Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Malay Mody Date

A meta-analysis of the association between CYP2D6 genotype and resistance to tamoxifen treatment in breast cancer patients

By

Malay Mody
Master of Public Health

Epidemiology

_____

Timothy L. Lash
Committee Chair

A meta-analysis of the association between CYP2D6 genotype and resistance to tamoxifen treatment in breast cancer patients

By

Malay Mody

Bachelor of Science
University of Michigan
2019

Thesis Committee Chair: Timothy L. Lash, DSc, MPH

An abstract of
A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Epidemiology
2021

Abstract

A meta-analysis of the association between CYP2D6 genotype and resistance to tamoxifen treatment in breast cancer patients

By Malay Mody

Objective: To obtain summary measures of association between CYP2D6 genotype and breast cancer mortality or recurrence after tamoxifen treatment from published studies each using unique cohorts. Results will be used to investigate potential sources of heterogeneity in CYP2D6-tamoxifen studies, including the variants genotyped in a study, the DNA source, ethnic descent of the study cohort, study participants' menopausal status, or the estrogen receptor-status of their tumor.

Methods: This meta-analysis will include only original research and stratify groups based on their study characteristics. Analyses will be performed using Ahern et al.'s web tool on the Shiny platform, which runs the 'metafor' R package and provides measures of association and 95% confidence intervals. These values will then be compared in to evaluate which categories account for substantial heterogeneity.

Results: Of CYP2D6 variants genotyped, DNA source, ethnic descent of study cohort, menopausal status, and estrogen receptor status, only ethnic descent accounted for substantial heterogeneity. Studies using cohorts of patients of Asian descent resulted in RR (95% CI) = 2.39 (1.82,3.15), while those using cohorts of European patients resulted in RR (95% CI) = 1.25 (1.09, 1.43).

Conclusions: This meta-analysis demonstrates a heterogeneity in relative risks reported by manuscripts using Asian cohorts when compared to European cohorts. Future research may include further research on immortal person-time bias in CYP2D6-tamoxifen studies, research on the role of genes other than CYP2D6 in tamoxifen treatment outcomes, and clinical trials to investigate outcomes after using CYP2D6 genotyping to inform treatment decisions.

A meta-analysis of the association between CYP2D6 genotype and resistance to tamoxifen treatment in breast cancer patients

By

Malay Mody

Bachelor of Science
University of Michigan
2019

Thesis Committee Chair: Timothy L. Lash, DSc, MPH

A thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements for the degree of
Master of Public Health
in Epidemiology
2021

A meta-analysis of the association between CYP2D6 genotype and resistance to tamoxifen treatment in breast cancer patients

Introduction

A current point of contention in cancer epidemiology is the role that a breast cancer patient's CYP2D6 genotype may play in the efficacy of their tamoxifen treatment, and in turn, should their genotype be considered when constructing treatment options for the patient. Studies thus far have presented heterogeneous findings on the topic, suggesting both that CYP2D6 genotype can play a key role in a patient's response to tamoxifen therapy and that the impact of CYP2D6 is negligible and therefore not a factor in a patient's resistance to therapy. Some potential study characteristics causing this heterogeneity could be the CYP2D6 variants genotyped, the source of genotyped DNA, ethnic descent of the study cohort, the menopausal status of the study subjects, or the estrogen receptor status of the study subjects' tumors. This analysis seeks to elucidate potential sources of bias in CYP2D6-tamoxifen studies by focusing on those specific factors.

Variants Genotyped:

The most genotyped variants included CYP2D6*1, *2, *4, *10, and *41. In Asian cohorts, it is notable that the *10 variant is the most prevalent. Meanwhile in European cohorts, the *1, *2, and *4 variants are more prevalent. This difference is important because the CYP2D6*10 variant confers reduced tamoxifen metabolism while the CYP2D6*4 variant eliminates gene function (1). To study potential associations, the selected studies will be split into the following categories: CYP2D6*4 only, CYP2D6*4 + other CYP2D6 variants (but not CYP2D6*10), CYP2D6*10 only, and studies including

either CYP2D6*4 or CYP2D6*10. Since reduced CYP2D6 function suggests inhibited tamoxifen metabolism, it is expected that treatment would be less effective in CYP2D6*4 patients than CYP2D6*10 patients.

DNA Source:

Studies mainly used blood or tumor tissue samples as the source of DNA extracted for genotyping. This could be biasing results due to genotypical differences in germline tissue, such as blood, versus somatic tissue, such as a tumor. These differences are caused by chromosomal instability found in breast cancer tissue, causing a loss of an allele from the germline DNA and therefore potentially a loss of heterozygosity (LOH). The hypothesized bias would stem from the fact that tamoxifen is metabolized in the liver, not in the tumor, therefore resulting in genotype misclassification bias. With evidence of LOH at CYP2D6 appearing in approximately 40% of estrogen receptor-positive breast cancers, there is potential for LOH and misclassified genotypes to be affecting the validity of many studies (2). The selected set of studies will be sorted based on whether the genotyped DNA was extracted from tumor tissue/somatic genotype or non-neoplastic tissue/genomic genotype.

