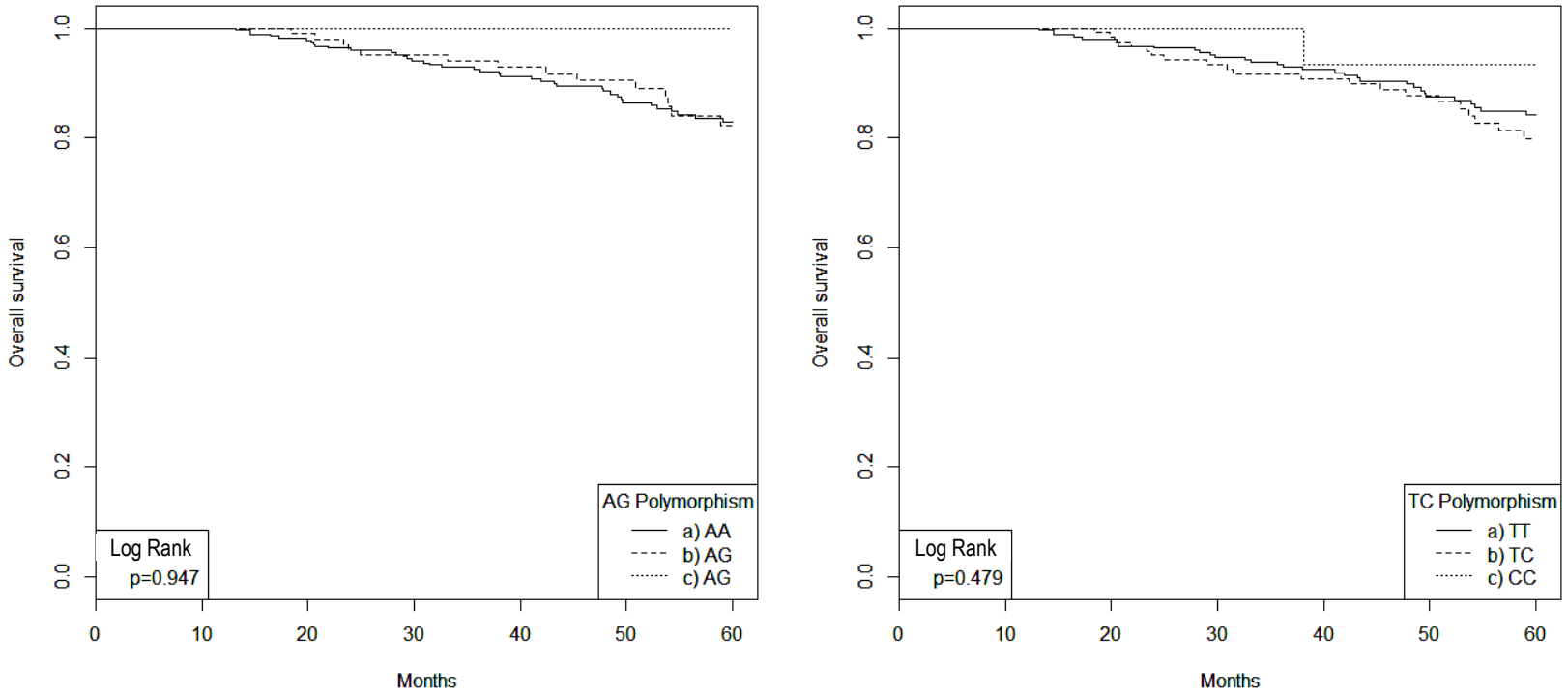


Figure 1. Kaplan-Meyer depiction of the overall survival as related to the *A4889G* (left) and *T6235C* (right) genotypes.

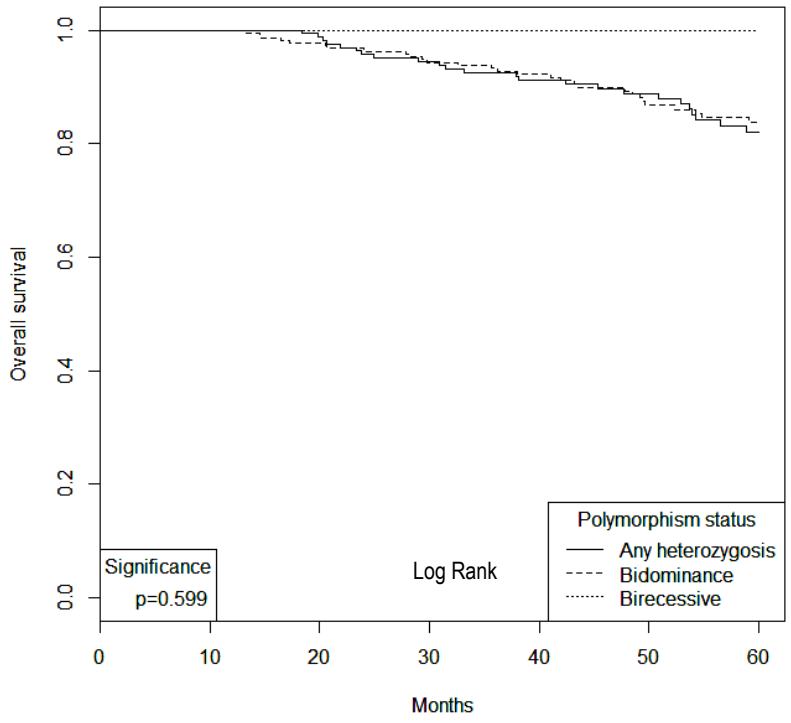


Figure 2. Kaplan-Meyer depiction of the overall survival as related to the combined *A4889G* and *T6235C* genotypes

