Polymorphisms in estrogenic metabolic pathway genes: clinical significance and lifetime outcome.

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Polymorphisms in estrogenic metabolic pathway genes: clinical significance and lifetime outcome.

**Graphical Abstract**

- Estrogen exposure through lifetime and estrogen biosynthetic and metabolic pathway enzyme’s deregulation
- Breast cancer development
- Age at diagnosis and clinical parameters

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERα</td>
<td>XbaI and PvuII</td>
</tr>
<tr>
<td>CYP17A1</td>
<td>T27C</td>
</tr>
<tr>
<td>CYP19A1</td>
<td>codon 39 Trp/Arg</td>
</tr>
<tr>
<td>CYP1A1</td>
<td>Ile462Val</td>
</tr>
<tr>
<td>CYP1B1</td>
<td>Val432Leu</td>
</tr>
<tr>
<td>COMT</td>
<td>Val158Met</td>
</tr>
<tr>
<td>MTHFR</td>
<td>C677T</td>
</tr>
<tr>
<td>NRF2</td>
<td>rs35652124</td>
</tr>
<tr>
<td>KEAP1</td>
<td>rs1048290</td>
</tr>
<tr>
<td>GSTM1</td>
<td>null</td>
</tr>
<tr>
<td>GSTT1</td>
<td>null</td>
</tr>
<tr>
<td>GSTP1</td>
<td>Ile105Val</td>
</tr>
<tr>
<td>TP53</td>
<td>Arg72Pro</td>
</tr>
</tbody>
</table>

- GSTM1*1/0 and cumulative presence of rs35652124 or rs1048290
- GSTM1 null + CYP1B1Val
- GSTT1 null + CYP1B1Val
- Breast cancer diagnosis at later ages
- Inefficient estrogen detoxification

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Abstract: Polymorphisms in estrogenic metabolic pathway genes: clinical significance and lifetime outcome.

Lifetime exposure to high estrogens levels and deregulation of enzymes of estrogen biosynthetic and metabolic pathway are considered breast cancer risk factors. Our research team has previously evaluated, in women with hormone-dependent breast cancer, NRF2 and KEAP1 polymorphisms (alone or in association), with breast cancer prognosis, in cases with GSTM1-present genotype. We identified that GSTM1*1/0 genotype and cumulative presence of at least one allele mutated in KEAP1 and/or NRF2 polymorphisms might be associated with worse prognosis. Moreover, older women were carriers of both GSTM1 null and CYP1B1Val genotypes, similar results were found for the cumulative presence of GSTT1 null and CYP1B1Val genotypes and for MTHFR C677T and GSTT1 null polymorphism. These results lead us to genotype other enzymes involved in estrogen biosynthetic/metabolic pathways, namely: CYP17A1 T27C, CYP19A1 codon 39 Trp/Arg, COMT Val158Met, CYP1A1 Ile462Val, GSTP1 Ile105Val, ERα Pvull and Xbal polymorphisms and TP53 Arg72Pro (DNA damage signalling and repair pathway) polymorphism. The genotyping analysis was evaluated, alone or in association, with breast cancer prognosis and with age at breast cancer diagnosis, we found no association (p-value>0.05). These results emphasize that polymorphisms in NRF2/KEAP1 complex and the null polymorphisms of GSTM1/GSTT1 should be further investigated as targets for breast cancer therapy. In addition, polymorphisms related to inefficient estrogen detoxification might be a trigger to hormone-dependent breast cancer development at later ages, and should be considered in breast cancer risk assessment models.

Keywords: Breast Cancer, estrogens, polymorphisms, risk model, targeted therapy
Introduction

Estrogens have been identified as carcinogens, and most of the risk factors for breast cancer are related to an increased or prolonged exposure to estrogens.
Introduction

Biosynthetic and metabolic pathways
Introduction

Our research team correlated:

- GSTM1 and GSTT1 null
- CYP19A1 arginine (C) allele
- TP53 proline allele

Increased risk of breast cancer development.

Reduced risk of breast cancer development.

Carriers of xx genotype of Erα XbaI
Introduction

Framework

GSTM1 null polymorphism taking into concern the three genotypes:
- GSTM1*1/1 (present)
- GSTM1*1/0 (heterozygous)
- GSTM1*0/0 (null)

NRF2 35652124 and KEAP1 rs1048290

Clinical parameters in order to evaluate breast cancer prognosis

GSTM1 and GSTT1 null polymorphism with CYP1B1 Val432Leu and MTHFR C677T.

Age at diagnosis of breast cancer

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Introduction

Framework

Clinical parameters in order to evaluate the breast cancer prognosis

Age at diagnosis

Genes

TP53
Arg72Pro

CYP17A1
T27C

CYP19A1
codon 39
Trp/Arg

CYP1A1
Ile462Val

CYP1B1

COMT
Val158Met

MTHFR

GSTP1
Ile105Val

GSTT1

GSTM1

Keap1

NRF2

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Results and discussion

Metabolism regulators, phase II enzymes and breast cancer prognosis

- **GSTM1** present genotypes to withdraw the risk of **GSTM1** null genotype

  - $n=52$ Women with hormone-dependent (ER+) breast cancer

  - **GSTM1**:*1/1**
  - **GSTM1**:*1/0**

- **KEAP1** and **NRF2** polymorphisms

  - **KEAP1** rs1048290
  - **NRF2** rs35652124 are related to ↑NRF2 levels in cytoplasm, might lead to resistance to therapeutic

