



LUND UNIVERSITY  
Faculty of Medicine

---

# LUP

*Lund University Publications*

Institutional Repository of Lund University

---

This is an author produced version of a paper published in *Growth Hormone & IGF Research* : official journal of the Growth Hormone Research Society and the International IGF Research Society. This paper has been peer-reviewed but does not include the final publisher proof-corrections or journal pagination.

Citation for the published paper:  
Maria Henningson, Maria Hietala, Erika Bågeman, Håkan Olsson, Helena Jernström

"Interactions between oral contraceptive status and GSTM1 and GSTT1 deletions on insulin-like growth factor-1 (IGF-1) plasma levels in young healthy women."

*Growth Hormone & IGF Research* : official journal of the Growth Hormone Research Society and the International IGF Research Society 2010 Dec

<http://dx.doi.org/10.1016/j.ghir.2010.10.003>

Access to the published version may require journal subscription.  
Published with permission from: Elsevier

# Interactions between oral contraceptive status and *GSTM1* and *GSTT1* deletions on insulin-like growth factor-1 (IGF-1) plasma levels in young healthy women

M CK Henningson<sup>1</sup>, M Hietala<sup>1</sup>, E Bågeman<sup>1</sup>, H Olsson<sup>1,2</sup>, H Jernström<sup>1</sup>

1. Department of Oncology, Clinical Sciences, Lund University, Sweden
2. Department of Cancer Epidemiology, Clinical Sciences, Lund University, Sweden

## Abstract

**Objective:** Insulin-like growth factor-1 (IGF-1) is essential for the pubertal growth spurt and for normal mammary gland development. IGF-1 increases premenopausal breast cancer risk. Oral contraceptives (OCs) decrease IGF-1 in most women. The endogenous estrogens and their metabolites also influence IGF-1 levels. Glutathione S-transferases (GSTs) are involved in estrogen metabolism. We aimed to study IGF-1 levels and body size in relation to *GSTM1* and *GSTT1* deletions, and *GSTP1\*1B* and current oral contraceptive (OC) status. **Design:** Questionnaires on reproductive factors and OC use were completed and blood samples were obtained during menstrual cycle day 18-23 in healthy women ( $\leq 40$  years) from breast cancer high-risk families. IGF-1 was analyzed with radioimmunoassay. Genetic analyses were done with PCR based methods. Initially 258 women were included. After exclusion 229 women were finally included in the analyses of IGF-1 in relation to *GSTM1* and *GSTT1*. **Results:** Among the 142 non-OC users, *GSTM1\*0/0* or *GSTT1\*0/0* alone were associated with lower IGF-1 levels while homozygous *GSTM1\*0/0/GSTT1\*0/0* carriers had higher IGF-1 levels ( $P_{interaction}=0.024$ ). In the 87 OC users, *GSTM1\*0/0* or *GSTT1\*0/0* alone were associated with higher IGF-1 levels while homozygous *GSTM1\*0/0/GSTT1\*0/0* carriers had lower IGF-1 levels ( $P_{interaction}=0.010$ ). Among all 229 women, a three-way interaction model showed an interaction between the *GSTM1\*0/0/GSTT1\*0/0* genotype and OC use on IGF-1 levels ( $P_{interaction}=0.003$ ). *GSTP1\*1B* was not associated with IGF-1. The *GSTM1\*1/GSTT1\*0/0* genotype was associated with high body weight ( $P=0.003$ ) and *GSTM1\*0/0/GSTT1\*0/0* was associated with early growth ( $P=0.003$ ). **Conclusion:** Both OC use and *GSTT1* and *GSTM1* genotypes may influence IGF-1 levels. Further studies are warranted to confirm our finding and elucidate the clinical importance.

**Correspondance:** Helena Jernström, Department of Oncology, Clinical Sciences, Lund University  
Barngatan 2B, SE-221 85 Lund, Sweden  
Fax +46-46-147327, E-mail:Helena.Jernstrom@med.lu.se

**Key terms:** oral contraception; IGF-1; *GSTM1*; *GSTT1*; *GSTP1*; gene mutations; metabolism

## Introduction

Insulin-like growth factor-1 (IGF-1) regulates cell proliferation and apoptosis and plays a key role in the development of the female breast<sup>1,2</sup>. High circulating IGF-1 levels have been associated with increased risk of premenopausal breast cancer<sup>3</sup>. Although the results of epidemiological studies are not totally consistent<sup>4,5</sup>, a recent pooled analysis of individual data showed a positive association between higher IGF-1 levels and breast cancer<sup>6</sup>.