Ethnic Descent:

The two predominant study cohort locations are Europe and Asia. It is expected that the European cohorts are associated with normal to rapid tamoxifen metabolism due to greater prevalence of *1 and *2 variants. However, it is important to note that *4 variants are prevalent in Europeans as well, which confers elimination of CYP2D6 function. To the contrary, Asian cohorts are expected to be associated with inhibited

tamoxifen metabolism due to the large proportion of the population carrying the CYP2D6*10 variant (1). When comparing studies on Asian cohorts versus European cohorts, the hypothesized bias may skew relative risks to be higher in Asian cohorts where inhibited CYP2D6 function is prevalent throughout a large portion of the population due to the high prevalence of the CYP2D6*10 variant.

Menopausal status:

As tamoxifen is a treatment currently recommended for pre-menopausal women with estrogen receptor-positive breast tumors, study results may not be generalizable to premenopausal women if including predominantly postmenopausal study subjects. Therefore, it is pertinent to study biases presented by study cohorts predominantly (>90%) made up by postmenopausal women. This is because postmenopausal women already have reduced estrogen levels, potentially lessening the need for full tamoxifen doses. Meanwhile, premenopausal women demonstrate higher levels of estrogen and thereby have a greater need for full tamoxifen treatments and metabolic activation (1). Selected studies will be split based on if the study cohort was composed of over 90% postmenopausal/perimenopausal women or over 10% premenopausal women. The hypothesized bias may suggest a lower association between CYP2D6 genotype and effectiveness of tamoxifen treatment in cohorts with high prevalence of post and peri-menopausal women.

ER Status:

Analysis will be conducted to study if the estrogen receptor (ER) status makeup of cohorts may introduce bias by including mixed ER statuses as opposed to exclusively

ER-positive tumors. As tamoxifen is recommended for treatment of ER-positive tumors, this could be a potential source of bias causing heterogenous study results (1). If a study includes ER-negative tumors, we should expect to see a bias towards the null due to less-effective treatment outcomes.

To perform the analysis, the selected studies have been integrated into an online meta-analysis tool created by Dr. Thomas Ahern and based on the Shiny platform, which allows R functions to be performed via a web interface. By sorting studies into specific categories for each characteristic of interest, the aim is to compare relative risks and confidence intervals to determine which study characteristics may be responsible for heterogeneous results in CYP2D6-tamoxifen studies.

Methods

Literature Review:

The literature review included 99 studies, all of which were original research articles investigating potential resistance to tamoxifen treatment conferred by CYP2D6 gene variants in cohorts of breast cancer patients. For each study, several characteristics were extracted and recorded, including DNA source, CYP2D6 variants genotyped, Ethnic descent of patients in the cohort, menopausal status of the subjects, and ER status of the subjects. The criteria for including studies in analysis will be that they must be comparing breast cancer-specific mortality or recurrence based on homozygous CYP2D6 genotype, and each study included must be from a distinct cohort.

Meta-Analysis Tool:

Analysis was conducted using the meta-analysis tool previously described in Ahern et al., 2020 (3). This online tool allowed for utilization of a "conventional model" and fixed-effects framework, which also can be achieved by using the "metafor" package in R (3). This tool is essentially a user interface for this R package, allowing for selection of desired papers and then automatically including the tool's background information on CYP2D6/tamoxifen, objectives for meta-analysis, literature search and selection criteria, and study characteristics for each manuscript. The result is displayed as a forest plot, accompanied by an adjusted relative risk and its 95% confidence interval (3).

Analysis:

Analysis will focus on DNA source (tumor, non-neoplastic), CYP2D6 variants genotyped (CYP2D6*4 only, CYP2D6*10 only, CYP2D6 *4 and others no CYP2D6*10 , and CYP2D6*4 and CYP2D6*10 together), ethnic descent of study population (European, Asian), estrogen receptor status (ER-positive only, mixed ER status), and percentage of study population that is perimenopausal/postmenopausal (>90%, <90%). Using data previously extracted from each paper, each set of studies was input into the meta-analysis tool, yielding a forest plot, an adjusted relative risk, a 95% confidence interval for relative risk, and a p-value for a test of heterogeneity. Adjusted relative risks and confidence intervals will allow for comparison between groups and therefore greater insight into potential sources of heterogeneity.