- HER2 status

  - HER2+ breast cancers are related to chemotherapy resistance and worse prognosis

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# Results and discussion

Metabolism regulators, phase II enzymes and breast cancer prognosis

## Association of KEAP1 and NRF2 polymorphisms in cases with GSTM1 present genotype (*1/1 or *1/0) and correlation with HER2 status

<table>
<thead>
<tr>
<th>Genotype</th>
<th>HER2 status n (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>KEAP1 rs1048290</td>
<td>GSTM1</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>1/1</td>
<td>0</td>
</tr>
<tr>
<td>CC</td>
<td>1/0</td>
<td>4 (15.4)</td>
</tr>
<tr>
<td>CG/GG</td>
<td>1/1</td>
<td>0</td>
</tr>
<tr>
<td>CG/GG</td>
<td>1/0</td>
<td>18 (69.2)</td>
</tr>
<tr>
<td>NRF2 rs35652124</td>
<td>GSTM1</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>1/1</td>
<td>0</td>
</tr>
<tr>
<td>AA</td>
<td>1/0</td>
<td>9 (34.6)</td>
</tr>
<tr>
<td>AG/GG</td>
<td>1/1</td>
<td>0</td>
</tr>
<tr>
<td>AG/GG</td>
<td>1/0</td>
<td>13 (50)</td>
</tr>
</tbody>
</table>

HER2+ → Resistance to therapy

GSTM1*1/0 → Low levels of GSTM1

↑ NRF2 levels in cytoplasm, might lead to resistance to therapeutic.

Worse prognosis

KEAP1 rs1048290

NRF2 rs35652124
## Results and discussion

### Polymorphisms in estrogen metabolic pathway and age at diagnosis

n=157

<table>
<thead>
<tr>
<th>GSTM1</th>
<th>CYP1B1 Val432Leu</th>
<th>Age, n (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>Leu/Leu (WT)</td>
<td>&lt;50: 8 (5.1)</td>
<td>≥50: 10 (6.4)</td>
</tr>
<tr>
<td>Present</td>
<td>Leu/Val + Val/Val</td>
<td>&lt;50: 9 (5.7)</td>
<td>≥50: 38 (24.2)</td>
</tr>
<tr>
<td>Null</td>
<td>Leu/Leu (WT)</td>
<td>&lt;50: 2 (1.3)</td>
<td>≥50: 19 (12.1)</td>
</tr>
<tr>
<td>Null</td>
<td>Leu/Val + Val/Val</td>
<td>&lt;50: 12 (7.6)</td>
<td>≥50: 59 (37.6)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GSTT1</th>
<th>CYP1B1 Val432Leu</th>
<th>Age, n (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>Leu/Leu (WT)</td>
<td>&lt;50: 10 (6.4)</td>
<td>≥50: 21 (13.4)</td>
</tr>
<tr>
<td>Present</td>
<td>Leu/Val + Val/Val</td>
<td>&lt;50: 17 (10.8)</td>
<td>≥50: 62 (39.5)</td>
</tr>
<tr>
<td>Null</td>
<td>Leu/Leu (WT)</td>
<td>&lt;50: 0</td>
<td>≥50: 8 (5.1)</td>
</tr>
<tr>
<td>Null</td>
<td>Leu/Val + Val/Val</td>
<td>&lt;50: 4 (2.5)</td>
<td>≥50: 35 (22.3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GSTT1</th>
<th>MTHFR C677T</th>
<th>Age, n (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present</td>
<td>CC (WT)</td>
<td>&lt;50: 10 (6.4)</td>
<td>≥50: 30 (19.1)</td>
</tr>
<tr>
<td>Present</td>
<td>CT+TT</td>
<td>&lt;50: 17 (10.8)</td>
<td>≥50: 53 (33.8)</td>
</tr>
<tr>
<td>Null</td>
<td>CC (WT)</td>
<td>&lt;50: 2 (1.3)</td>
<td>≥50: 13 (8.3)</td>
</tr>
<tr>
<td>Null</td>
<td>CT+TT</td>
<td>&lt;50: 2 (1.3)</td>
<td>≥50: 30 (19.1)</td>
</tr>
</tbody>
</table>

Breast cancer diagnosis at ≥ 50 years old

*GSTM1 null + CYP1B1Val

*GSTT1 null + CYP1B1Val

*GSTT1 null + MTHFR T allele
Results and discussion

Polymorphisms in estrogen synthetic and metabolic pathway: age at diagnosis and prognosis

Significant results allow to emphasize that polymorphisms in NRF2/KEAP1 complex and the null polymorphisms of GSTM1/GSTT1 should be considered prognostic markers for prognosis of the disease and cancer therapy.
Conclusions

Cumulative presence of polymorphisms in *NRF2/KEAP1* and *GSTM1*1/0 genotype might be associated with worse prognosis in breast cancer patients and with resistance to therapy.

Cumulative presence of polymorphisms in genes related to estrogen metabolism and lifetime exposure to estrogens might lead to breast cancer development at later ages.

Polymorphisms in *NRF2* and *KEAP1*, also as the null polymorphisms of *GSTM1* and *GSTT1* should be further investigated as possible targets for breast cancer therapy.
Acknowledgments

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