4. Discussão

No presente estudo, quando se avaliou toda a população de mulheres, sejam elas com tumores positivos ou não para receptores esteróides, os polimorfismos *A4889G* e *T6235C* do gene *CYP1A1* estiveram associados a menor agressividade do tumor, mas este achado não se correlacionou com a maior sobrevida global. Os polimorfismos também foram mais prevalentes em mulheres caucasianas, as quais formam a maioria da amostra deste estudo. Quando restringimos as análises ao grupo de mulheres com tumores positivos para receptores esteróides e que fizeram uso de tamoxifeno como hormonioterapia adjuvante, a presença dos polimorfismos não esteve associada a nenhuma das características clínicas e histopatológicas estudadas. Além disso, os dois polimorfismos não estiveram correlacionados, isoladamente ou de forma combinada, com a sobrevida global.

As possíveis implicações clínicas dos efeitos laboratorialmente comprovados dos polimorfismos do *CYP1A1* não foram confirmadas neste estudo, sobretudo no grupo de mulheres em que era esperado encontrar as associações mais

importantes, ou seja, aquelas com tumores RE/RP positivos. Em tese, uma maior exposição desses tumores aos estrógenos e uma menor proteção conferida pelo tamoxifeno poderiam levar a maior taxa de progressão e redução da sobrevida, mas isto não foi detectado na amostra deste estudo. Em futuros estudos pretende-se contar com medições dos níveis plasmáticos das substâncias potencialmente afetadas pela CYP, o que daria uma informação importante sobre a penetrância dos polimorfismos nesta amostra.

Estudou-se uma população de mulheres brasileiras do Sudeste, com grande número de descendentes de europeus (72, 74). Em estudos anteriores, foi detectada uma prevalência maior dos polimorfismos *A4889G* e *T6235C* em caucasianos. Ao estudar mulheres nigerianas, Okobia e cols (60) encontraram uma única pessoa com o polimorfismo *A4889G*, heterozigoto, entre 250 pacientes com câncer. Este mesmo polimorfismo tem frequências relatadas de alelos de 0,69 e 0,31 entre as mulheres japonesas (75), e estudos em mulheres asiáticas têm indicado que mais de 10% da população possui genótipos variantes (76). No entanto, em caucasianos, as frequências alélicas dos polimorfismos *A4889G* e *T6235C* são relativamente baixas (77). A maioria das pesquisas sobre os polimorfismos do gene *CYP1A1* enfocam a associação destes com o risco para o desenvolvimento do câncer de mama. Esse não foi o objetivo deste estudo, pois procuramos avaliar as implicações clínicas e patológicas dos polimorfismos do gene *CYP1A1* em mulheres que já haviam desenvolvido a doença. Entretanto, vale comentar que o gene *CYP1A1* é altamente polimórfico e, portanto, as estimativas de risco de câncer de mama e as manisfestações

clínicas e patológicas dos tumores nos portadores de seus polimorfismos variam entre estudos (78). É bom ressaltar que, em populações caucasianas semelhantes à que estudamos, nenhuma relação entre os polimorfismos *A4889G* e *T6235C* e risco de câncer de mama foi encontrado. O único estudo onde os autores relataram um aumento do risco de desenvolver câncer de mama em mulheres com polimorfismos do *CYP1A1* foi baseado em uma população de mulheres africanas, em que a prevalência do SNP é sabidamente mais elevada do que aquela em mulheres brancas e asiáticas (79).

Retornando ao objetivo deste estudo, que foi analisar as associações entre a presença dos polimorfismos e as características patológicas e clínicas dos tumores de mama, existe apenas uma pesquisa semelhante à esta, onde os autores investigaram a associação dos polimorfismos com as características morfológicas dos tumores de mama (55). Naquele estudo, o polimorfismo *A4889G* foi associado com tumores de alto grau, um achado contrário ao deste estudo. No entanto, naquele estudo, o polimorfismo *T6235C* não esteve associado com nenhuma das características do tumor.

Todo o embasmento teórico desta tese baseia-se no fato conhecido de que o gene *CYP1A1* participa da regulação da hidroxilação do estradiol e estrona em metabólitos que subsequentemente sofrerão metilação. É por essa via que se formam metabólitos com fortes propriedades estrogênicas, os quais têm sido associados à carcinogênese induzida por estrogênio em animais de laboratório e humanos (80,81,82). Em modelos animais, tumores com receptores de estrógenos positivos progridem de baixo a alto grau, por causa da aquisição de

instabilidade genética e acúmulo de eventos genéticos estocásticos, devido à presença de estrogênio e seus metabólitos (83). O que nunca se demonstrou, entretanto, é se os metabólitos de estrogênio produzidos a partir da ocorrência dos polimorfismos do sistema citocromo P-450 poderiam induzir a formação de tumores bem diferenciados, a exemplo do que ocorre em anciãs e tumores decorrentes do uso de estrógenos exógenos. No presente estudo, corroborando esta tese, foi encontrada prevalência discretamente maior de tumores de baixo grau em mulheres portadoras da alteração genômica.