Results

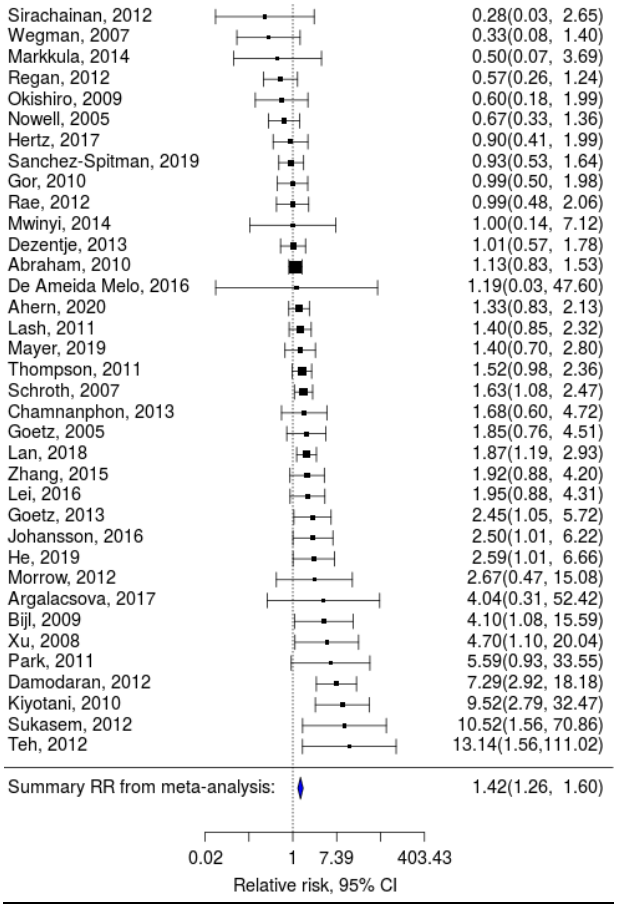
Summary:

After literature review, 36 papers comparing disease-specific mortality or recurrence rates between women with homozygous wild-type CYP2D6 and those homozygous for variants in unique cohorts were used for analysis. The 'Shiny' meta-analysis tool for CYP2D6 and tamoxifen produced the following results for each of our potential sources of heterogeneity in study results.

Category	# of Studies	Relative Risk	95% Confidence Interval
All	36	1.42	1.26, 1.80
CYP2D6*4 only	5	1.28	0.95, 1.71
CYP2D6*4, not CYP2D6*10	17	1.35	1.17, 1.56
CYP2D6*10 only	7	1.98	1.27, 3.08
CYP2D6*4 or CYP2D6*10	36	1.42	1.26, 1.60
Tumor DNA	10	1.22	1.00, 1.49
Non-neoplastic DNA	26	1.55	1.33, 1.81
European population	23	1.25	1.09, 1.43
Asian population	12	2.39	1.82, 3.15
>90% postmenopausal/perimenopausal	11	1.30	1.03, 1.65
>10% premenopausal	24	1.51	1.30, 1.74
Estrogen-positive tumor only	13	1.38	1.08, 1.76
Mixed estrogen-receptor statuses	23	1.43	1.25, 1.64

All Studies:

Studies in the meta-analysis include those cited by Ahern et al, 2017 (2) with the addition of (4-14). When including all studies, the summary relative risk was 1.42, with 95% confidence interval between 1.26 and 1.80. The test for heterogeneity produced p-value = 0.0001.

