The insulin-like growth factor-1 pathway mediator genes: SHC1 Met300Val shows a protective effect in breast cancer

Kerstin Wagner1,6, Kari Hemminki1,2, Ewa Grzybowska3, Rüdiger Klæs4, Dorota Butkiewicz1, Jolanta Panula3, Wioletta Pekala3, Helena Zientek3, Danuta Mielżynska5, Ewa Siwinska5 and Asta Forsti1,2

1Division of Molecular Genetic Epidemiology C050, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 280, 69120 Heidelberg, Germany, 2Department of Biosciences at Novum, Karolinska Institute, Huddinge, Sweden, 3Department of Tumor Biology, Centre of Oncology, Maria Skłodowska-Curie Institute, Gliwice, Poland, 4Institute of Human Genetics, University of Heidelberg, Heidelberg, Germany and 5Department of Genetic Toxicology, Institute of Occupational Medicine and Environmental Health, Sosnowiec, Poland

6To whom correspondence should be addressed. Tel: +49 6221 421811; Fax: +49 6221 421810;
Email: k.wagner@dkfz.de

The insulin-like growth factor 1 (IGF-1) pathway plays an important role in regulating cell proliferation, differentiation and apoptosis. IRS1, IRS2 and SHC1 are the key mediators for the downstream pathway processes. Genetic variation within these genes may lead to altered signalling. We screened IRS1, IRS2 and SHC1 for published coding region polymorphisms and choose five of them, IRS1 Ala804Ala and Gly972Arg, IRS2 Cys816Cys and Gly1057Asp and SHC1 Met300Val, for further analysis. We studied the association of the polymorphisms with breast cancer risk using a case-control design with Polish familial breast cancer cases and respective controls. For the polymorphisms in IRS1 and IRS2 no differences in the allele, genotype or haplotype distributions could be detected between the case and control subjects. Carriers of the variant allele of the SHC1 polymorphism were at decreased risk of breast cancer (OR 0.54, 95% CI 0.32–0.90, P = 0.016). A non-significant trend for a protective effect of the SHC1 Val300 allele was also seen in an independent population consisting of German familial breast cancer cases and matched controls. The joint analysis after Mantel-Haenszel adjustment of the two populations gave an OR of 0.62 (95% CI 0.41–0.93, P = 0.02) for the SHC1 Val300 carriers. A stronger effect was detected in women diagnosed below the age of 50 (OR 0.54, 95% CI 0.32–0.89, P = 0.01). A genotype combination analysis of the non-synonymous polymorphisms in the IRS1, IRS2 and SHC1 genes did not show any effect on breast cancer risk.

Introduction

One of the major health problems for women in the Western world is breast cancer. Both genetic and non-genetic factors are involved in breast cancer development. Twin studies have shown that about one-third of the causes of breast cancer can be explained by heritability, the rest by random and shared environmental causes (1). About 10% of all breast cancer cases have a family history of breast cancer (2). Mutations in the high penetrance genes BRCA1 and BRCA2 are well known, but they only explain a small proportion of the family aggregation of breast cancer (3). Genetic variants in low penetrance genes may moderately increase the risk of breast cancer and in combination they may have a high impact on cancer formation (3–5).