Apesar de a proporção de tumores de alto grau nas portadoras de polimorfismos do *CYP1A1* ter sido menor na amostra deste estudo, não observamos diferenças na sobrevivência global das mulheres. Vale notar que o grau do tumor é um fator de risco bem estabelecido para a recorrência da doença e menor sobrevida (84). É possível que a coorte deste estudo não tenha sido suficientemente numerosa, e que o tempo de seguimento de cinco anos não tenha sido suficientemente longo para permitir a detecção de pequenas diferenças na sobrevivência relacionadas com a presença dos SNP. Contribuiu para isso a relativamente baixa prevalência dos polimorfismos na população analisada neste estudo, preponderantemente caucasiana. Futuros trabalhos derivados desta casuística poderão mensurar, a longo prazo, este impacto suspeitado na sobrevida global.

Em conclusão, não foi detectada qualquer diferença no comportamento clínico do câncer de mama em portadoras de polimorfismos do gene *CYP1A1* em comparação com não portadoras. Se a falta de associação entre estes

polimorfismos e prognóstico da doença no presente estudo é conseqüência da baixa prevalência do SNP na população estudada ou outro artefato metodológico desconhecido, não sabemos. Os dados laboratoriais que apoiaram esta pesquisa apontam para uma relação plausível entre os polimorfismos *A4889G* e *T6235C* do gene *CYP1A1* e carcinomas ductais invasivos bem-diferenciados, o que foi demonstrado nesta casuística.

5. Conclusões

- **Artigo 1:** Os polimorfismos do gene *CYP1A1* estiveram positivamente associados com câncer de mama esporádico de baixo grau histológico. Entretanto, esta associação não se traduziu em melhor sobrevida global em cinco anos.

- **Artigo 2:** Em mulheres usuárias de tamoxifeno (como opção de hormonioterapia adjuvante) por câncer de mama esporádico receptor de esteroides positivos, os polimorfismos do gene *CYP1A1* não se associaram com as características patológicas do tumor e com a sobrevida global.

6. Referências Bibliográficas

1. Howlader N, Noone AM, Krapcho M, Neyman N, Aminou R, Altekruse SF et al. SEER Cancer Statistics Review, 1975-2009 (Vintage 2009 Populations), National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2009 _pops09/, based on November 2011 SEER data submission, posted to the SEER web site, 2012 (http://seer.cancer.gov/statfacts/html/breast.html)

2. Brasil, Ministério da Saúde, Instituto Nacional do Câncer. Estimativas 2012, Incidência de Câncer no Brasil, pág. 33. INCA [on line]. Rio de Janeiro. http://www1.inca.gov.br/estimativa/2012/estimativa20122111.pdf [acesso em 24 de setembro de 2012]

3. Kelsey JL, Gammon MD, John EM. Reproductive factors and breast cancer. Epidemiol Rev. 1993;15(1):36-47.

4. Pike MC, Krailo MD, Henderson BE, Casagrande JT, Hoel DG. Hormonal risk factors, breast tissue age, and the age-incidence of breast cancer. Nature 1983;303(5920):767-70.

5. RussoJ, Russo IH. Biological and molecular bases of mammary carcinogenesis. Lab Invest 1987;57(2):112-37.

6. Hardy EE, Pinotti JA, Osis MJ, Faundes A. Reproductive variables and risk of breast cancer: a case-control study carried out in Brazil. Bol Oficina Sanit Panam 1993;115(2):93-102.

7. Dumitrescu RG, Cotarla I. Understanding breast cancer risk -- where do we stand in 2005? J Cell Mol Med. 2005;9(1):208-21.

8. Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML et al; Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial. JAMA. 2002;288(3):321-33.

9. Chlebowski RT, Anderson GL, Gass M, Lane DS, Aragaki AK, Kuller LH et al; WHI Investigators. Estrogen plus progestin and breast cancer incidence and mortality in postmenopausal women. JAMA. 2010;304(15):1684-92.

10. Singletary KW, Gapstur SM. Alcohol and breast cancer: review of epidemiologic and experimental evidence and potential mechanisms. JAMA. 2001;286(17): 2143-51.

11. Hamajima N, Hirose K, Tajima K, Rohan T, Calle EE, Heath CW Jr et al. Alcohol, tobacco and breast cancer -- collaborative reanalysis of individual data from 53 epidemiological studies, including 58,515 women with breast cancer and 95,067 women without the disease. Br J Cancer. 2002;87(11): 1234-45.

12. Terry PD, Rohan TE. Cigarette smoking and the risk of breast cancer in women: a review of the literature. Cancer Epidemiol Biomarkers Prev. 2002;11(10Pt1):953-71.

