**SULT1A1 gene deletion in BRCA2-associated male breast cancer: a link between genes and environmental exposures?**

Domenico Palli a, Piera Rizzolo b, Ines Zanna a, Valentina Silvestri b, Calogero Saieva a, Mario Falchetti b, Anna Sara Navazio b, Veronica Graziano b, Giovanna Masala a, Simonetta Bianchi c, Antonio Russo d, *, Stefania Tommasi e, Laura Ottinib, *

a Molecular and Nutritional Epidemiology Unit, Cancer Research and Prevention Institute (ISPO), Florence, Italy
b Department of Molecular Medicine, “Sapienza” University of Rome, Roma, Italy
c Division of Pathological Anatomy, Department of Medical and Surgical Critical Care, AOU Careggi, University of Florence, Florence, Italy
d Section of Medical Oncology, Department of Surgical and Oncological Sciences, University of Palermo, Palermo, Italy
e Clinical Experimental Oncology Laboratory, National Cancer Centre of Bari, Bari, Italy

Received: November 3, 2012; Accepted: January 31, 2013

**Abstract**

SULT1A1, a member of the sulfotransferase superfamily, is a drug and hormone metabolizing enzyme involved in the metabolism of a variety of potential mammary carcinogens of endogenous and exogenous origin. Interestingly, the metabolic activity of SULT1A1 can be affected by variations in gene copy number. Male Breast Cancer (MBC) is a rare disease and less investigated disease compared to female BC (FBC). As in FBC, the concurrent effects of genetic risk factors, particularly BRCA2 mutations, increased exposure to estrogens and environmental carcinogens play a relevant role in MBC. By quantitative real-time PCR with TaqMan probes, we investigated the presence of SULT1A1 gene copy number variations (CNVs) in a series of 72 MBCs. SULT1A1 gene deletion was observed in 10 of the 72 MBCs (13.9%). In a multivariate analysis association between BRCA2 mutation and SULT1A1 gene deletion emerged ($p = 0.0005$). Based on the evidence that the level of SULT1A1 enzyme activity is correlated with CNV, our data suggest that in male breast tumors SULT1A1 activity may be decreased. Thus, it can be hypothesized that in a proportion of MBCs, particularly in BRCA2-associated MBCs, the level of estrogens and environmental carcinogens exposure might be increased suggesting a link between gene and environmental exposure in the pathogenesis of MBC.

**Keywords:** SULT1A1 ● copy number variations (CNVs) ● BRCA2 ● male breast cancer

SULT1A1, a member of the sulfotransferase superfamily, is a drug and hormone metabolizing enzyme involved in the metabolism of a variety of potential mammary carcinogens of endogenous and exogenous origin, including oestrogens and polycyclic aromatic hydrocarbons (PAHs) [1].

Gene copy number variations (CNVs) are increasingly recognized to play a relevant role in the expression of drug metabolizing genes and in their respective enzymatic activities. In particular, the metabolic activity of SULT1A1 can be affected by variations in gene copy number [2].

Male breast cancer (MBC) is a rare disease, compared with female BC. The concurrent effects of genetic risk factors, particularly BRCA2 mutations, increased exposure to oestrogens and environmental carcinogens play a relevant role in MBC [3]. MBC is unaffected by the strong confounding effects of high disease frequency and of reproduction-related variables, thus, the complex effects of genetic, hormonal and environmental factors, involved in the pathogenesis of BC in both genders, can be better investigated in MBC.

On the basis of SULT1A1 biochemical properties, we investigated the presence of SULT1A1 CNVs in a series of 72 MBCs characterized for relevant clinical-pathologic features, including BRCA1/2 mutation origin, including oestrogens and polycyclic aromatic hydrocarbons (PAHs) [1].

Gene copy number variations (CNVs) are increasingly recognized to play a relevant role in the expression of drug metabolizing genes and in their respective enzymatic activities. In particular, the metabolic activity of SULT1A1 can be affected by variations in gene copy number [2].