The insulin-like growth factor 1 (IGF-1) pathway plays an important role in regulating cell proliferation, differentiation and apoptosis (6–8). The interaction between IGF-1 and its receptor is regulated by insulin-like growth factor binding proteins (IGFBPs). Clinical and epidemiological studies have suggested an association between the levels of circulating IGF-1 and IGFBP3 and the risk of breast cancer (6–8). A recent meta-analysis of 16 published studies showed that the increased risk associated with high IGF-1 and IGFBP3 levels is only seen in premenopausal women (9). Insulin receptor substrates (IRS) and Src homology 2 domain-containing transforming protein 1 (SHC1) are key mediators of the IGF-1 pathway (6,10,11). They act as docking proteins between the activated receptor and further downstream signalling proteins. IRS1 is a major substrate for the IGF-1 receptor while IRS2 and SHC1 are proteins that, beside the IGF-1 pathway, also are substrates for other tyrosine kinase growth factors. After IGF-1 binding and autophosphorylation of the IGF-1 receptor, the IRS and SHC1 proteins are tyrosine phosphorylated and can therefore interact and activate especially Src homology 2 (SH2) domain-containing proteins. Both IRS and SHC1 can bind to growth factor receptor bound protein 2 (GRB2) to activate the Ras/MAP kinase pathway that regulates cell proliferation. The IRS proteins can also bind to the p85 subunit of phosphatidylinositol (PI)-3 kinase and activate the serine kinase PKB/Akt pathway that regulates apoptosis. Changes in the activity of the IRS and SHC1 proteins may thus have an effect both on cell proliferation and apoptosis. It has been shown that IRS1 and SHC1 are constitutively activated in a high number of human tumours, including breast tumours, and may explain the overexpression of MAP kinase detected in many breast cancers (12–14). Polymorphisms that are located in important regulatory regions, such as in the promoter region or functionally active domains, may affect the function of the protein (4). In IRS1, IRS2 and SHC1 polymorphisms that are located close to the tyrosine phosphorylation sites may cause a change in the tertiary structure of these proteins and therefore show an effect on cell proliferation and apoptosis. This has been shown for the IRS1 Gly972Arg polymorphism (15).

Abbreviations: 95% CI, 95% confidence interval; GRB2, growth factor receptor bound protein 2; IGF-1, insulin-like growth factor-1; IGFBPs, insulin-like growth factor binding proteins; IRS, insulin receptor substrate; OR, odds ratios; PI, phosphatidylinositol; RFLP, restriction fragment length polymorphism; SH2, Src homology 2; SHC1, Src homology 2 domain-containing transforming protein 1; SNPs, single nucleotide polymorphisms.
In this paper, we have sequenced the coding region of the IRS1, IRS2 and SHC1 genes for polymorphisms. Of the confirmed polymorphisms, we chose three for further analysis because of their putative functional effects (IRS1 Gly972Arg, IRS2 Gly1057Asp and SHC1 Met300Val) and two polymorphisms because of their usefulness in haplotype analysis (IRS1 Ala804Ala and IRS2 Cys816Cys). To our knowledge, the role of these polymorphisms has never been tested in breast cancer. We carried out a case–control study on the effects of the polymorphisms on breast cancer risk among familial breast cancer cases and controls.

Materials and methods

Subjects

The initial analysis was performed on 23 breast cancer cases to confirm the presence of the polymorphisms by DNA sequencing. Further analyses were performed on genomic DNA from 357 Polish familial breast cancer cases (mean age 46 years, range 22–81) and 450 controls. Additionally, the IRS2 Met300Val polymorphism was analysed in 217 German familial breast cancer cases (mean age 42 years, range 17–68) and 243 controls. The study population consisted of cases collected during the years 1997–2003 according to criteria described earlier (16–18) through Chemotherapy Clinics and the Genetic Counseling (Gliwice, Poland) and the Institute of Human Genetics, University Heidelberg (Germany). About 90% of the patients approved participation in the study. All the familial cases were unrelated and they did not carry BRCA1/2 mutations. The controls were recruited to earlier studies with a comparable participation rate. They represented the same ethnic group and came from the same geographic area as the breast cancer cases. The study was approved by the ethical committee of the University of Heidelberg.