The endogenous estrogen level is positively associated with IGF-1<sup>7</sup>, while oral contraceptive (OC) use has been associated with lower IGF-1 levels in most, but not all women<sup>8-11</sup>. The mechanism behind the effect of exogenous estrogen on IGF-1 levels is still not fully understood. Polymorphisms in genes involved in steroid hormone metabolism, such as glutathione S-transferase genes (*GST*), may influence the IGF-1 response to endogenous as well as exogenous estrogens.

The *GST* gene products are phase II metabolic enzymes involved in the metabolism of environmental carcinogens and steroid hormones such as estrogens. Deletions in *GST* mu class (*GSTM1*) and theta class (*GSTT1*) genes are found in approximately 50% and 10 to 20% of Caucasian populations, respectively<sup>12</sup>. Double homozygous deletions (*GSTM1\*0\*0* and *GSTT1\*0\*0*) are present in approximately ten percent of Caucasian populations and may be somewhat lower in Scandinavian populations<sup>13</sup>. For individuals who carry homozygous deletions in *GSTM1* or *GSTT1*, no enzymatic activity for that enzyme can be measured and the ability to detoxify *GST* substrates may be impaired. However, the *GSTM1* and *GSTT1* enzymes have partly overlapping substrate specificity. One study found the *GSTM1\*0\*0* genotype to be associated with a significant increase in *GSTT1* activity after exposure to xenobiotics. Subjects with the *GSTM1\*0\*0/GSTT1\*1* genotype had higher enzyme activity than subjects with *GSTM1\*1/GSTT1\*1* indicating potential overcompensation<sup>14</sup>. Whether overcompensation also takes place during OC exposure in women lacking one of the *GSTM1* or *GSTT1* genes is currently unknown.

The *GST* pi class enzyme (*GSTP1*) is the predominant *GST* expressed in breast tissue<sup>15</sup>. The *GSTP1\*1B* polymorphism of A313G encodes the substitution of the amino acid isoleucine for valine at position 105. The *GSTP1* Val allele is found in 40% of European populations<sup>13</sup>, and the Val allele gene product was found to alter substrate specific activity and affinity compared to that of the Ile allele<sup>16</sup>. The *GSTP1* Val allele was found to be a breast cancer risk allele in a sub-analysis of this gene in a larger meta-analysis<sup>17</sup>. This polymorphism may influence the IGF-1 response to estrogens.

The combination of *GSTM1\*0\*0* and *GSTT1\*0\*0* as well as the combination of the three risk genotypes, *GSTM1\*0\*0*, *GSTT1\*0\*0* and *GSTP1 Ile/Ile*, has been associated with increased breast cancer risk among premenopausal women<sup>18</sup>. However, other studies have yielded conflicting results<sup>17-25</sup>. Whether any association between *GSTM1*, *GSTT1* and *GSTP1* genotypes and breast cancer is mediated through the IGF-1 axis is currently unknown.

We hypothesized that the *GST* polymorphisms influence the efficiency of estrogen metabolism and thereby the effect of estrogens on IGF-1 levels. Endogenous and exogenous estrogens have opposite effects on IGF-1 levels in most women<sup>8-11</sup>. IGF-1 may also influence body size and growth during puberty<sup>26</sup>. Lack of estrogen metabolizing *GSTM1* and *GSTT1* enzymes may allow endogenous estrogens to have a stronger influence on IGF-1 levels, which then would be higher compared with those in a woman with functional enzymes. OCs may have a stronger impact on IGF-1 levels among women with no functional enzymes, since

these women would be expected to have lower IGF-1 levels compared with women with functional enzymes.

To our knowledge, there are no studies examining potential associations between polymorphisms in the *GST* genes and IGF-1 plasma levels in healthy young women in relation to OC status. Our aims were to study whether the *GSTM1*, *GSTT1* and *GSTP1* genotypes were associated with body sizes and early growth during puberty in all women, and whether the *GSTM1*, *GSTT1* and *GSTP1* genotypes were associated with IGF-1 levels in OC users and non-users.