13. Coyle YM. The effect of environment on breast cancer risk. Breast Cancer Res Treat. 2004;84(3):273-88.

14. Nagata C, Mizoue T, Tanaka K, Tsuji I, Wakai K, Inoue M, et al [Research Group for the Development and Evaluation of Cancer Prevention Strategies in Japan]. Tobacco smoking and breast cancer risk: an evaluation based on a systematic review of epidemiological evidence among the Japanese population. Jpn J Clin Oncol. 2006;36(6):387-94.

15. Maxwell GL, Risinger JI. Racial disparities research: it's not just black and white. Gynecol Oncol. 2006;101(2):194-7.

16. Tammemagi CM. Racial/ethnic disparities in breast and gynecologic cancer treatment and outcomes. Curr Opin Obstet Gynecol. 2007;19(1):31-6.

17. Maskarinec G, Sen C, Koga K, Conroy SM. Ethnic differences in breast cancer survival: status and determinants. Womens Health (Lond Engl). 2011;7(6):677-87.

18. Newman B, Austin MA, Lee M, King MC. Inheritance of human breast cancer: evidence for autosomal dominant transmission in high-risk families. Proc Natl Acad Sci USA. 1988;85(9):3044-8.

19. Peto J, Easton DF, Matthews FE, Ford D, Swerdlow AJ. Cancer mortality in relatives of women with breast cancer: the OPCS Study. Office of Population Censuses and Surveys. Int J Cancer. 1996;65(3):275-83.

20. Levy-Lahad E, Friedman E. Cancer risks among BRCA1 and BRCA2 mutation carriers. Br J Cancer. 2007;96(1):11-5.

21. Tempfer CB, Hefler LA, Schneeberger C, Huber JC. How valid is single nucleotide polymorphism (SNP) diagnosis for the individual risk assessment of breast cancer? Gynecol Endocrinol. 2006;22(3):155-9.

22. Paik S, Shak S, Tang G, Kim C, Baker J, Cronin M et al. A multigene assay to predict recurrence of tamoxifen-treated, node-negative breast cancer. N Engl J Med. 2004;351(27):2817-26.

23. Kaklamani V. A genetic signature can predict prognosis and response to therapy in breast cancer: Oncotype DX. Expert Rev Mol Diagn. 2006;6(6):803-9.

24. Kaklamani VG, Gradishar WJ. Gene expression in breast cancer. Curr Treat Options Oncol. 2006;7(2):123-8.

25. Paik S, Tang G, Shak S, Kim C, Baker J, Kim W et al. Gene expression and benefit of chemotherapy in women with node-negative, estrogen receptor-positive breast cancer. J Clin Oncol. 2006;24(23):3726-34.

26. Dunning AM, Healey CS, Pharoah PD, Teare MD, Ponder BA, Easton DF. A systematic review of genetic polymorphisms and breast cancer risk. Cancer Epidemiol Biomarkers Prev. 1999;8(10):843-54.

27. Ramalhinho AC, Fonseca-Moutinho JA, Breitenfeld Granadeiro LA. Positive association of polymorphisms in estrogen biosynthesis gene, CYP19A1, and metabolism, GST, in breast cancer susceptibility. DNA Cell Biol. 2012;31(6):1100-6

28. Zheng T, Holford TR, Zahm SH, Owens PH, Boyle P, Zhang Y et al. Cigarette smoking, glutathione-s-transferase M1 and T1 genetic polymorphisms, and breast cancer risk (United States). Cancer Causes Control. 2002;13(7):637-45.

29. AJCC: Breast. In: Edge SB, Byrd DR, Compton CC, et al., eds.: AJCC Cancer Staging Manual. 7th ed. New York, NY: Springer, 2010, pp 347-76.

30. Cianfrocca M, Goldstein LJ. Prognostic and predictive factors in early-stage breast cancer. Oncologist. 2004;9(6):606-16.

31. Rosen PP, Oberman HA. Tumors of the mammary gland. Armed Forces Institute of Pathology, Washington D.C., 1993.

32. Bloom HJ, Richardson WW. Histological grading and prognosis in breast cancer; a study of 1409 cases of which 359 have been followed for 15 years. Br J Cancer. 1957;11(3):359-77.

33. Scarff RW, Torloni H. Histological typing of breast tumors. International histological classification of tumors, No.2. World Health Organization, Geneva, 1968:510-13.

34. Frierson HF Jr, Wolber RA, Berean KW, Franquemont DW, Gaffey MJ, Boyd JC et al. Interobserver reproducibility of the Nottingham modification of the Bloom and Richardson histologic grading scheme for infiltrating ductal carcinoma. Am J Clin Pathol. 1995;103(2):195-8.

35. Fisher B, Bauer M, Wickerham DL, Redmond CK, Fisher ER, Cruz AB et al. Relation of number of positive axillary nodes to the prognosis of patients with primary breast cancer. An NSABP update. Cancer. 1983;52(9):1551-7.

36. Mittra I, MacRae KD. A meta-analysis of reported correlations between prognostic factors in breast cancer: does axillary lymph node metastasis represent biology or chronology? Eur J Cancer. 1991;27(12):1574-83.