PCR amplification

Amplification was performed with 5 ng genomic DNA in a 10 μl reaction volume using 1× PCR buffer, 1.5 mM MgCl2, 0.11 μM dNTP Mixture (Invitrogen, Paisley, UK), 0.15 μM each primer (MWG Biotech AG, Ebersberg, Germany) and 0.3 U Platinum Taq DNA polymerase (Invitrogen). The primer and probe sequences for detecting single nucleotide polymorphisms (SNPs) in each gene are available on request from the corresponding author. For the IRS2 Gly1057Asp polymorphism 5% DMSO was added to the reaction mixture. The PCR programme was: 94°C for 2 min; 3 cycles of 94°C for 1 min, the optimum annealing temperature for the gene (61, 65 and 59°C for IRS1, IRS2 and SHC1, respectively) for 1 min; 72°C for 1 min; 32 cycles of 94°C for 30 s, the optimum annealing temperature minus 1°C for 30 s; final extension at 72°C for 6 min. PCR was performed in GeneAmp PCR System 9700 thermocyclers (Applied Biosystems, Foster City, USA).

DNA sequencing

PCR was performed in 10 μl using the same primers and conditions as mentioned above. The PCR product was cleaned up using 0.75 μl ExoSapIT (USB Amersham, Uppsala, Sweden) for 40 min at 37°C followed by 15 min at 85°C. The sequencing reaction was carried out using a Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). The sequencing reaction was performed using forward and reverse primers separately with the following PCR conditions: 96°C for 1 min; 27 cycles of 96°C for 16 s, 54°C for 5 s and 60°C for 4 min. The sequencing products were precipitated with isopropanol, resuspended in 25 μl of water and loaded onto an ABI Prism 3100 Genetic analyser (Applied Biosystems). The original data were analysed using Sequencing Analysis 3.7 software (Applied Biosystems) for base calling. The obtained sequences were aligned using DNAStar Lasergene 5.0 software (DNAStar Inc., Madison, WI).

Genotyping using the Taq-Man assay

The polymorphisms Ala804Ala and Gly972Arg in IRS1 and Cys816Cys in IRS2 were investigated using the allelic discrimination method. Taq-Man primers and probes were ordered from Applied Biosystems. The reaction was performed in 10 μl using 225 nM each primer, 30 nM each probe and 5 μl Taq-Man Universal 2× PCR Master Mix (Applied Biosystems) per reaction. PCR was performed at 50°C for 2 min, 95°C for 10 min and for 35–45 cycles at 92°C for 15 s and 60°C for 1 min. PCR was performed in GeneAmp PCR System 9700 thermocyclers and the number of cycles was dependent on the genotype clustering. The samples were read and analysed in an ABI Prism 7900HT sequence detection system using SDS 1.2 software (Applied Biosystems). We confirmed ~10% of the results by sequencing.

Genotyping using RFLP assay

For the IRS2 Gly1057Asp and SHC1 Met300Val polymorphisms we were not able to design Taq-Man probes and therefore restriction fragment length polymorphism analysis (RFLP) was used. The IRS2 polymorphism was digested with 4 U Smal directly after PCR. The PCR fragment had an additional restriction site for SmaI that we used as an internal control for digestion. The resulting fragments (GG, 124 + 115 + 54 bp; AA, 178 + 115 bp; GA, 178 + 124 + 115 + 54 bp) were analyzed on ethidium bromide stained 15% PAGE mini-gels (Gel Casting System; Bio-Rad, Hercules, CA). For the SHC1 polymorphism we used a long tail forward primer for the PCR which contained a second restriction site for BshNI (MBI Fermentas, St Leon-Rot, Germany) in addition to the polymorphic site, to be used as an internal digestion control. The resulting fragments (AA, 262 + 21 bp; GG, 146 + 108 + 21 bp; AG, 262 + 146 + 108 + 21 bp) were analyzed on ethidium bromide stained 8% PAGE mini-gels (Gel Casting System). The temperatures and digestion times used were as recommended by the manufacturers. About 10% of the RFLP assays were randomly repeated and the results checked for concordance. Additionally, we confirmed ~5% of the RFLP results by DNA sequencing.

Haplotype analysis

Haplotypes for the IRS1 and IRS2 genes were determined using PHASE 2 software, created by Stephens et al. (19) (http://archimedes.well.ox.ac.uk/pise/ PHASE-simple.html).