## Materials and Methods

### *Study population*

This is an ongoing prospective study of young healthy women from Swedish high-risk breast cancer families. The material and methods have been described in detail elsewhere<sup>10</sup>. Two hundred and fifty-eight women from 158 families were included. They did not have any type of cancer at the time of enrolment between 1996 and 2004. Only women who were menstruating and who had not undergone a prophylactic mastectomy were eligible to participate. Potential participants were identified from charts and pedigrees from the Lund Oncogenetic Clinic and were contacted, first by a letter including brief information on the study, then by phone. A package including an extensive epidemiologic questionnaire and a written consent form was mailed to women who verbally agreed to participate. The questionnaire requested information regarding reproductive factors, the use of OCs and other medications, smoking etc. Women were also asked about their height relative to classmates (shorter, average or taller) when they were 11 to 13 years old (5<sup>th</sup> and 6<sup>th</sup> grade in Sweden). All women signed a written informed consent. The Ethics Committee of Lund University approved the study.

Blood samples and body measurements were taken once during the follicular phase, i.e. menstrual cycle days 5 to 10, and once again during the luteal phase 5 to 10 days before the predicted onset of the following menstrual period, i.e. cycle days 18 to 23 in most women. All women were asked to call back with the date of the first day of their next menstrual period. The plasma and blood cells were separated and frozen at minus 70 degrees Celsius at the laboratory of the Department of Oncology, Lund. Body measurements included height, weight, waist and hip circumferences, and breast volume.

### *Genetic analyses*

DNA was extracted from 300 $\mu$ L of peripheral blood using Wizard, Genomic DNA Purification Kit (Promega, Madison, WI, USA). The *GSTM1* and *GSTT1* deletions were evaluated in a multiplex reaction. The PCR primers for *GSTM1* and *GSTT1* yielded products when wild type alleles were present. The technique did not distinguish between heterozygote and homozygote *GSTM1*\*1 or *GSTT1*\*1 genotypes, but conclusively identified null genotypes.  $\beta$ -globulin was used as a positive control to conclude that absence of a PCR product was the result of a deletion. PCR primers *GSTM1*-Fw: 5'-GAACTCCCTGAAAAGCTAAAGC-3' and *GSTM1*-Re: 5'-GTTGGGCTCAAATATACGGTGG-3' (Invitrogen, Carlsbad, CA, USA) yielded a 219 bp nucleotide sequence. PCR primers *GSTT1*-Fw: 5'-TTCCTTACTGGTCCTCACATCTC-3' and *GSTT1*-Re: 5'-TCACCGGATCATGGCCAGCA-3' (Invitrogen, Carlsbad, CA, USA) yielded a 459 bp nucleotide sequence and  $\beta$ -globulin-Fw: 5'-

CAACTTCATCCACGTTCCACC-3' and  $\beta$ -globulin-Re: 5'-GAAGAGCCAAGGACAGGTAC-3' yielded a 268bp nucleotide sequence. PCR was performed in 12.5 $\mu$ l reactions using 0.6 $\mu$ M of each primer, 0.2mM of each deoxynucleotide (Amersham Biosciences, Buckinghamshire, UK), 2.5mM MgCl<sub>2</sub> (Applied Biosystems, Foster City, CA, USA), 1 x PCR Gold Buffer (Applied Biosystems, Foster City, CA, US5A) and 0.25U AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA). PCR products were analyzed by High Performance Liquid Chromatography (HPLC) performed in a 25 $\mu$ l reaction volume.

The *GSTP1* exon 5 A to G (Ile 105 Val) SNP (rs1695) was genotyped at Region Skåne Competence Centre (RSKC Malmö), Malmö University Hospital, Malmö, Sweden. The SEQUENOM massARRAY® designer software was used for multiplex SNP analysis design. The analysis was performed on a matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (SEQUENOM MassArray) using iPLEX reagents according to the manufacturers' protocol. For quality control thirty-two samples were run in duplicate and the concordance was 100%.

Mutation testing of the *BRCA1* and *BRCA2* genes was not performed as part of this study. Carrier status was obtained from the clinical records. Further information on mutation testing procedures has been published elsewhere<sup>27</sup>.

