Confirmation of the reduction of hormone replacement therapy-related breast cancer risk for carriers of the *HSD17B1_937_G* variant

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1   **Abstract**

2   17β-hydroxysteroid dehydrogenase type 1 (HSD17B1) plays an important role in the biosynthesis

3   of 17β-estradiol. The current study aimed at confirming the reduced risk of breast cancer in

4   carriers of the non-synonymous *HSD17B1*_937_A>G (rs605059) polymorphism who used any

5   hormone replacement therapy (HRT) for ten years or longer. We performed an independent

6   association study using four breast cancer case-control studies from Australia, Germany and

7   Sweden. In all, 5,777 cases and 8,189 age-matched controls of European descent were genotyped

8   by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF

9   MS) and TaqMan. Risk estimates were calculated by interaction analysis and main effect analysis

10   adjusted for age and study. Main effect analyses for women using any HRT for 10 years or longer

11   (1,428 cases versus 1,724 controls) revealed a protective effect of the *HSD17B1*_937_G allele on

12   breast cancer risk (OR 0.86, 95% CI: 0.73-0.99; p = 0.048). Thus, our previous finding of a

13   protective effect of the *HSD17B1*_937_G allele on HRT-associated breast cancer risk has now

14   been confirmed both in independent large patient cohorts and a comprehensive pooled analysis

15   supporting the hypothesis, that a HSD17B1-mediated decreased conversion of estrone to the more

16   potent 17β-estradiol may reduce the estrogenic effects, thereby reducing the risk of developing

17   breast cancer during long-term HRT use.

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1 Introduction

2 The 17β-hydroxysteroid dehydrogenase type 1 (HSD17B1) is a key enzyme in the sex steroid

3 hormone metabolism pathway that mediates the catalytic conversion of less active estrone to its

4 most potent form, 17β-estradiol [1]. Since elevated steroid hormone levels can result from

5 variations in genes encoding enzymes of the steroid metabolism pathway, such genetic variations

6 have been suggested to contribute to an increased breast cancer risk especially in postmenopausal

7 women [2]. Notably, HSD17B1, a member of this pathway, is predominantly expressed in

8 steroidogenic tissues and its over-expression has been observed in estrogen-sensitive cancers.

9 Moreover, increased HSD17B1 activity has been observed in breast tumors of postmenopausal

10 but not premenopausal women [3-5]. Interestingly, *HSD17B1* polymorphisms have not been

11 prioritized among the over 20 novel breast cancer susceptibility genes reported from genome-

12 wide association studies (GWAS) conducted with large global case-control collections [6-8].

13 Despite a lack of evidence but due to the HSD17B1 involvement in steroid metabolism, its

14 polymorphisms may likely contribute to breast cancer risk, however there is a possibility that this

15 may become only evident for the risk associated with hormone replacement therapy (HRT). HRT

16 is commonly prescribed as estrogen plus progestin or estrogen monotherapy for the relief from

17 menopausal symptoms such as hot flushes and has been commonly accepted as a breast cancer

18 risk factor particularly when used for more than 5 years [9, 10]. The number of studies that have

19 previously examined the relevance of *HSD17B1* polymorphisms and HRT use are rather limited

20 and provide inconsistent results [3, 11-15]. Our follow-up investigation of whether or not a

21 genetic *HSD17B1* polymorphism contributes to an HRT-associated breast cancer risk is based on

22 a German molecular epidemiology study MARIE-GENICA [16] which suggested a reduced

23 breast cancer risk for female carriers of the *HSD17B1*_937_G allele having used any long-term

24 HRT.

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1   The non-synonymous polymorphism rs605059 at nucleotide position 937 of the *HSD17B1* gene

2   is located at chromosome 17q12 with a minor allele frequency of 48% in Europeans [17]. The

3   polymorphism causes an amino acid exchange from Serine to Glycine at position 313 in exon 6

4   [3, 15] and the absence of other frequent non-synonymous polymorphisms within the *HSD17B1*

5   coding region made this the polymorphism of interest. Following the hypothesis that genetic risk

6   modifiers could aid in the stratification of patients into those with and without a confounding risk

7   for breast cancer in HRT users, and based on the previous finding of an *HSD17B1_937_G*

8   association with HRT-related breast cancer risk, we set out to validate this association using two

9   independent breast cancer case-control collections with available HRT information (any HRT

10   use) from Sweden (SASBAC) and Australia (MCCS). Here we report the confirmation of the

11   reduced risk effect of the *HSD17B1_937_G* allele in the context of long-term HRT use both in

12   the independent studies and in the pooled analysis including the MARIE-GENICA study.

