Null Results in Brief

CYP2D6 Gene Variants and Their Association with Breast Cancer Susceptibility

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Abstract

Background: The gene encoding the phase I enzyme cytochrome P4502D6 (CYP2D6) has been previously investigated for its potential predictive role in the efficacy of breast cancer treatments such as tamoxifen, but its role in breast cancer susceptibility is unclear. This study aims to evaluate the association between germ line variations in CYP2D6 and breast cancer susceptibility.

Methods: DNA samples from 13,472 cases and controls were genotyped for seven known functional variants [minor allele frequency (MAF) ≥ 0.01] and five single nucleotide polymorphisms (SNP) that tag common genetic variation (MAF > 0.05) in CYP2D6.

Results: One relatively rare functional variant, CYP2D6*6, (MAF = 0.01) showed a modest increased association with breast cancer susceptibility (Ptrend = 0.02; OR = 1.32; 95% CI = 1.04–1.68). All other functional and tagSNPs showed no association with breast cancer susceptibility.

Conclusions: Common variants of CYP2D6 do not play a significant role in breast cancer susceptibility. However, this study raises questions regarding the role of rare variants, such as CYP2D6*6, in breast cancer susceptibility which merit further investigation.

Impact: This large case–control study, involving 13,472 women, found no evidence of any association between common CYP2D6 gene variants and breast cancer susceptibility. However, one relatively rare functional variant CYP2D6*6 showed a modest association with breast cancer susceptibility, indicating that the role of rare CYP2D6 variants in breast cancer risk is unclear and requires further investigation in an adequately powered study. Cancer Epidemiol Biomarkers Prev; 20(6); 1255–8. ©2011 AACR.

Introduction

Genome-wide association studies provide empirical evidence that part of the inherited genetic component of breast cancer risk is due to common alleles (1). Coverage of common genetic variation by the various genome-wide single nucleotide polymorphism (SNP) arrays is incomplete. Coverage of variants with a minor allele frequency (MAF) of less than 0.05 is very poor. Breast cancer development is multifactorial, involving genetic and environmental factors.

Genes encoding enzymes within metabolic pathways may be important because of their role in detoxification of xenobiotic compounds. Conversely, they may potentiate the risk of cancer by activating carcinogens. Environmental exposures can affect cancer susceptibility genes (2). Cytochrome P4502D6 (CYP2D6) is a polymorphic gene involved in phase I metabolism (3). Many CYP2D6 variants have altered function which may influence cancer susceptibility. Variants are categorized into poor (PM); intermediate (IM); extensive (EM); and ultra (UM) metabolizers.

The role of CYP2D6 in breast cancer susceptibility is uncertain. Nine published studies, with heterogeneous methodology, study design, and analysis, have given conflicting results. Four studies found an association between CYP2D6 variants and breast cancer susceptibility (4). However, 5 studies showed either no supportive evidence or contradictory evidence (5).

Methods and Materials

These methods and materials have previously been published (3).
Cases and controls
The SEARCH breast cancer case–control study and the characteristics of the cases used in this research have been described previously (3, 6). The total number of cases and controls available for analysis were 6,640 and 6,832, respectively. Eastern Region Multicentre Research Ethics Committee approved the study. Participants provided written informed consent.

Selection of tagging and functional SNPs
Selected tagSNPs tagged all known common SNPs (MAF ≥ 0.05) in the CYP2D6 gene, with a pairwise correlation of $r^2 > 0.8$. Seven functional SNPs were successfully genotyped (3).

Genotyping
Genotyping was conducted using TaqMan (Applied Biosystems) according to manufacturer’s instructions. Primers and probes were supplied by Applied Biosystems as Assays-by-Design. Plates were read on the ABI Prism 7900 using the Sequence Detection Software.

A nested PCR approach was used for CYP2D6*4 and a biplex TaqMan real-time quantification assay was used to identify the CYP2D6*5 (gene deletion) and CYP2D6*UM (gene duplication) variants (3).

Statistics
For each polymorphism, deviation of the genotype frequencies from those expected under Hardy–Weinberg equilibrium was assessed using a $\chi^2$ test. Genotype