37. Clark GM, McGuire WL. New biologic prognostic factors in breast cancer. Oncology (Williston Park). 1989;3(5):49-54; discussion 58,61,64.

38. Gasparini G, Weidner N, Bevilacqua P, Maluta S, Dalla-Palma P, Caffo O et al. Tumor microvessel density, p53 expression, tumor size, and peritumoral lymphatic vessel invasion are relevant prognostic markers in node-negative breast carcinoma. J Clin Oncol. 1994;12(3):454-66.

39. Mirza AN, Mirza NQ, Vlastos G, Singletary SE. Prognostic factors in node-negative breast cancer: a review of studies with sample size more than 200 and follow-up more than 5 years. Ann Surg. 2002;235(1):10-26.

40. Cattoretti G, Rilke F, Andreola S, D'Amato L, Delia D. P53 expression in breast cancer. Int J Cancer. 1988;41(2):178-83.

41. Lovekin C, Ellis IO, Locker A, Robertson JF, Bell J, Nicholson R et al. c-erbB-2 oncoprotein expression in primary and advanced breast cancer. Br J Cancer. 1991;63(3):439-43.

42. Gasparini G, Gullick WJ, Bevilacqua P, Sainsbury JR, Meli S, Boracchi P et al. Human breast cancer: prognostic significance of the c-erbB-2 oncoprotein compared with epidermal growth factor receptor, DNA ploidy, and conventional pathologic features. J Clin Oncol. 1992;10(5):686-95.

43. Toikkanen S, Helin H, Isola J, Joensuu H. Prognostic significance of HER-2 oncoprotein expression in breast cancer: a 30-year follow-up. J Clin Oncol. 1992;10(7):1044-8.

44. Martinazzi M, Crivelli F, Zampatti C, Martinazzi S. Relationship between p53 expression and other prognostic factors in human breast carcinoma. An immunohistochemical study. Am J Clin Pathol. 1993;100(3):213-7.

45. Tsuchiya Y, Nakajima M, Yokoi T. Cytochrome P450-mediated metabolism of estrogens and its regulation in human. Cancer Lett. 2005;227(2):115-24.

46. Aynacioglu AS, Cascorbi I, Mrozikiewicz PM, Roots I. High frequency of CYP1A1 mutations in a Turkish population. Arch Toxicol. 1998;72:215-218.

47. Wang J, Deng Y, Li L, Kuriki K, Ding J, Pan X et al. Association of GSTM1, CYP1A1 and CYP2E1 genetic polymorphisms with susceptibility to lung adenocarcinoma: a case-control study in Chinese population. Cancer Sci. 2003;94:448-452.

48. Canalle R, Burim RV, Tone LG, Takahashi CS. Genetic polymorphisms and susceptibility to childhood acute lymphoblastic leukemia. Environ Mol Mutagen. 2004;43(2):100-109.

49. Hefler LA, Tempfer CB, Grimm C, Lebrecht A, Ulbrich E, Heinze G et al. Estrogen-metabolizing gene polymorphisms in the assessment of breast carcinoma risk and fibroadenoma risk in Caucasian women. Cancer. 2004;101(2):264-269.

50. Joseph T, Kusumakumary P, Chacko P, Abraham A, Radhakrishna Pillai M. Genetic polymorphisms of CYP1A1, CYP2D6, GSTM1, GSTT1 and susceptibility to acute lymphoblastic leukaemia in Indian children. Pediatr Blood Cancer. 2004;43:560-567.

51. Li Y, Millikan RC, Bell DA, Cui L, Tse CK, Newman B et al. Cigarette smoking, cytochrome P4501A1 polymorphisms, and breast cancer among African-American and white women. Breast Cancer Res. 2004;6:R460-R473.

52. Li Y, Millikan RC, Bell DA, Cui L, Tse CK, Newman B et al. Polychlorinated biphenyls, cytochrome P450 1A1 (CYP1A1) polymorphisms, and breast cancer risk among African American women and white women in North Carolina: a population-based case-control study. Breast Cancer Res. 2005;7(1):R12-8.

53. Seremak-Mrozikiewicz A, Drews K, Semczuk A, Jakowicki JA, Mrozikiewicz PM. CYP1A1 alleles in female genital cancers in the Polish population. Eur J Obstet Gynecol Reprod Biol. 2005;118(2):246-50.

54. Shin A, Kang D, Choi JY, Lee KM, Park SK, Noh DY et al. Cytochrome P450 1A1 (CYP1A1) polymorphisms and breast cancer risk in Korean women. Exp Mol Med. 2007;39(3):361-6.

55. Singh N, Mitra AK, Garg VK, Agarwal A, Sharma M, Chaturvedi R et al. Association of CYP1A1 polymorphisms with breast cancer in North Indian women. Oncol Res. 2007;16(12):587-97.

56. Diergaarde B, Potter JD, Jupe ER, Manjeshwar S, Shimasaki CD, Pugh TW et al. Polymorphisms in genes involved in sex hormone metabolism, estrogen plus progestin hormone therapy use, and risk of postmenopausal breast cancer. Cancer Epidemiol Biomarkers Prev. 2008;17(7):1751-9.