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15   **Materials and methods**

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17   Study populations

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19   The study sample included 5,777 breast cancer cases and 8,189 controls from four studies from

20   Australia, Germany and Sweden. All women were of European descent. The Australian

21   prospective Melbourne Collaborative Cohort Study (MCCS) is a prospective study including

22   41,514 residents of the city of Melbourne (24,469 women) aged between 40 and 69 years at base-

23   line out of which 677 breast cancer cases diagnosed during follow-up and 778 controls were

24   randomly selected [18]. The population-based Singapore and Swedish Breast Cancer Study

1   (SASBAC) comprised 3,345 breast cancer cases and 3,454 age-matched controls from Sweden,

2   aged between 50 and 74 years [19]. For a pooled analysis, data from the previously published

3   MARIE-GENICA study on these subjects were included [16]. The latter is a joint investigation of

4   two population-based German breast cancer case-control studies: MARIE (MAmmakarzinom

5   RIsikofaktor Erhebung) [20] and GENICA (Gene ENvironment Interaction and Breast CAncer in

6   Germany) [21, 22] which together included 3,149 breast cancer patients with primary breast

7   cancer and 5,489 age-matched controls. Participants were aged between 50 and 80 years. All

8   studies were approved by respective ethical review committees and participants gave written

9   informed consent.

10   Data on any HRT use were available for 4,912 patients and 7,137 controls, of which 1,428 cases

11   and 1,724 controls used HRT for 10 or more years.

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13   Genotyping and statistical analysis

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15   Genotyping of blood-derived DNA from SASBAC participants was done using Sequenom®

16   Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)

17   (Sequenom, San Diego, CA, USA) as previously done for the MARIE-GENICA participants [16].

18   Genotyping of blood-derived DNA from MCCS participants was done by TaqMan® analysis

19   using the Genotyping Assay, Human SM, C___2350902_10 (Applied Biosystems, Foster City,

20   CA, USA).

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22   Statistical analysis was done by logistic regression with adjustment for study and age: a) main

23   effect analysis of *HSD17B1*_937 A>G and use of HRT for 10 years or longer. (Pooled: 1,411

24   cases and 1,715 controls successfully genotyped); b) interaction analysis between *HSD17B1*_937

A>G and duration of HRT use (Pooled: 4,824 cases and 7,041 controls successfully genotyped). Risk estimates are given as odd ratios (OR) along with 95% confidence interval (CI). The validation study had an 80% power to detect a minimum OR of 0.84 for the main effect analysis of the *HSD17B1* polymorphism under investigation (α = 0.05, two-sided test). Analysis for a potential association between genotype and respective tumor steroid hormone receptor status was done also for ER-positive cases versus ER-negative cases and PR-positive cases versus PR-negative cases. All statistical analyses were performed using the freely available R-package R-2.11.1 (SNPassoc-1.8-5) (www.r-project.org) and SPSS v. 15.0 (SPSS Inc., IBM Corporation, NY, USA), respectively. Statistical significance was defined as $p < 0.05$.

Results

*HSD17B1*_937_A>G genotype frequencies of cases and controls met Hardy-Weinberg equilibrium. Call rates were greater than 98% and repeated analyses of the duplicate samples (10%) showed 100% concordance. After adjusting for potential confounders (age and study), we observed no statistically significant association between any of the *HSD17B1*_937_A>G genotypes and overall or hormone receptor-dependent breast cancer risk (data not shown). In the previous MARIE-GENICA study, interaction analysis showed a reduced breast cancer risk for women carrying the homozygous *HSD17B1*_937_GG genotype with long-term HRT use ($p_{interaction} = 0.032$, Table 1). This effect was not seen in the interaction analysis of the MCCS and SASBAC collections (data not shown).

To compare the effects of each genotype on an HRT associated breast cancer risk we performed a main effect analysis of the subgroup of long-term HRT users (10 years or longer). Within the MCCS and SASBAC studies, we observed a protective effect for carriers of the GG genotype

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1 with an OR of 0.56 (95% CI: 0.32-0.96; $p = 0.041$; Table 1). When we looked at women with

2 HRT use 10 years and longer, the main effect analysis of the MARIE-GENICA study did not

3 show a significant protective effect (Table 1). The pooled main effect analysis of the MCCS,

4 SASBAC, MARIE-GENICA confirmed the significant decreased breast cancer risk for carriers of

5 the *HSD17B1*_937_G variant (OR 0.86, 95% CI 0.73-0.99; $p = 0.048$; Table 1).

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8 **Discussion**

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10 Our pooled analysis of four international breast cancer case-control studies with available data on

11 any HRT use confirmed the protective effect of the *HSD17B1*_937_G allele on breast cancer risk

12 in women using HRT for 10 years or longer. Although the functional effect of the non-

13 synonymous *HSD17B1*_937_A>G polymorphism is largely unknown, it has been suggested that

14 the variant G allele may lead to decreased enzyme activity *in vitro* [3, 15]. Accordingly, the

15 observed decreased risk effect is in line with the current knowledge of the role of HSD17B1 in

16 the biosynthesis of 17β-estradiol [1] whereby lower activity of the G variant may lead to

17 decreased estrogenic effects on breast epithelial tissue during HRT use, thus lowering the risk of

18 developing breast cancer.