Statistical analysis

The observed genotype frequencies in the breast cancer cases and controls were tested for Hardy–Weinberg equilibrium and the difference between the observed and expected frequencies was tested for significance using the χ² test. Statistical significance for the differences in the genotype and haplotype frequencies was determined by the χ² test. The joint analysis was carried out using Mantel–Haenszel adjustment. Whenever the expected number of cases was smaller than five, Fisher’s exact test was used. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated for associations between genotypes and breast cancer. With the present sample size we had a power of 90% to detect an OR of 0.5 for polymorphisms with a 5% rare allele frequency in the control population. For the more common polymorphisms with a variant allele frequency of 35% we had a 90% power to detect an OR of 0.7. All calculations were carried out using the Hardy–Weinberg equilibrium test tool offered by the Institute of Human Genetics, Technische Universität Munich (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl) and Epi Info 2000 software.

Results

We sequenced 23 familial breast cancer samples (46 alleles) for published polymorphisms in the coding regions of the IRS1, IRS2 and SHC1 genes (Table I). Of the 36 polymorphisms screened, 26 did not exist or were too rare to be found in our 23 samples. Four unpublished silent and one intronic polymorphism were detected (Table I).

From the existing polymorphisms we chose three for further analyses because of their closeness to tyrosine phosphorylation sites and, therefore, their putative functional effects (Table I). Two were chosen because of their suitability for haplotype analysis. All the other detected polymorphisms were either too rare to be included in the analysis or seemed to be in linkage disequilibrium with those chosen. The genotype distributions of all five polymorphisms were consistent with the Hardy–Weinberg equilibrium.

IRS1 variants are not associated with breast cancer risk

The Ala804Ala and Gly972Arg polymorphisms were used for a case–control study and haplotype analysis of the IRS1 gene. The allele frequencies were concordant with so far published Caucasian studies (20–22). For the Ala804Ala polymorphism, the allele and genotype frequencies did not differ between the breast cancer cases and controls (Table II) and no homozygous individuals for the GG genotype could be detected. For the Gly972Arg polymorphism, the GA frequency in controls was slightly higher than in cases (10.5 and 8.5%, respectively), but the homozygous AA genotype was only detected in two breast
IRS2 variants do not show any effect on breast cancer risk

The Cys816Cys and Gly1057Asp polymorphisms were used for further analyses in the IRS2 gene. In the Polish population these SNPs were very common, with rare allele frequencies of 35.4 and 39.7%, respectively, frequencies concordant with so far published Caucasian studies (23,24). No differences in the allele or genotype frequencies between cases and controls could be observed (Table II). Age of diagnosis, <50 (GA, OR 1.03, 95% CI 0.73–1.46, \( P = 0.87 \)) or \( \geq 50 \) years (GA, OR 1.46, 95% CI 0.90–2.35, \( P = 0.12 \)) did not have any effect on breast cancer risk. However, when mutually adjusted for the other SNP in the IRS2 gene no changes in the ORs were observed (data not shown). The haplotype distribution also did not show any effect on breast cancer risk (Table II).

SHC1 Val300 reduces breast cancer risk

We continued to investigate the Met300Val polymorphism in the SHC1 coding region because of its possible functional activity. Initially, only the Polish population was screened. The allele frequency was in concordance with the literature for Caucasian populations (25,26). The results showed a significant difference in the genotype frequency which led to a decreased risk of breast cancer with an OR of 0.54 (95% CI 0.32–0.90, \( P = 0.016 \)) (Table III). The protective effect was observed in women diagnosed both below and over the age of 50, but the effect was significant only in women below the age of 50 (OR 0.51, 95% CI 0.28–0.93, \( P = 0.03 \)). Heterozygotes were more common in controls than in cases. The only homozygote was found among the control population. Because of this finding we included 217 German familial breast cancer cases (Table II). The genotypes did not show any significant relationship to breast cancer risk, not even when stratified according to age of diagnosis, <50 (OR 0.69, 95% CI 0.39–1.22, \( P = 0.20 \)) or \( \geq 50 \) years (OR 1.09, 95% CI 0.90–1.35, \( P = 0.12 \)) did not have any effect on the risk. When mutually adjusted for the other SNP in the IRS2 gene no changes in the ORs were observed (data not shown). The haplotype distribution also did not show any effect on breast cancer risk (Table II).