57. Surekha D, Sailaja K, Rao DN, Padma T, Raghunadharao D, Vishnupriya S. Association of CYP1A1*2 polymorphisms with breast cancer risk: a case control study. Indian J Med Sci. 2009;63(1):13-20.

58. Parl FF; Dawling S, Roodi N, Crooke PS. Estrogen metabolism and breast cancer: a risk model. Ann NY Acad Sci. 2009;1155:68-75.

59. da Fonte de Amorim L, Rossini A, Mendonça G, Lotsch P, de Almeida Simão T, de Moura Gallo C et al. CYP1A1, GSTM1, and GSTT1 polymorphisms and breast cancer risk in Brazilian women. Cancer Lett. 2002;181(2):179-186.

60. Okobia M, Bunker C, Zmuda J, Kammerer C, Vogel V, Uche Eet al. Cytochrome P4501A1 genetic polymorphisms and breast cancer risk in Nigerian women. Breast Cancer Res Treat. 2005;94(3):285-93.

61. Shen Y, Li DK, Wu J, Zhang Z, Gao E. Joint effects of the CYP1A1 MspI, ERalpha PvuII, and ERalpha XbaI polymorphisms on the risk of breast cancer: results from a population-based case-control study in Shanghai, China. Cancer Epidemiol Biomarkers Prev. 2006;15(2):342-7.

62. Zhu K, Hunter S, Payne-Wilks K, Sutcliffe C, Bentley C, Roland CL et al. Potential differences in breast cancer risk factors based on CYP1A1 MspI and African-American-specific genotypes. Ethn Dis. 2006;16(1):207-15.

63. Gulyaeva LF, Mikhailova ON, PustyInyak VO, Kim IV 4th, Gerasimov AV, Krasilnikov SE et al. Comparative analysis of SNP in estrogen-metabolizing enzymes for ovarian, endometrial, and breast cancers in Novosibirsk, Russia. Adv Exp Med Biol. 2008;617:359-66.

64. Shimada N, Iwasaki M, Kasuga Y, Yokoyama S, Onuma H, Nishimura H et al. Genetic polymorphisms in estrogen metabolism and breast cancer risk in case-control studies in Japanese, Japanese Brazilians and non-Japanese Brazilians. J Hum Genet. 2009;54(4):209-15.

65. Yamaguti GG, Lourenço GJ, Costa FF, Lima CS. High risk of 'de novo' acute myeloid leukaemia in individuals with cytochrome P450 A1 (CYP1A1) and NAD(P)H:quinone oxidoreductase 1 (NQO1) gene defects. Eur J Haematol. 2009;83(3):270-2.

66. Sreerama L, Sladek NE. Cellular levels of class 1 and class 3 aldehyde dehydrogenases and certain other drug-metabolizing enzymes in human breast malignancies. Clin Cancer Res. 1997;3(11):1901-14.

67. Montero R, Serrano L, Dávila VM, Ito A, Plancarte A. Infection of rats with Taenia taeniformis metacestodes increases hepatic CYP450, induces the activity of CYP1A1, CYP2B1 and COH isoforms and increases the genotoxicity of the procarcinogens benzo[a]pyrene, cyclophosphamide and aflatoxin B(1). Mutagenesis. 2003;18(2):211-6.

68. Oesch-Bartlmowicz B, Oesch F. Modulation of mutagenicity by phosphorylation of mutagen-metabolizing enzymes. Arch Biochem Biophys. 2004;423(1):31-6.

69. Sharma M, Shubert DE, Sharma M, Lewis J, McGarrigle BP, Bofinger DP et al. Biotransformation of tamoxifen in a human endometrial explant culture model. Chem Biol Interact. 2003;146(3):237-49.

70. Parte P, Kupfer D. Oxidation of tamoxifen by human flavin-containing monooxygenase (FMO) 1 and FMO3 to tamoxifen-N-oxide and its novel reduction back to tamoxifen by human cytochromes P450 and hemoglobin. Drug Metab Dispos. 2005;33(10):1446-52.

71. van Schaik RH. CYP450 pharmacogenetics for personalizing cancer therapy. Drug Resist Updat. 2008;11(3):77-98.

72. Alves-Silva J, da Silva Santos M, Guimarães PE, Ferreira AC, Bandelt HJ, Pena SD et al. The ancestry of Brazilian mtDNA lineages. Am J Hum Genet. 2000;67(2):444-61.

73. Carvalho-Silva DR, Santos FR, Rocha J, Pena SD. The phylogeography of Brazilian Y-chromosome lineages. Am J Hum Genet. 2001;68(1):281-6.

74. Gonçalves FT, Francisco G, de Souza SP, Luiz OC, Festa-Neto C, Sanches JA et al. European ancestry and polymorphisms in DNA repair genes modify the risk of melanoma: a case-control study in a high UV index region in Brazil. J Dermatol Sci. 2011;64(1):59-66.

75. Klauber N, Parangi S, Flymn E, Hamel E, D'Amato RJ: Inhibition of angiogenesis and breast cancer in mice by the microtubulin inhibitors 2-methoxyestradiol and taxol. Cancer Res. 1997;57:81–86.