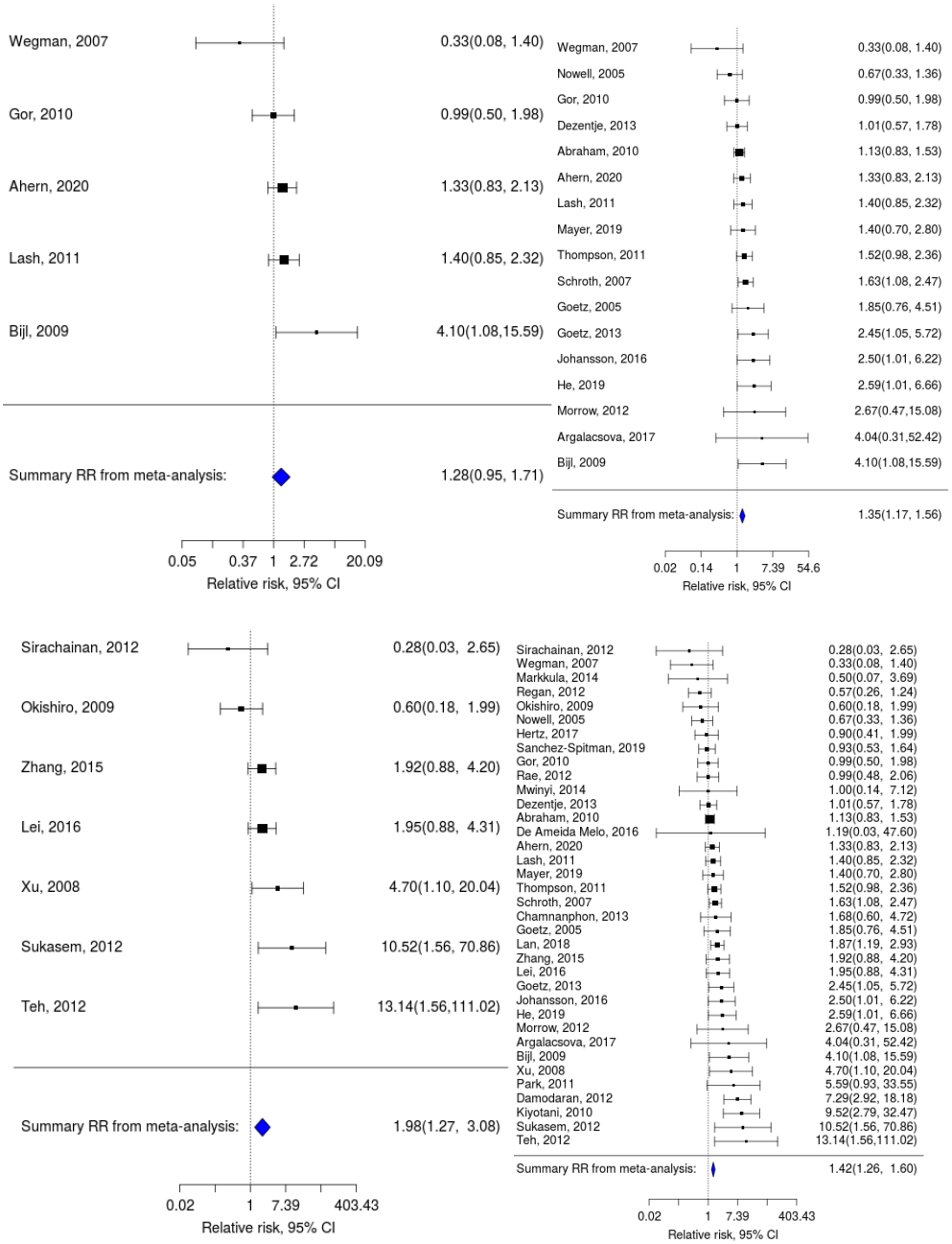


n = 36; RR (95% CI) = 1.42 (1.26, 1.80); Test for Heterogeneity p-value = 0.0001

Variants Genotyped:

When including 5 studies specifically including CYP2D6*4 genotype, Relative risk (95% confidence interval) was 1.28 (.95, 1.71). The p-value for the test for heterogeneity was 0.14. For the 17 studies including CYP2D6*4 and excluding CYP2D6*10, the resulting

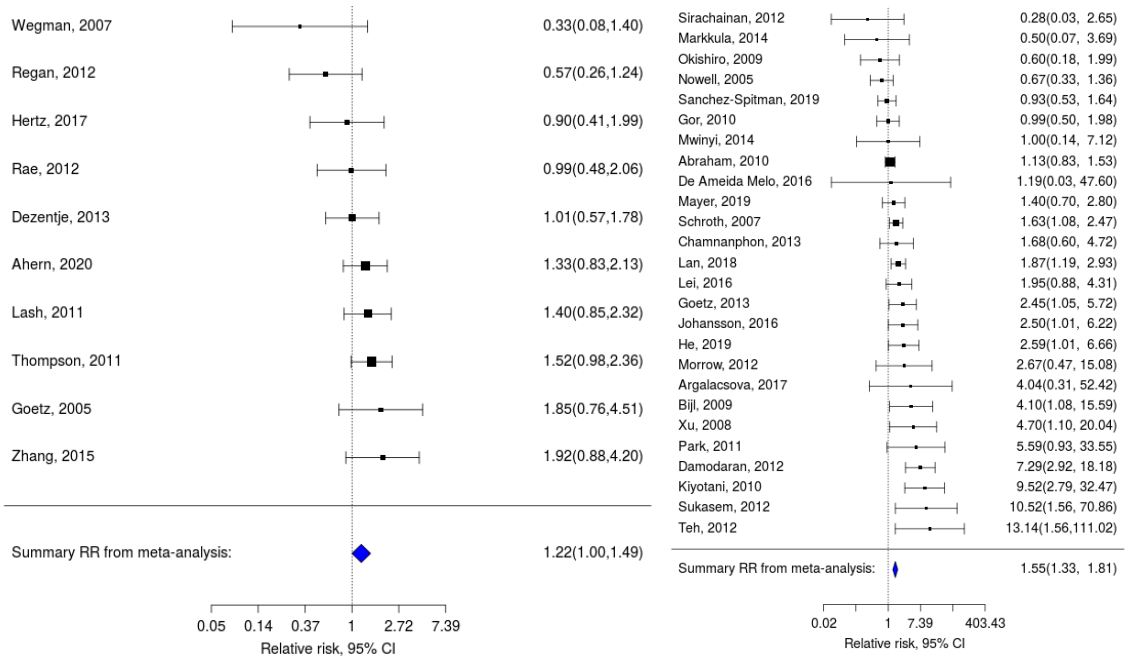
RR (95% CI) is 1.35 (1.17, 1.56); p-value for heterogeneity = 0.16. 7 studies exclusively with CYP2D6*10 had RR (95% CI) = 1.98 (1.27, 3.08) with p-value for heterogeneity = 0.03. 36 with at least 1 of CYP2D6 *4 and CYP2D6*10 resulted in 1.42 (1.26, 1.60) and p-value for heterogeneity = 0.0001.