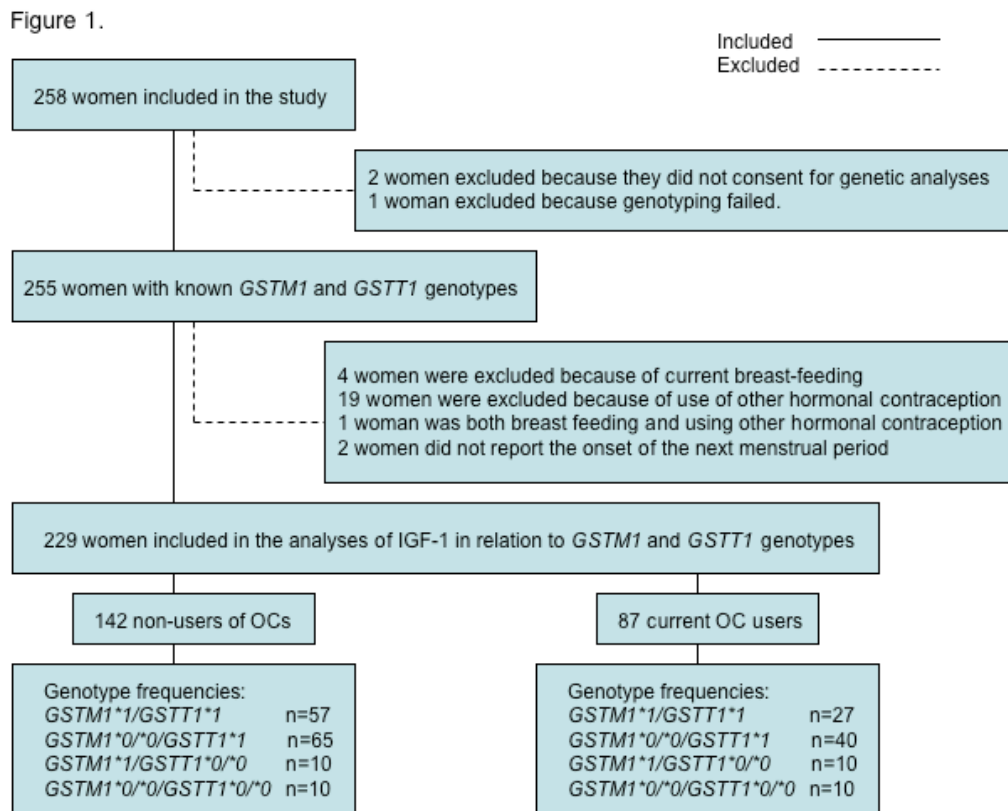
#### *Insulin-like growth factor-1*

IGF-1 was analyzed in EDTA-plasma with a radioimmunoassay (RIA) method at the Endocrinological Laboratory of Karolinska Hospital, Stockholm, Sweden as previously described by Bang *et al.*<sup>28</sup>. Acid ethanol extraction followed by cryo-precipitation was used to separate insulin-like binding proteins (IGFBPs) from IGF-1 and decrease the interference of IGFBPs in the IGF-1 RIA. Recombinant human IGF-1 (Kabi-Pharmacia, Stockholm, Sweden) was used as standard. The international IGF-1 standard was delivered by the National Institute of Biological Standards and Control, London, Great Britain. The intra assay variation was 4% and the inter assay variation was 11%. The limit of detection was 6  $\mu$ g/L. IGF-1 levels were missing during cycle days 5-10 for one woman. For several of the OC users, the samples obtained during cycle days 5-10 were collected during their pill-free week. Therefore, we only assessed the association between *GSTM1* and *GSTT1* genotypes and IGF-1 during menstrual cycle days 18-23.

#### *Data analyses*

The statistical software program PASW 17.0 (former SPSS) was used for all statistical analyses. Weight and BMI in relation to *GSTM1/GSTT1* genotypes were compared in multivariate linear regression models. Chi-square was used to compare relative height at ages 11 to 13 years in relation to *GSTM1/GSTT1* genotypes. IGF-1 levels were not normally distributed and were therefore transformed using the natural logarithm (ln). Twenty six women were excluded from the IGF-1 analyses because of current breast-feeding (n=4), use of hormonal contraception other than combined estrogen plus progestin oral contraceptives (n=19), or both (n=1), as well as two women who had not reported the onset of the next menstrual period, figure 1. Multivariate linear regression analyses were performed to evaluate the relationship between *GST* genotypes and ln-transformed IGF-1 levels. Adjustments were made for potentially confounding factors, age, body weight, height, parity, and menstrual cycle day. Body weight was not normally distributed and the values were transformed using