76. Huang CS, Shen CY, Chang KJ, Hsu SM, Chern HD: Cytochrome P4501A1 polymorphism as a susceptibility factor for breast cancer in postmenopausal Chinese women in Taiwan. Br J Cancer. 1999;80(11):1838–1843.

77. Rebbeck TR, Rosvold EA, Duggan DJ, Zhang J, Buetow KH: Genetics of CYP1A1: Coamplification of specific alleles by Polymerase Chain Reaction and association with breast cancer. Cancer Epidemiol Biomarkers Prev. 1994;3:511–514.

78. Taioli E, Trachman J, Chen X, Toniolo P, Garte SJ. A CYP1A1 restriction fragment length polymorphism is associated with breast cancer in African-American women. Cancer Res. 1995;55:3757–3758.

79. Kato I, Cichon M, Yee CL, Land S, Korczak JF. African American-preponderant single nucleotide polymorphisms (SNPs) and risk of breast cancer. Cancer Epidemiol. 2009;33(1):24-30.

80. Schneider J, Huh MM, Bradlow LH, Fishman J: Antiestrogen action of 2-hydrxyestrone on MCF-7 human breast cancer cells. J Biol Chem. 1984;259:4840–4845.

81. Fotsis T, Zhang Y, Pepper MS, Aldercreutz H, Montesano R, Nawroth PP, Schwelgerer L: The endogenous estrogen metabolite 2-methoxyestradiol inhibits angiogenesis and suppresses tumor growth. Nature. 1994;368:237–239.

82. Cushman M, He HM, Katzenellenbogan JA, Lin CM, Hamel E: Synthesis, antitubulin and antimitotic activity, and cytotoxicity of analogs of 2-methoxyestradiol, an endogenous mammalian metabolite of estradiol that inhibits tubulin polymerization by binding to the colchicines binding site. J Med Chem. 1995;38:2041–2049.

83. Lopez-Garcia MA, Geyer FC, Lacroix-Triki M, Marchió C, Reis-Filho JS.Breast cancer precursors revisited: molecular features and progression pathways. Histopathology. 2010;57(2):171-92.

84. Elston CW, Ellis IO: Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. Histopathology 1991;19:403–410.

7. Anexos

7.1. Anexo 1 – Check List

ASSOCIAÇÃO DOS POLIMORFISMOS *A4889G* E *T6235C* DO GENE *CYP1A1* COM CARACTERÍSTICAS CLÍNICAS E EPIDEMIOLÓGICAS DE CARCINOMAS DA MAMA

Pesquisador: Cassio Cardoso Filho

	SIM	**NÃO**
Sexo feminino	❑	❑
Idade $\geq$ 25 e $\leq$ 90 anos	❑	❑
Antecedente pessoal negativo para câncer de mama	❑	❑
Conhece seus antecedentes familiares	❑	❑
Antecedente familiar de primeiro grau negativo para câncer de mama	❑	❑
Grau de compreensão adequado frente aos questionamentos do pesquisador	❑	❑

Se todas as questões forem respondidas como “SIM”, o sujeito será incluído no projeto sob o número: _____

7.2. Anexo 2 – Ficha para coleta de dados

ASSOCIAÇÃO DOS POLIMORFISMOS *A4889G* E *T6235C* DO GENE *CYP1A1* COM CARACTERÍSTICAS CLÍNICAS E EPIDEMIOLÓGICAS DE CARCINOMAS DA MAMA

Pesquisador: Cassio Cardoso Filho

Número na pesquisa: _____ Data programada da cirurgia: ___ / ___ / _____

1. Idade:_____ anos ❑ ign
2. Idade à menarca:_____ anos ❑ ign
3. Idade à menopausa:_____ anos ❑ não se aplica ❑ ign
4. Idade à primeira gravidez a termo: _____ anos ❑ não se aplica ❑ ign
5. Lactação (por tempo maior do que 06 meses): ❑ sim ❑ não
6. TRH por mais de 05 anos: ❑ sim ❑ não ❑ ign
7. Hábito tabágico:
   ❑ nunca fumou, ou parou há mais de 10 anos
   ❑ fumante, ou parou há menos de 10 anos (inclusive)
8. Etilismo: ❑ sim ❑ não
9. Etnia:
   [1] Branca, sem antecedente de miscigenação com outras etnias
   [2] Parda, ou branca com antecedente de miscigenação com etnia negra
   [3] Negra
   [4] Amarela
   [7] Outros
10. GH ❑ 1 ❑ 2 ❑ 3 ❑ ign ❑ não se aplica
11. GN ❑ 1 ❑ 2 ❑ 3 ❑ ign ❑ não se aplica
12. RE ❑ (+) ❑ (–) ❑ ign ❑ não se aplica
13. RP ❑ (+) ❑ (–) ❑ ign ❑ não se aplica
14. Estadiamento ❑ 0 ❑ Ia ❑ Ib ❑ IIa ❑ IIb ❑ IIIa ❑ IIIb ❑ IIIc ❑ IV
    ❑ ignorado ❑ não se aplica

Nome: _____ HC:_____

7.3. Anexo 3 – Carta de aprovação do projeto CP – DTG/FCM/UNICAMP

UNICAMP

Comissão de Pesquisa do DTG / CAISM

Campinas, 18 de outubro de 2011.