Top Left) *4 Only: n=5; RR (95% CI) = 1.28 (.95, 1.71); test for heterogeneity p-value = 0.14
Top Right) *4 + others, not *10: n= 17; RR (95% CI) = 1.35 (1.17,1.56); p-value = 0.16
Bottom Left) *10 only: n= 7; RR (95%CI) = 1.98 (1.27,3.08); p-value: .03
Bottom Right) *4 + *10: n = 36; RR (95%CI) = 1.42(1.26,1.60); p-value = 0.0001

DNA Source:

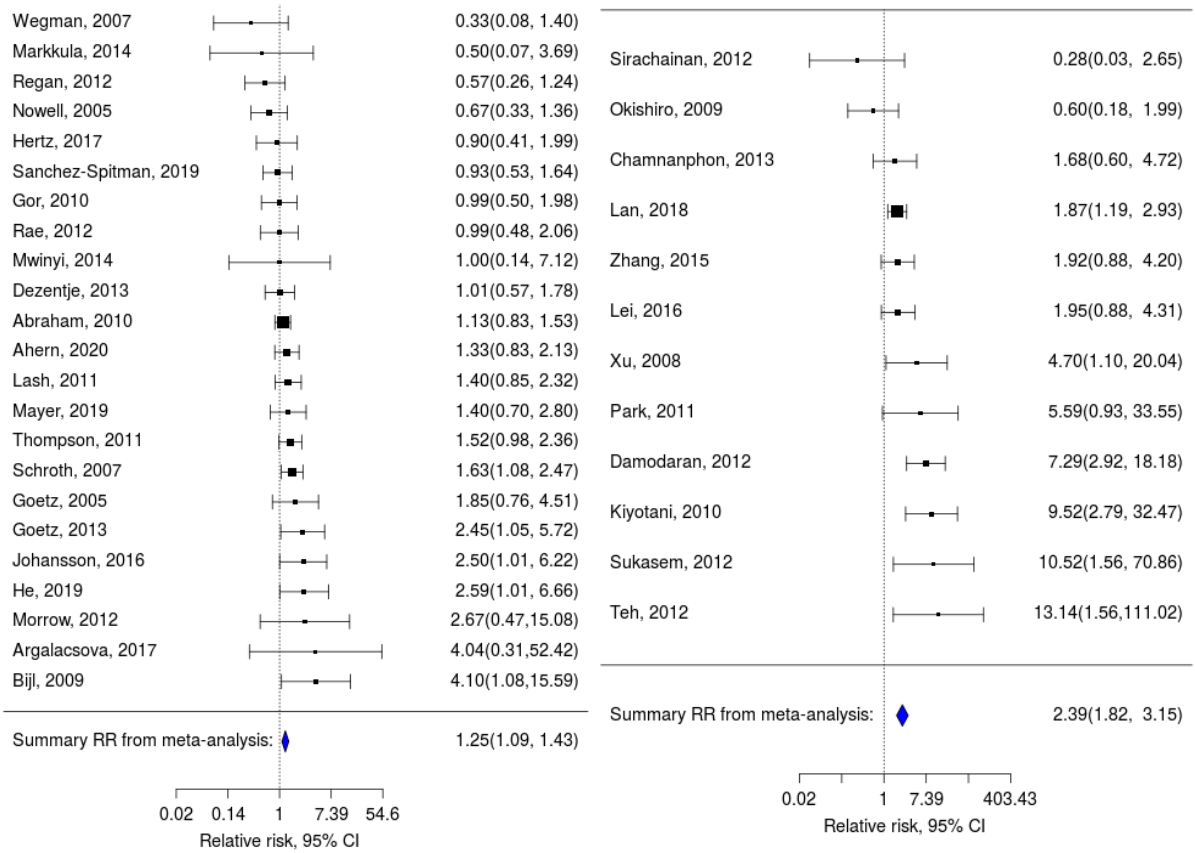
10 studies using DNA extracted from tumor tissue resulted in relative risk (95% confidence interval) = 1.22 (1.00, 1.49) with test for heterogeneity p-value = 0.23. 26 studies using non-tumor tissues resulted in 1.55 (1.33, 1.81), p-value for heterogeneity = 0.0001.



Left) Extracted from tumor: n=10; 1.22 (1.00, 1.49); p-value = 0.23
Right) Extracted from non-neoplastic (blood, lymph nodes, others): n=26; 1.55 (1.33, 1.81); p-value = 0.0001

Ethnic descent of study population:

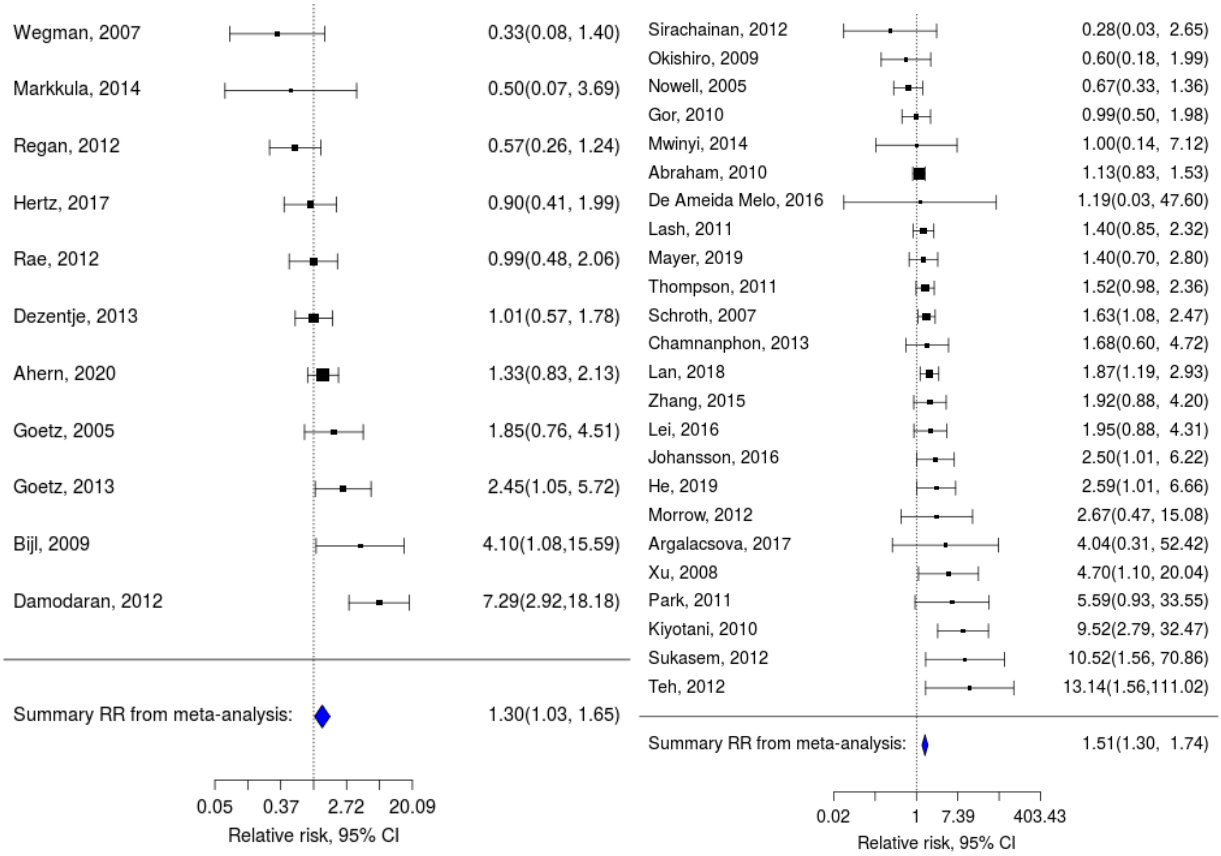
23 studies predominately using European populations had an adjusted relative risk of 1.25 with 95% confidence interval = 1.09-1.43) and p-value for heterogeneity = 0.13. 12 studies using Asian women had relative risk = 2.39 (1.82, 3.15) with p-value for heterogeneity = 0.004.



Left) White/European: n=23; RR (95% CI) = 1.25 (1.09, 1.43); p-value = 0.13
Right) Asian: n=12; RR (95% CI) = 2.39 (1.82, 3.15); p-value = 0.004

Menopausal Status:

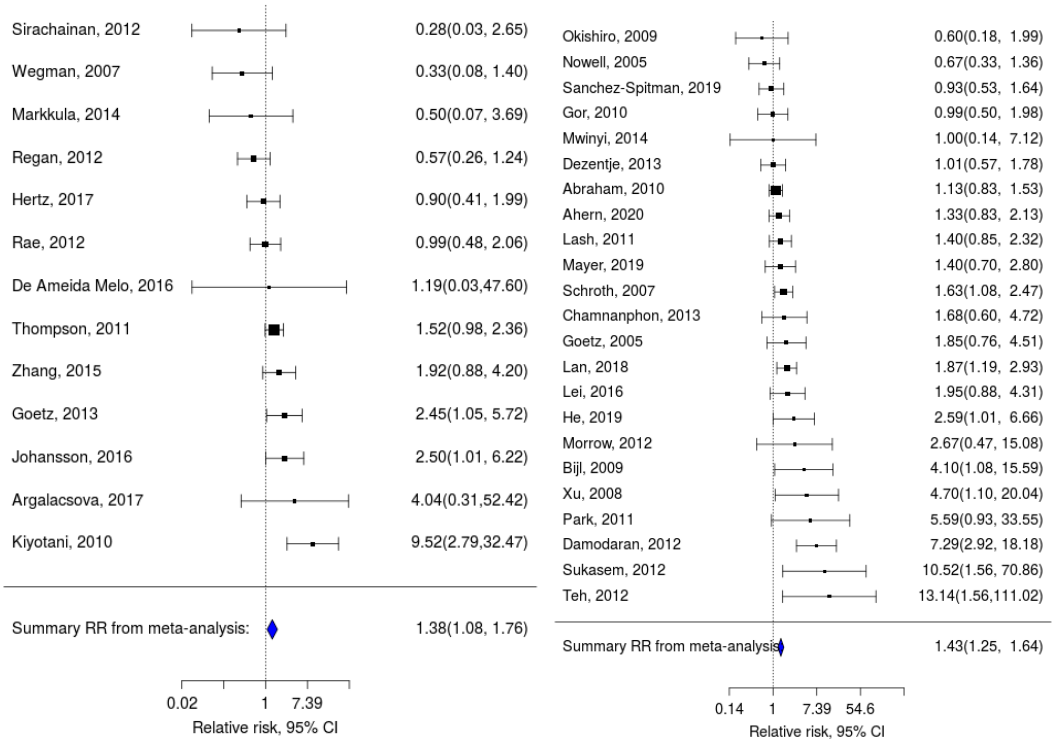
11 studies using over 90% proportion of perimenopausal and postmenopausal women had a relative risk of 1.30 with 95% confidence interval = 1.03-1.65 and p-value for heterogeneity = 0.0008. The 24 studies using 10% or more premenopausal women resulted in a RR (95% CI) = 1.51 (1.30, 1.74) and p-value for heterogeneity = 0.01.