SHC1 Met300Val protects against breast cancer

We continued to investigate the Met300Val polymorphism in the SHC1 coding region because of its possible functional activity. Initially, only the Polish population was screened. The allele frequency was in concordance with the literature for Caucasian populations (25,26). The results showed a significant difference in the genotype frequency which led to a decreased risk of breast cancer with an OR of 0.54 (95% CI 0.32–0.90, \( P = 0.016 \)) (Table III). The protective effect was observed in women diagnosed both below and over the age of 50, but the effect was significant only in women below the age of 50 (OR 0.51, 95% CI 0.28–0.93, \( P = 0.03 \)). Heterozygotes were more common in controls than in cases. The only homozygote was found among the control population. Because of this finding we included 217 German familial breast cancer cases (Table II). The genotypes did not show any significant relationship to breast cancer risk, not even when stratified according to age of diagnosis, <50 (OR 0.69, 95% CI 0.39–1.22, \( P = 0.20 \)) or \( \geq 50 \) years (OR 1.09, 95% CI 0.90–1.35, \( P = 0.12 \)) did not have any effect on the risk. When mutually adjusted for the other SNP in the IRS2 gene no changes in the ORs were observed (data not shown). The haplotype distribution also did not show any effect on breast cancer risk (Table II).
cases and 243 German controls in our study. In this population only a small difference in the frequency of the AG genotype between the case and control subjects was observed (7.4 and 9.1%, respectively), however, a trend for a lower breast cancer risk in heterozygotes could still be seen. No homozygous frequencies of the different genotype combinations between cases and controls were detected (data not shown). There was no trend for a decreased risk of breast cancer with increased number of variant alleles ($\chi^2 = 2.77, P = 0.6, df = 4$).

### Discussion

We used familial breast cancer cases to study the influence of polymorphisms in the IRS1, IRS2 and SHC1 genes on breast cancer. We chose familial cases for our study because it is expected that the use of familial cases significantly increases the power to detect rare alleles contributing to risk or protective effects in breast cancer (27,28). To achieve the same power a substantially lower sample size is required for a study using familial breast cancer cases compared with a study using unselected cases. Interestingly, a significant risk for breast cancer in carriers of the CHEK2*1100delC allele could only be detected in familial cases (29).

The $\text{IRS1}$ Gly972Arg polymorphism may change the function of the IRS1 protein. The arginine variant has been shown to have decreased binding to the p85 regulatory subunit of PI-3 kinase, resulting in decreased kinase activity and, finally, increased apoptosis (15,30,31). This impaired interaction may be due to an altered tertiary structure of the variant IRS1. The variant is located close to Tyr939 and Tyr987, both included in a YMXM motif which, upon phosphorylation, binds to the SH2 domain of PI-3K. Compared with the wild-type protein, the IRS1 variant may also show decreased binding to GRB2 and thus lower MAP kinase activity and impaired proliferation (15). The Gly972Arg polymorphism also influences insulin secretion and insulin signalling. It therefore plays an important role in insulin resistance disorders (20,21,30). Transgenic mice lacking IRS1 show a 50% growth retardation and resistance to insulin and IGF-1; insulin action can be partly compensated by IRS2 (30,32). In the case of cancer the arginine allele would be expected to have a protective effect. However, in a recent population-based study the arginine allele was associated with an increased risk of colon cancer (22). In our study no effect on breast cancer risk was observed. The synonymous change Ala804Ala showed no difference in the allele or genotype frequencies between cases and controls. The haplotype frequencies were also similar among cases and controls.