Protocolo nº: 048/2011

A Comissão de Pesquisa do DTG/CAISM tomou ciência do protocolo de pesquisa *"Polimorfismos CYP1A1 T6235C e A4889G e NQO229 bp-I/D e A237C no risco e prognóstico do câncer de mama esporádico"* do pesquisador Cássio Cardoso Filho, orientado pela Profa. Dra. Maria Salete Costa Gurgel, aprovado anteriormente pelo Comitê de Ética em Pesquisa, parecer CEP nº 581/02.

Atenciosamente,

PROF. DR. JOSÉ GUILHERME CECATTI
Presidente da Comissão de Pesquisa do DTG/CAISM

Rua Alexander Flemming, n.º101 – Cidade Universitária Zeferino Vaz – Campinas-SP
Fone: (19) 3521-9400
comissaopesquisa@caism.unicamp.br

7.4. Anexo 4 – Carta de aprovação do projeto no CEP – FCM/UNICAMP

FACULDADE DE CIÊNCIAS MÉDICAS
COMITÊ DE ÉTICA EM PESQUISA

www.fcm.unicamp.br/fcm/pesquisa

CEP, 24/05/11.
(PARECER CEP: N° 581/2002)

PARECER

I – IDENTIFICAÇÃO:

PROJETO: **"INFLUÊNCIA DO POLIMORFISMO D104N DO GENE COL18A1 NA SUSCEPTIBILIDADE AO CÂNCER DE MAMA ESPORÁDICO".**

PESQUISADOR RESPONSÁVEL: Carmen Sílvia Passos Lima

II – PARECER DO CEP.

O Comitê de Ética em Pesquisa da Faculdade de Ciências Médicas da UNICAMP tomou ciência e aprovou o adendo que inclui o projeto **"POLIMORFISMOS CYP1A1 T6235C E A4889G E NQO229 BP-I/D E A237C NO RISCO E PROGNÓSTICO DO CÂNCER DE MAMA ESPORÁDICO"**, referente ao protocolo de pesquisa supracitado.

O conteúdo e as conclusões aqui apresentados são de responsabilidade exclusiva do CEP/FCM/UNICAMP e não representam a opinião da Universidade Estadual de Campinas nem a comprometem.

III – DATA DA REUNIÃO.

Homologado na V Reunião Ordinária do CEP/FCM, em 24 de maio de 2011.

Prof. Dr. Carlos Eduardo Steiner
PRESIDENTE do COMITÊ DE ÉTICA EM PESQUISA
FCM / UNICAMP

Comitê de Ética em Pesquisa - UNICAMP
Rua: Tessália Vieira de Camargo, 126
Caixa Postal 6111
13083-887 Campinas – SP

FONE (019) 3521-8936
FAX (019) 3521-7187
cep@fcm.unicamp.br

7.5. Anexo 5 – Termo de Consentimento Livre e Esclarecido

ASSOCIAÇÃO DOS POLIMORFISMOS *A4889G* E *T6235C* DO GENE *CYP1A1* COM CARACTERÍSTICAS CLÍNICAS E EPIDEMIOLÓGICAS DE CARCINOMAS DA MAMA

Pesquisador: Cassio Cardoso Filho

Nome:_____

Idade:_____anos **RG**:_____ **HC**:_____

Endereço:_____

_____

Nome do responsável legal (se aplicável):_____

RG:_____ **Grau de parentesco**:_____

Endereço:_____

Aceito participar de um estudo no CAISM – UNICAMP sobre alterações genéticas que podem estar envolvidas no aparecimento do câncer de mama, e no seu tratamento. Isto ainda não está comprovado, e a análise do meu DNA poderá ajudar a esclarecer essa dúvida. Assim eu contribuirei para um melhor entendimento dos fatores que levam ao surgimento do câncer de mama, para o progresso de exames diagnósticos e para novas formas de tratamento. Minha contribuição será autorizar a utilização de:

- uma parte do sangue colhido antes da cirurgia ou da quimioterapia para a análise de DNA. Compreendo que não terei prejuízos com a realização desta análise.
- informações do meu prontuário médico, sabendo que meus dados pessoais de identificação serão mantidos em sigilo pelo pesquisador.

Fui informada que posso sair do estudo a qualquer momento e que isto não vai prejudicar o meu tratamento no CAISM. Se tiver qualquer dúvida sobre o estudo poderei procurar o Dr. Cassio Cardoso Filho, ou o Prof. Dr. Luís Otávio Zanatta Sarian, ou a Dra. Maria Salete Costa-Gurgel, Tel: (19) 3521-9305. Em caso de reclamações sobre qualquer procedimento do estudo, poderei procurar a secretaria do Comitê de Ética da FCM - UNICAMP, Tel: (19) 3521-8936. Eu li/ouvi o conteúdo deste termo e recebi esclarecimentos sobre as minhas dúvidas oralmente.

_____ _____

Assinatura do sujeito ou do responsável legal Assinatura do pesquisador

Campinas, ____ / _____ / _____