Male breast cancer (MBC) is a rare disease, compared with female BC. The concurrent effects of genetic risk factors, particularly BRCA2 mutations, increased exposure to oestrogens and environmental carcinogens play a relevant role in MBC [3]. MBC is unaffected by the strong confounding effects of high disease frequency and of reproduction-related variables, thus, the complex effects of genetic, hormonal and environmental factors, involved in the pathogenesis of BC in both genders, can be better investigated in MBC.

On the basis of SULT1A1 biochemical properties, we investigated the presence of SULT1A1 CNVs in a series of 72 MBCs characterized for relevant clinical-pathologic features, including BRCA1/2 mutation origin, including oestrogens and polycyclic aromatic hydrocarbons (PAHs) [1].

**Correspondence to:** Laura OTTINI, M.D., Department of Molecular Medicine, “Sapienza” University of Rome, Viale Regina Elena, 324, Roma 00161, Italy.
Tel.: +39-0649973009
Fax: +39-0649973004
E-mail: laura.ottini@uniroma1.it
Antonio RUSSO, M.D., Ph.D., Section of Medical Oncology, Department of Surgical and Oncological Sciences, University of Palermo, Via del Vespro 129, 90127 Palermo, Italy.
Tel.: +39 0916552500
Fax: +(011) 39-091-6554529
E-mail: antonio.russo@usa.net

© 2013 The Authors. Published by Foundation for Cellular and Molecular Medicine/Blackwell Publishing Ltd.
This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

doi: 10.1111/jcmm.12043
**Table 1** Distribution of 72 MBC cases according to **SULT1A1** gene deletion and clinical-pathologic features

| **Parameter** | **Deletion (%)** | **No deletion (%)** | **P value** |
|---------------|------------------|---------------------|-------------|
| **Family history of breast/ovarian cancer** | | | |
| Negative      | 4 (8.2)          | 45 (91.8)           | 0.06†       |
| Positive      | 6 (27.3)         | 16 (72.7)           |             |
| **Personal history of cancer** | | | |
| Negative      | 7 (11.7)         | 53 (88.3)           | 0.18        |
| Positive      | 3 (27.3)         | 8 (72.7)            |             |
| **BRCA1 status** | | | |
| BRCA1 wt      | 10 (14.5)        | 59 (85.5)           |             |
| BRCA1 mutation | 0 (0)           | 2 (100.0)           | 1.0         |
| **BRCA2 status** | | | |
| BRCA2 wt      | 4 (6.3)          | 60 (93.7)           | <0.0001     |
| BRCA2 mutation | 6 (85.7)        | 1 (14.3)            |             |
| **ER**        | | | |
| Negative      | 4 (40)           | 6 (60.0)            | 0.03‡       |
| Positive      | 6 (9.7)          | 56 (90.3)           |             |
| **PR**        | | | |
| Negative      | 4 (25.0)         | 12 (75.0)           | 0.12        |
| Positive      | 6 (10.7)         | 50 (89.3)           |             |
| **HER2**      | | | |
| Negative      | 5 (9.4)          | 48 (90.6)           | 0.12        |
| Positive      | 4 (22.2)         | 14 (77.8)           |             |
| **Ki-67**     | | | |
| Low           | 4 (10.0)         | 36 (90.0)           | 0.2         |
| High          | 5 (16.1)         | 26 (83.9)           |             |
| **Histological grade** | | | |
| G1/G2         | 3 (6.8)          | 41 (93.2)           | 0.06        |
| G3            | 6 (25.0)         | 18 (75.0)           |             |
| **Lymph node status** | | | |
| Negative      | 1 (3.9)          | 25 (96.2)           | 0.35        |
| Positive      | 4 (14.3)         | 24 (85.7)           |             |
| Total         | 10 (13.9)        | 62 (86.1)           |             |

*Some data for each parameter are not available.
†From Fisher exact test.
‡This association was not evident in a multivariate analysis.
status [4]. SULT1A1 CNVs were analysed by quantitative real-time PCR with TaqMan probes (Life Technologies, Carlsbad, CA, USA) comparing DNAs from tumour and blood from each MBCs included in the study. Normal breast tissue was used as calibrator sample. The fold change in studied gene copy number, normalized to endogenous control, was calculated using Relative Quantity (RQ) = 2−ΔΔCt

SULT1A1 gene copy number differences were found to occur in tumour compared with matched blood samples in 10 of the 72 MBCs (13.9%). In particular, SULT1A1 gene deletion (RQ = 0.5) was observed indicating the presence of a single copy of SULT1A1 gene in the 10 tumour samples compared with two copies (RQ = 1) detected in the corresponding blood samples. The results of blood and normal breast tissue paired samples were comparable in each patient.