The $\text{IRS2}$ Gly1057Asp polymorphism is located close to two putative tyrosine phosphorylation sites at positions 1042 and 1072 and may change the tertiary structure and function of the protein, similar to the $\text{IRS1}$ Gly972Arg polymorphism (23,33). However, in the only functional study of the IRS2 variant, no altered binding to the p85 regulatory subunit of PI-3 kinase could be detected (23). In mice lacking IRS2 insulin resistance has been shown to prevail because no other protein can compensate for the altered insulin action. However, only minimal growth retardation was observed (30,34). Thus, IRS2 seems to be more important for regulating carbohydrate metabolism while IRS1 promotes somatic growth (30,32,34,35). Polymorphisms affecting the function of IRS2 would be expected to have a bigger effect in insulin resistance disorders than in cancer. The effects of the $\text{IRS2}$ Gly1057Asp polymorphism on insulin resistance disorders have been intensively studied, with controversial results (21,23,24,33,36,37). In a recent study heterozygotes for the Gly1057Asp polymorphism were shown to be at decreased risk of colon cancer (22). However, no effect on homozygotes for the Asp allele was observed.

### Table II. Genotype, allele and haplotype distributions of the polymorphisms in the genes for $\text{IRS1}$ and $\text{IRS2}$ in Polish breast cancer patients and controls

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genotype</th>
<th>BC (%)</th>
<th>Control (%)</th>
<th>OR (95% CI)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\text{IRS1}$ Ala804Ala</td>
<td>AA</td>
<td>300 (85.0)</td>
<td>380 (84.8)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AG</td>
<td>53 (15.0)</td>
<td>68 (15.2)</td>
<td>0.99 (0.67–1.46)</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gly972Arg</td>
<td>GG</td>
<td>323 (90.1)</td>
<td>400 (89.5)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>30 (8.5)</td>
<td>47 (10.5)</td>
<td>0.79 (0.49–1.28)</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>2 (0.6)</td>
<td>0 (0)</td>
<td>6.19 (0.3–129.4)</td>
<td>0.12</td>
</tr>
<tr>
<td>Haplotype*</td>
<td>AG</td>
<td>635 (88.9)</td>
<td>790 (88.0)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>33 (4.6)</td>
<td>41 (4.6)</td>
<td>1.00 (0.63–1.60)</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>46 (6.4)</td>
<td>67 (7.5)</td>
<td>0.85 (0.58–1.26)</td>
<td>0.43</td>
</tr>
<tr>
<td>$\text{IRS2}$ Cys816Cys</td>
<td>CC</td>
<td>152 (42.7)</td>
<td>183 (40.9)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CT</td>
<td>161 (45.2)</td>
<td>207 (46.2)</td>
<td>0.94 (0.71–1.26)</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>TT</td>
<td>43 (12.1)</td>
<td>58 (12.9)</td>
<td>0.89 (0.57–1.4)</td>
<td>0.62</td>
</tr>
<tr>
<td>Gly1057Asp</td>
<td>GG</td>
<td>129 (36.4)</td>
<td>177 (39.3)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>161 (45.5)</td>
<td>199 (44.2)</td>
<td>1.11 (0.82–1.51)</td>
<td>0.51</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>64 (18.1)</td>
<td>74 (16.4)</td>
<td>1.19 (0.79–1.78)</td>
<td>0.41</td>
</tr>
<tr>
<td>Haplotype*</td>
<td>AG</td>
<td>40.8</td>
<td>38.6</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>183 (25.7)</td>
<td>241 (26.8)</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>280 (39.3)</td>
<td>336 (37.3)</td>
<td>1.10 (0.86–1.41)</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>TG</td>
<td>238 (33.4)</td>
<td>314 (34.9)</td>
<td>1.00 (0.77–1.29)</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>TA</td>
<td>11 (1.6)</td>
<td>9 (1.0)</td>
<td>1.61 (0.65–3.4)</td>
<td>0.30</td>
</tr>
</tbody>
</table>

*Bases are cited in the order Ala804Ala and Gly972Arg.