Left) >90% Postmenopausal/Perimenopausal: n=11; RR (95% CI) = 1.30 (1.03, 1.65); p-value = 0.0008
Right) >10% Premenopausal: n=24; RR (95% CI) = 1.51 (1.30, 1.74); p-value = 0.01

ER Status:

13 studies using only women with ER+ tumors yielded a relative risk of 1.38 and confidence interval of 1.08 to 1.76, with p-value for heterogeneity = 0.005. For the 23 studies using mixed ER status cohorts, RR = 1.43 and confidence interval = (1.25, 1.64) with p-value for heterogeneity = 0.002.



Left) Entirely ER+: n=13; RR (95% CI) = 1.38 (1.08, 1.76); p-value = 0.005
Right) Mixed ER Status: n=23; RR (95% CI) = 1.43 (1.25, 1.64); p-value = 0.002

Discussion

This meta-analysis revealed overlapping confidence intervals for all categories (CYP2D6 variants genotyped, DNA source menopausal status of cohort, and estrogen receptor status) except for the ethnic descent of the cohort used. The distinct relative risk confidence intervals between studies using cohorts of European descent and Asian descent demonstrate ethnic descent may be an important source of heterogeneity.

The observed heterogeneity between studies in Asian and European cohorts could be explained by immortal person-time bias as described by Cronin-Fenton et al. (15) The authors noted that a study by Kiyotani et al. of CYP2D6 genotype and tamoxifen effectiveness was limited by immortal person-time, contributing to an extremely high observed relative risk. Immortal person-time bias comes into play when

person-time reported is higher than the actual person-time spent at risk. This hypothesized bias in studies of some Asian cohorts could help to explain the higher relative risk observed in the meta-analysis (16).

Strengths of this meta-analysis include the large, heterogeneous evidence base. 36 studies all utilizing separate cohorts makes for a comprehensive set of data used in the analysis. Cohorts come from around the world, including Asia, Europe, the United States, and South America. Such a large dataset inspires confidence in the results of the meta-analysis.

Potential limitations stem from the hypothesized immortal person-time bias, lack of knowledge on the completeness of genotyping, lack of research on association between non-CYP2D6 genotype and tamoxifen effectiveness, lack of data on tamoxifen adherence, and publication bias. Immortal person-time bias, as previously noted, may be playing a role in impacting observed relative risks in the meta-analysis (15, 16). Furthermore, we do not know that the completeness of the genotyping in each of the cohorts is consistent, potentially posing limitations on the analysis of a CYP2D6-tamoxifen association. Additionally, much is unknown about other genes' potential relationship with tamoxifen effectiveness, meaning that any potential observed associations may be impacted by factors that have yet to be studied. Another limitation may be presented by a lack of adherence to tamoxifen treatment, an extremely prevalent behavior that may impact the effectiveness of tamoxifen more than CYP2D6 genotype (2). Lastly, a meta-analysis can only include published data. This allows for

potential publication bias, where unpublished data goes unaccounted for in the analysis and may bias results away from null associations.

Clinical trials could be the next step in investigating the relationship between CYP2D6 genotype and tamoxifen effectiveness. This trial would begin by randomizing participants to genotyping or non-genotyping groups. Those assigned to genotyping would then be assigned tamoxifen treatment or aromatase inhibitors based on their CYP2D6 genotype. Those assigned to the non-genotyping group would all be given tamoxifen treatment. The intent-to-treat analysis would compare the outcomes of the genotyping versus non-genotyping group. This trial would allow for more causal inferences to be made on the CYP2D6-tamoxifen association, as randomized clinical trials are the gold standard for establishing causation instead of correlation.