As shown in Table 1, statistically significant association emerged between SULT1A1 gene deletion and BRCA2 mutations (P < 0.0001) and ER-negative status (P = 0.03). However, in a multivariate analysis only the association for BRCA2 status persisted (P = 0.0005).

To date, there are no data on CNVs of SULT1A1 gene in BC. We found that a quite relevant proportion of MBCs (about 14%) showed SULT1A1 gene deletion and, interestingly, that the deletion was significantly found in BRCA2-associated tumours. Based on the evidence that the level of SULT1A1 enzyme activity is correlated with CNV [2], our data suggest that in male breast tumours SULT1A1 activity may be decreased.

It has been reported that oestrogen sulfotransferases are frequently decreased in breast carcinomas and this may result in an increased exposure of mammary tissue to oestrogens [5]. Very recently, SULT1A1 has been shown to play an important role in the detoxication of PAHs in lung cells [6]. Thus, based on our results, it can be suggested that in a proportion of MBCs, particularly in BRCA2-associated MBCs, the level of oestrogens and environmental carcinogens exposure might be increased. This could be particular relevant considering the important role of oestrogens in MBC pathogenesis and the molecular crosstalk between oestrogens and BRCA2 gene [7]. Intriguingly, we have previously shown an interaction between BRCA carrier status and occupational exposure to chemicals, such as PAHs, in MBC patients [8]. Thus, our present results may help to clarify possible pathogenetic mechanisms underlying this interaction.

Overall, our data suggest that MBCs, particularly BRCA2-associated MBCs, may be characterized by low SULT1A1A enzymatic activity thus suggesting a link between gene and environmental exposure and opening interesting questions on clinical settings.

Acknowledgement

The study was supported by a grant from Associazione Italiana per la Ricerca sul Cancro (AIRC IG 8713).

Conflict of interest

The authors indicate no potential conflict of interest.

References

1. Hempel N, Gamage N, Martin JL, et al. Human cytosolic sulfotransferase SULT1A1. Int J Biochem Cell Biol. 2007; 39: 685–9.
2. Hebrbing SJ, Adjei AA, Baer JL, et al. Human SULT1A1 gene: copy number differences and functional implications. Hum Mol Genet. 2007; 16: 463–70.
3. Brinton LA, Richesson DA, Gierach GL, et al. Prospective evaluation of risk factors for male breast cancer. J Natl Cancer Inst. 2008; 100: 1477–81.
4. Ottini L, Rizzolo P, Zanna I, et al. BRCA1/BRCA2 mutation status and clinical-pathologic features of 108 male breast cancer cases from Tuscany: a population-based study in central Italy. Breast Cancer Res Treat. 2009; 116: 577–86.
5. Suzuki T, Miki Y, Nakata T, et al. Steroid sulfatase and estrogen sulfotransferase in normal human tissue and breast carcinoma. J Biol Chem. 2003; 278: 29909–29914.
6. Zhang L, Huang M, Blair IA, et al. Detoxication of benzo[a]pyrene-7,8-dione by sulfotransferases (SULTs) in human lung cells. J Biol Chem. 2012; 287: 29909–29914.
7. Malone JL, Nelson AC, Lieberman R, et al. Oestrogen-mediated phosphorylation and stabilization of BRCA2 protein in breast. J Pathol. 2009; 217: 380–8.
8. Palli D, Pascale G, Mariani-Costantini R, et al. A gene-environment interaction between occupation and BRCA1/BRCA2 mutations in male breast cancer? Eur J Cancer. 2004; 40: 2474–9.