Genotyping analyses

Lymphocyte DNA (100 ng) extracted by standard methods was used as a template in PCR-based restriction fragment length polymorphism (RFLP) assays. For the *XRCC1 Arg280His* genotype determination, a 282 base pair (bp) fragment was amplified using the primers described earlier [18]. After digestion of the PCR product with 3 U of *Rsa*I, the *280Arg* wild-type allele was revealed by two 141 bp fragments, while an intact 282 bp fragment indicated the presence of the *280His* variant allele.

In the *XRCC1 Arg399Gln* genotype analysis, a 615 bp fragment amplified using the primers described in [20] was digested with 3 U of *Msp*I restriction enzyme; the *XRCC1-399 Arg* wild-type allele was revealed by the presence of 221 bp and 374 bp fragments while the *399Gln* variant allele remained intact (615 bp).

The *XPD Lys751Gln* genotypes were determined by amplifying a 324 bp fragment with the primers described in [50]. The resulting fragment was digested with 3 U of *Pst*I; the amplicon from the *751Lys* wild-type allele was cut into 220 bp and 104 bp fragments while the amplicon from the *751Gln* variant allele was cut into 157 bp, 104 bp and 63 bp fragments.

The genotype analyses were performed unaware of the case-control status. Two positive controls with known genotype and two negative controls were used within each PCR amplification batch, and two independent researchers interpreted the gel images to ensure the validity of genotyping for each polymorphism. The PCR for samples with divergent results was repeated and an additional 10% of all samples were reanalysed for each polymorphism for quality control. No discrepancies were found in the replicate tests. The *XRCC1 Arg280His* genotype could not be determined for three cases and three controls, the *Arg399Gln* genotype for four cases and four controls, and the *XPD Lys751Gln* genotype for two cases and two controls.

Statistical analyses

Association between genotypes and risk of breast cancer were evaluated by unconditional logistic regression to calculate multivariate adjusted odds ratios (ORs) and 95% confidence intervals (CIs) using SPSS version 11.5 (SPSS Inc., Chicago, Illinois, USA). Calendar age, age at menarche, age at first full term pregnancy, number of children, history of benign breast diseases, first degree (mother, sister, daughter) family history of breast cancer, waist-to-hip ratio and use of alcohol and smoking were used as adjusting variables in all analyses. Subjects with missing values in any of the adjusting variables were excluded from the analysis. When the adjusted ORs differed significantly from the unadjusted ORs, both are shown.

Women who had smoked daily for longer than three months were considered as smokers. Never smokers were catego-

Table 1

Selected characteristics of the study subjects

Characteristic	Case/control[a]	OR[b] (95% CI)
Age at menarche		
≤12	98/101	1.0
13–14	219/251	0.82 (0.59–1.16)
≥15	150/127	0.99 (0.68–1.46)
Age at first full-term pregnancy		
Nulliparous	102/57	1.0
≤25	237/263	0.55 (0.38–0.81)
26–30	94/122	0.44 (0.29–0.69)
≥31	47/40	0.64 (0.36–1.12)
Number of full-term pregnancies		
Nulliparous	102/57	1.0
1	68/64	0.59 (0.36–0.98)
2	141/181	0.50 (0.33–0.76)
3+	171/180	0.54 (0.36–0.80)
Waist-to-hip ratio		
≤0.91	187/236	1.0
>0.91	291/243	1.38 (1.06–1.81)
First-degree family history of breast cancer		
No	424/459	1.0
Yes	54/22	2.53 (1.48–4.31)
History of benign breast disease		
No	296/313	1.0
Yes	180/167	1.33 (1.01–1.75)
Current alcohol intake		
Never	271/206	1.0
Once a month or less	134/187	0.74 (0.54–1.01)
Daily-weekly	75/89	0.87 (0.59–1.29)
Smoking habits		
Never active or passive	210/182	1.0
Passive only	153/169	0.78 (0.58–1.06)
Ever active	112/130	0.91 (0.65–1.28)
<5 pack-years	45/63	0.75 (0.48–1.16)
≥5 pack-years	67/67	1.08 (0.72–1.62)

[a] Total number of cases and controls does not correspond because of missing values.
[b] Adjusted for age. CI, confidence interval; OR, odds ratio.