Table 1. Breast cancer risk associated with CYP2D6 functional and tagSNPs

<table>
<thead>
<tr>
<th>SNPs</th>
<th>Metabolizer status</th>
<th>MAF</th>
<th>$OR$ (95% CI; heterozygotes vs. common homozygotes)</th>
<th>OR (95% CI; rare homozygotes vs. common homozygotes)</th>
<th>Heterogeneity P (2df)</th>
<th>Trend test P (1df)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functional SNPs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2D6*41</td>
<td>(IM)</td>
<td>0.09</td>
<td>0.98 [0.89–1.07]</td>
<td>0.95 [0.71–1.28]</td>
<td>0.85</td>
<td>0.58</td>
</tr>
<tr>
<td>CYP2D6*04</td>
<td>(PM)</td>
<td>0.2</td>
<td>1.00 [0.92–1.07]</td>
<td>0.86 [0.73–1.02]</td>
<td>0.23</td>
<td>0.26</td>
</tr>
<tr>
<td>CYP2D6*05a</td>
<td>(PM)</td>
<td>0.04</td>
<td>N/A</td>
<td>3.08 [0.83–11.37]</td>
<td>0.07</td>
<td>0.09</td>
</tr>
<tr>
<td>CYP2D6*06</td>
<td>(PM)</td>
<td>0.01</td>
<td>1.33 [1.05–1.69]</td>
<td>N/A</td>
<td>0.02</td>
<td>0.02</td>
</tr>
<tr>
<td>CYP2D6*09</td>
<td>(IM)</td>
<td>0.03</td>
<td>1.04 [0.90–1.21]</td>
<td>N/A</td>
<td>0.59</td>
<td>0.45</td>
</tr>
<tr>
<td>CYP2D6*10</td>
<td>(IM)</td>
<td>0.02</td>
<td>1.07 [0.85–1.36]</td>
<td>N/A</td>
<td>0.55</td>
<td>0.55</td>
</tr>
<tr>
<td>CYP2D6*UMb</td>
<td>(UM)</td>
<td>0.08</td>
<td>N/A</td>
<td>1.25 [0.79–1.98]</td>
<td>0.35</td>
<td>0.35</td>
</tr>
<tr>
<td>CYP2D6*PM Model 1d</td>
<td>(PM)</td>
<td>1/N/A</td>
<td>Trend test only: h</td>
<td>0.99 [0.89–1.10]</td>
<td>N/A</td>
<td>0.89</td>
</tr>
<tr>
<td>CYP2D6*PM Model 2d</td>
<td>(PM)</td>
<td>1/N/A</td>
<td>Trend test only: h</td>
<td>0.98 [0.89–1.07]</td>
<td>N/A</td>
<td>0.65</td>
</tr>
<tr>
<td>TagSNPs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2D6 01t</td>
<td>N/A</td>
<td>0.32</td>
<td>0.92 [0.86–0.99]</td>
<td>1.04 [0.93–1.18]</td>
<td>0.03</td>
<td>0.53</td>
</tr>
<tr>
<td>CYP2D6 02t</td>
<td>N/A</td>
<td>0.46</td>
<td>1.04 [0.96–1.13]</td>
<td>1.06 [0.96–1.17]</td>
<td>0.43</td>
<td>0.2</td>
</tr>
<tr>
<td>CYP2D6 03t</td>
<td>N/A</td>
<td>0.24</td>
<td>0.95 [0.89–1.02]</td>
<td>1.03 [0.88–1.21]</td>
<td>0.31</td>
<td>0.48</td>
</tr>
<tr>
<td>CYP2D6 04t</td>
<td>N/A</td>
<td>0.08</td>
<td>0.99 [0.90–1.08]</td>
<td>0.98 [0.65–1.48]</td>
<td>0.98</td>
<td>0.83</td>
</tr>
<tr>
<td>CYP2D6 05t</td>
<td>N/A</td>
<td>0.22</td>
<td>0.98 [0.91–1.06]</td>
<td>0.86 [0.73–1.01]</td>
<td>0.19</td>
<td>0.15</td>
</tr>
</tbody>
</table>

NOTE: Abbreviation: N/A, not applicable.
Cases $n = 6,640$; Controls $n = 6,832$. $a$CYP2D6*05 - gene deletion
$A$CYP2D6*05 - gene deletion and CYP2D6*UM expression levels assessed using real-time PCR, but expression levels could not adequately distinguish between heterozygotes and common homozygotes.
$c$The PM group are classified as carriers of 2 variant alleles (rare homozygote alleles) for at least 1 of the functional SNPs associated with PM or IM status. Where IM = CYP2D6*41; CYP2D6*9; CYP2D6*10; and PM = CYP2D6*4; CYP2D6*5; CYP2D6*6. In CYP2D6* PM model 1: The PM group (classified as stated) is compared with individuals who carried 2 copies of the wild-type allele (EM) at all SNPs (i.e., common homozygotes) or individuals who carried a single variant allele at a single SNP (heterozygotes).
$e$The PM group was classified as individuals carrying at least 1 variant allele at 1 or more of the functional SNPs (heterozygotes and rare homozygotes), associated with PM or IM status. Where IM = CYP2D6*41; CYP2D6*9; CYP2D6*10; and PM = CYP2D6*4; CYP2D6*5; CYP2D6*6. In CYP2D6* PM model 2: The PM group (classified as stated) is compared with individuals who carried 2 copies of the wild-type allele (EM) at all SNPs (common homozygotes).
$g$M AF shown for controls
$g$N/A - Either no or very few homozygotes for this variant within study population.
$h$Trend test only given as these are combined variants.
frequencies in cases and controls were compared using a \( \chi^2 \) test with 2 degrees of freedom \( (df; P_{\text{heterogeneity}}) \) and the Cochrane–Armitage trend test \( (\chi^2 \text{ on 1 } df) \) for the per allele breast cancer risk \( (P_{\text{trend}}) \). The relative risks of breast cancer for heterozygotes and rare homozygotes, relative to common homozygotes, were estimated as ORs with associated 95% CIs.