*Haplotype frequencies were also similar among cases and controls.

Genotype combinations show no effect on breast cancer risk

Based on the functional indications for the $\text{IRS1}$ Gly972Arg polymorphism (15), a reported decreased risk of colon cancer in heterozygotes for the $\text{IRS2}$ Gly1057Asp polymorphism (22) and our finding of a protective effect of the SHC1 variant allele we hypothesized that the variant alleles of these genes would have a protective effect. Thus, the genotypes AA, AA and GG of the $\text{IRS1}$, $\text{IRS2}$ and $\text{SHC1}$ genes, respectively, would cause decreased signalling and accordingly decreased risk for breast cancer. We combined the genotypes according to the number of variant alleles and examined their effect on breast cancer risk. While the wild-type genotype combination (GGGGGAA) was very common in both cases and controls (30.8 versus 32.0%, respectively), the combinations containing at least 5 variant alleles (AAAGGG, GAAGGG and AAAAAAG) were not present at all (data not shown). No differences in the
our study we could not observe any differences between breast cancer cases and controls in allele or genotype frequencies. The Cys816Cys polymorphism also did not show any differences in the allele or genotype frequencies between breast cancer cases and controls. None of the haplotypes were associated with breast cancer risk.

SHC1 binds to the IGF-1 receptor upon stimulation and becomes phosphorylated so that it can bind to GRB2 and activate the Ras/MAPK pathway that leads to cell proliferation (11). A Met→Val variant at codon 300 (according to the p52 isoform) has been described (25,26). The only high affinity binding site for GRB2, a tyrosine phosphorylation site at codon 317, is close by, so we hypothesize that the amino acid exchange may alter the 3-dimensional structure of the protein, as in the case of the IRS1 Gly972Arg polymorphism (11,38). It may thus result in decreased binding to GRB2 and finally to impaired signalling through the Ras/MAPK pathway. However, no functional tests have been reported so far. We could observe a higher frequency of the G allele coding for valine in the Polish control group than in the breast cancer case group, which led to a significant (~50%) decreased risk for breast cancer (OR 0.53, 95% CI 0.32–0.90, P = 0.016). Because we used incident cases, it is unlikely that our results would have been biased due to differential survival of the variant allele carriers. We included 217 German cases and 243 controls in the study to confirm the results in an independent population. For this population we could detect the same protective trend, which, however, was not statistically significant. In each population the protective effect was stronger in women diagnosed for breast cancer below the age of 50. In the joint analysis the effect was significant in all women (OR 0.62, 95% CI 0.41–0.93, P = 0.02) and with a stronger effect in the younger women (OR 0.54, 95% CI 0.32–0.89, P = 0.01). This suggests that the protective effect is stronger in premenopausal women than postmenopausal. Because of the selection criteria in our study ~75% of women were under 50 years of age at diagnosis. Earlier, a higher risk for breast cancer with increasing circulating IGF-1 and IGFBP3 levels was observed only in premenopausal women (9). For a better functional understanding this variant needs to be tested in vitro and in vivo, particularly with regard to its interaction with GRB2.

Because of the potential effects of the polymorphisms and partly overlapping functions of these proteins (30,32,34), we performed a genotype combination analysis in each individual according to the number of observed variant alleles. No trend for a decreased or increased risk of breast cancer could be seen.

In summary, in this first study on polymorphisms in the IRS1, IRS2 and SHC1 genes in relation to breast cancer risk we could detect a significantly decreased risk for breast cancer in carriers of the variant Val allele at codon 300 in the SHC1 gene. The IRS1 and IRS2 polymorphisms did not show any influence on breast cancer risk nor did the haplotype or genotype combinations.

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