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20 In a previous study of the MARIE-GENICA breast cancer case-control collection, we observed

21 an interaction between the *HSD17B1*_937_GG genotype and reduced breast cancer risk with

22 duration of HRT [16]. This statistical modeling provided us with information on the effect of a

23 genotype on the breast cancer risk effect across all categories of duration of HRT use. Although

24 this interaction study suggested a link between the *HSD17B1* G allele and HRT-related breast

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1   cancer risk, it limits the direct comparison between patients with different genotypes. For that

2   reason, in the current investigation, we put emphasis on a main effect analysis to directly compare

3   the risk effect of the different *HSD17B1*_937 genotype groups, i.e. AA versus AG, AA versus

4   GG and AA versus AG+GG. This statistical modeling is commonly used in pharmacogenetic

5   studies for risk stratification [23]. Of note, the main effect analysis also enabled us to specifically

6   examine the subgroup of long-term HRT users (10 years or longer) without bias from HRT users

7   with shorter duration. Notably, large clinical and epidemiological studies have shown the HRT-

8   related breast cancer risk for only long-term use [9, 10]. Therefore, our findings are important and

9   provide a clue to better understand possible underlying mechanisms involved in HRT-related

10   breast cancer risk.

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12   The analysis of two independent study collections, MCCS and SASBAC, validated our

13   hypothesis of a protective effect of the *HSD17B1*_937_GG genotype derived from the initial

14   MARIE-GENICA study. The pooled analysis including all breast cancer case-control collections

15   MARIE-GENICA, MCCS and SASBAC confirmed this decrease in risk and moreover, suggests

16   a co-dominant effect, in that carriers of the *HSD17B1*_937_G variant have a decreased breast

17   cancer risk with long-term HRT use.

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19   The strength of our study is the confirmation of the hypothesis of a reduced breast cancer risk

20   effect for carriers of the G variant of the *HSD17B1*_937_A>G polymorphism in association with

21   long-term HRT use in independent, large, and age-matched pooled study collections. The study

22   size allowed for a well powered analysis. Although the main effect is not statistically significant

23   in MARIE-GENICA, the direction of the effect is in line with the MCCS and SASBAC results.

24   We suggest that the effect of this genetic variant is reproducible but small.

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In conclusion, women who are carriers of the *HSD17B1*_937_G variant and use any HRT for 10 years or longer are more likely to be diagnosed with breast cancer than non-carriers. The *HSD17B1*_937_G variant does not influence breast cancer risk in women not using HRT. This genetic predisposition should encourage further association studies towards a better understanding of the inter-individual differences in the HRT-related breast cancer risk eventually leading to safer HRT use in the future.

Conflict of interest

The authors have no conflict of interest.

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Table 1 Association between *HSD17B1*_937_A>G genotypes and hormone replacement therapy-related breast cancer risk

Study	*HSD17B1* Genotypes	Cases *n* (%)	Controls *n* (%)	OR (95% CI)	*p* value
Interaction Analysis					
Initial study MARIE-GENICA [1]	AA	916 (29.4)	1,613 (29.8)	1.17[a] (1.10-1.25)	
	AG	1,592 (51.0)	2,702 (49.9)	1.10[a] (1.05-1.16)	
	GG	610 (19.6)	1,102 (20.3)	1.11[a] (1.02-1.20)	0.032
Main Effect Analysis (subgroup of HRT users for 10 years or longer)					
MARIE-GENICA	AA	359 (30.3)	444 (27.7)	1.00[b]	
	AG	591 (49.8)	833 (52.0)	0.88[c] (0.74-1.05)	0.141
	GG	236 (19.9)	324 (20.2)	0.95[c] (0.85-1.06)	0.339
	AG+GG	827	1,157	0.88[c] (0.75-1.04)	0.142
Validation study MCCS and SASBAC	AA	84 (37.3)	44 (38.6)	1.00[b]	
	AG	108 (48.0)	50 (43.9)	0.86[c] (0.35-2.11)	0.741
	GG	33 (14.7)	20 (17.5)	0.56[c] (0.32-0.96)	0.041
	AG+GG	141	70	0.64[c] (0.28-1.47)	0.294
Pooled Analysis MCCS, SASBAC and MARIE-GENICA	AA	443 (31.4)	488 (28.5)	1.00[b]	
	AG	699 (49.5)	883 (51.5)	0.86[c] (0.73-1.01)	0.065
	GG	269 (19.1)	344 (20.1)	0.92[c] (0.83-1.02)	0.124
	AG+GG	968	1,227	0.86[c] (0.73-0.99)	0.048

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