the natural logarithm. A two-way interaction term between the *GSTM1*\*0/\*0 and *GSTT1*\*0/\*0 genotypes was calculated and used in a regression analysis. A three-way interaction term was calculated between the *GSTM1*\*0/\*0 and *GSTT1*\*0/\*0 genotypes and OC use. The magnitude of the interaction is presented as  $\beta$ . Adjustment for family clustering was done by using the cluster option of the regress command in Stata. No initial power calculation was made for analyses of three-way interactions. Nominal *P*-values are presented, all *P*-values were two-sided.



**Figure 1.** The flow-diagram shows the number of women included in (solid lines) and excluded from (broken lines) the analyses of IGF-1 levels in relation to *GSTM1* and *GSTT1* genotypes.

## Results

The characteristics for all 258 women, with and without stratification according to the *GSTM1* and *GSTT1* genotypes, are presented in table I. Two hundred and fifty six of the 258 women consented to genetic analyses and 229 women were either current users of combined OCs (n=87) or non-users (n=142). *GSTM1* and *GSTT1* were successfully genotyped in 255 women and *GSTP1* in 254 women, table II. *GST* genotype frequency did not differ according to *BRCA* mutation status. The 21 women with the *GSTM1*\*1/*GSTT1*\*0/\*0 genotype had higher body weight and BMI ( $P=0.003$  and  $P=0.005$ ) than women with other genotypes. The proportion of women who were relatively taller than their classmates at age 11 to 13 was higher among those with the *GSTM1*\*0/\*0/*GSTT1*\*0/\*0 genotype than among women with other genotypes (linear-by-linear association; df 1;  $P=0.003$ ). The other variables did not differ according to the *GST* genotypes.

Table I. Characteristics of all 258 women, and in relation to *GSTM1* and *GSTT1* genotypes†.

	All women	<i>GSTM1</i> *1/ <i>GSTT1</i> *1	<i>GSTM1</i> *1/ <i>GSTT1</i> *0/*0	<i>GSTM1</i> *0/0/ <i>GSTT1</i> *1	<i>GSTM1</i> *0/*0/ <i>GSTT1</i> *0/*0
	n=258	n=93	n=21	n=119	n=22
	Mean (±SD) or %	Mean (±SD) or %	Mean (±SD) or %	Mean (±SD) or %	Mean (±SD) or %
Year of birth	1970 (±7.0)	1969 (±7.0)	1971 (±6.9)	1970 (±6.9)	1970 (±7.1)
Age at baseline, years	29.2 (±6.4)	29.7 (±6.2)	28.0 (±6.1)	29.0 (±6.4)	29.2 (±6.5)
Age at menarche, years‡	12.8 (±1.3)	12.8 (±1.2)	12.5 (±1.3)	12.9 (±1.3)	12.2 (±1.3)
Nulliparous at baseline, %	51	48	52	54	46
Hormonal contraception, ever, %	92	95	90	90	95
OC-use, current, %	41	37	48	43	50
Height, cm	168 (±5.9)	168 (±6.3)	170 (±8.0)	167 (±5.2)	168 (±6.0)
Weight, kgs	67.2 (±12.8)	65.5 (±11.7)	74.9 (±15.0)	67.1 (±13.3)	67.1 (±11.3)
BMI kg/m <sup>2</sup>	23.8 (±4.3)	23.1 (±3.7)	26.0 (±5.3)	23.9 (±4.5)	23.7 (±3.7)
Waist to hip ratio	0.77 (±0.06)	0.78 (±0.06)	0.79 (±0.07)	0.76 (±0.05)	0.76 (±0.05)
Relative height at age 11 to 13	n=255 (%)	n=93 (%)	n=21 (%)	n=119(%)	n=22 (%)
Shorter	36 (14)	17 (18)	2 (10)	16 (13)	1 (5)
Average	151 (59)	50 (54)	12 (57)	80 (67)	9 (41)
Taller	68 (27)	26 (28)	7 (33)	23 (19)	12 (55)

†Genotypes were missing for three women.

‡One woman had not answered the question about age at menarche.