This meta-analysis consisted of 36 studies investigating the relationship between CYP2D6 and resistance to tamoxifen treatment in breast cancer patients. Factors considered include CYP2D6 variants genotyped, source of DNA genotyped, ethnic make-up of the study cohort, menopausal status of the women in the cohort (over or under 90% postmenopausal), and estrogen receptor status of the tumors in the cohort (ER-positive only or mixed status). Of these factors, the only with a distinct difference in relative risk is the studies of Asian-descent cohorts versus those of European-descent cohorts. While the analysis has a strong evidence base, limitations include the potential for immortal person-time bias and a lack of research on genes other than CYP2D6 that may have an association with tamoxifen resistance. Future work in this field may

include clinical trials investigating CYP2D6 and tamoxifen, along with further study of non-CYP2D6 genes.

References

1. Dean L. Tamoxifen Therapy and CYP2D6 Genotype. In: Pratt VM, Scott SA, Pirmohamed M, Esquivel B, Kane MS, Kattman BL, et al., editors. Medical Genetics Summaries. Bethesda (MD): National Center for Biotechnology Information (US); 2012.

2. Ahern TP, Hertz DL, Damkier P, Ejlertsen B, Hamilton-Dutoit SJ, Rae JM, et al. Cytochrome P-450 2D6 (CYP2D6) Genotype and Breast Cancer Recurrence in Tamoxifen-Treated Patients: Evaluating the Importance of Loss of Heterozygosity. Am J Epidemiol. 2017;185(2):75-85.

3. Ahern TP, MacLehose RF, Haines L, Cronin-Fenton DP, Damkier P, Collin LJ, et al. Improving the transparency of meta-analyses with interactive web applications. BMJ Evidence-Based Medicine. 2020:bmjebm-2019-111308.

4. Hertz DL, Kidwell KM, Hilsenbeck SG, Oesterreich S, Osborne CK, Philips S, et al. CYP2D6 genotype is not associated with survival in breast cancer patients treated with tamoxifen: results from a population-based study. Breast Cancer Res Treat. 2017;166(1):277-87.

5. Sanchez-Spitman A, Dezentjé V, Swen J, Moes D, Böhringer S, Batman E, et al. Tamoxifen Pharmacogenetics and Metabolism: Results From the Prospective CYPTAM Study. J Clin Oncol. 2019;37(8):636-46.

6. De Ameida Melo M, De Vasconcelos-Valença RJ, Neto FM, Borges RS, Costa-Silva DR, Da Conceição Barros-Oliveira M, et al. CYP2D6 gene polymorphisms in Brazilian patients with breast cancer treated with adjuvant tamoxifen and its association with disease recurrence. Biomed Rep. 2016;5(5):574-8.

7. Ahern TP, Collin LJ, Baurley JW, Kjærsgaard A, Nash R, Maliniak ML, et al. Metabolic Pathway Analysis and Effectiveness of Tamoxifen in Danish Breast Cancer Patients. Cancer Epidemiol Biomarkers Prev. 2020;29(3):582-90.

8. Mayer SE, Weiss NS, Chubak J, Doody DR, Carlson CS, Makar KW, et al. CYP2D6-inhibiting medication use and inherited CYP2D6 variation in relation to adverse breast cancer outcomes after tamoxifen therapy. Cancer Causes Control. 2019;30(1):103-12.

9. Lan B, Ma F, Zhai X, Li Q, Chen S, Wang J, et al. The relationship between the CYP2D6 polymorphisms and tamoxifen efficacy in adjuvant endocrine therapy of breast cancer patients in Chinese Han population. Int J Cancer. 2018;143(1):184-9.

10. Zhang X, Pu Z, Ge J, Shen J, Yuan X, Xie H. Association of CYP2D6*10, OATP1B1 A388G, and OATP1B1 T521C polymorphisms and overall survival of breast cancer patients after tamoxifen therapy. Med Sci Monit. 2015;21:563-9.

11. Lei L, Wang X, Wu XD, Wang Z, Chen ZH, Zheng YB, et al. Association of CYP2D6*10 (c.100C>T) polymorphisms with clinical outcome of breast cancer after tamoxifen adjuvant endocrine therapy in Chinese population. Am J Transl Res. 2016;8(8):3585-92.

12. Johansson H, Gandini S, Serrano D, Gjerde J, Lattanzi M, Macis D, et al. A pooled analysis of CYP2D6 genotype in breast cancer prevention trials of low-dose tamoxifen. Breast Cancer Res Treat. 2016;159(1):97-108.

13. He W, Grassmann F, Eriksson M, Eliasson E, Margolin S, Thorén L, et al. CYP2D6 Genotype Predicts Tamoxifen Discontinuation and Prognosis in Patients With Breast Cancer. J Clin Oncol. 2020;38(6):548-57.

14. Argalacsova S, Slanar O, Bakhouche H, Pertuzelka L. Impact of ABCB1 and CYP2D6 polymorphisms on tamoxifen treatment outcomes and adverse events in breast cancer patients. J buon. 2017;22(5):1217-26.

15. Cronin-Fenton DP, Damkier P, Lash TL. Metabolism and transport of tamoxifen in relation to its effectiveness: new perspectives on an ongoing controversy. Future Oncol. 2014;10(1):107-22.

16. Lash TL, Cole SR. Immortal Person-Time in Studies of Cancer Outcomes. Journal of Clinical Oncology. 2009;27(23):e55-e6.