The admixture maximum likelihood (AML) test (7) tests the null hypothesis that none of the SNPs are associated with disease compared with the alternative that 1 or more of SNPs are associated.

Comparison of haplotype frequencies by haplotype block, and subject-specific expected haplotype indicators were calculated using an in-house program (6). Haplotypes with greater than 2% frequency were considered "common." Rare haplotypes were pooled. Logistic regression generated haplotype specific risks with respect to all other common haplotypes present (and "rare" grouping) as ORs with 95% CIs. All analyses were conducted in Intercooled Stata version 10.

Results

Using HapMap data, 5 tagSNPs tagged 46 common SNPs \( (\text{MAF} \geq 0.05) \) in a 100-kb region centered on CYP2D6. Seven key functional SNPs, \( (\text{MAF} \geq 0.01) \), were identified. All variants were genotyped in 13,472 cases and controls.

The AML test did not indicate any evidence for an association with breast cancer susceptibility \( (P_{\text{trend}} = 0.24 \text{ linkage disequilibrium (LD) block1}; P_{\text{trend}} = 0.78 \text{ for LD block2}) \).

Only 1 SNP had a modest association with increased breast cancer susceptibility \( (P_{\text{trend}} \leq 0.05) \). CYP2D6*6 heterozygotes showed an increased breast cancer risk \( (P = 0.02; \text{OR} = 1.32; \text{95\% CI} = 1.04–1.68) \). No rare homozygotes for CYP2D6*6 \( (\text{MAF} = 0.01) \) were detected.

No association was seen with any other tagSNPs or functional SNPs (Table 1).

Discussion

There is little evidence to suggest that common or functional variants are associated with breast cancer risk. CYP2D6*6 was associated with a modestly increased breast cancer risk \( (P = 0.02) \). Given that there are many potential low to moderate penetrance breast cancer susceptibility genes and the prior probability of an underlying association for any particular gene is low, this finding is likely to represent a false positive. The SNPs in this study are in pairwise correlation \( (r^2 \geq 0.8) \) with 77% of all the common variants present in the resequenced CYP2D6 region available in the 1000 Genomes Project (8).

To assess if any, as yet undetermined, CYP2D6 variant could be associated with susceptibility, a global haplotype analysis was conducted (Table 2). No significant differences in haplotype frequencies were seen between cases and controls. The combined rare haplotype group was marginally statistically significant; however, this study does not have sufficient power to investigate rare variants.

In conclusion, there is limited evidence to suggest that CYP2D6 plays a significant role in breast cancer susceptibility. However, the role of rarer variants, such as CYP2D6*6, requires further investigation.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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Table 2. Global haplotype analysis of CYP2D6 functional and tag SNPs

<table>
<thead>
<tr>
<th>Gene</th>
<th>Haplotype(^b)</th>
<th>Frequency</th>
<th>( P )</th>
<th>( ^{d}\text{OR} )</th>
<th>( ^{d}\text{95% CIs} )</th>
<th>Global ( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2D6</td>
<td>h001000000</td>
<td>0.22</td>
<td>0.55</td>
<td>0.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Block 1</td>
<td>h001100000</td>
<td>0.09</td>
<td>0.47</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5t, 2t, 1t, *41, *9, *6, *4, *10</td>
<td>h01001000</td>
<td>0.03</td>
<td>0.62</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>h10000010</td>
<td>0.1</td>
<td>0.19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined rare(^d)</td>
<td>0.04</td>
<td>0.01</td>
<td>1.18</td>
<td>1.04–1.35</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CYP2D6(^+)</td>
<td>h01</td>
<td>0.08</td>
<td>0.73</td>
<td>0.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Block 2</td>
<td>h10</td>
<td>0.23</td>
<td>0.46</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3t, 4t</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( ^a\text{SNPs used for haplotype analysis are arranged in chromosome order; 0 = major allele; 1 = minor allele.} \)

\( ^b\text{The baseline for each test is the frequency of all the other haplotypes combined.} \)

\( ^c\text{Rare haplotypes (\( \leq 5\% \)) were pooled.} \)

\( ^d\text{OR and 95\% CI only stated if haplotypes show a difference between cases and controls at a significance level \( P \leq 0.05 \)).} \)

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