ORIGINAL ARTICLE

A common variant of CDKN2A (p16) predisposes to breast cancer

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Background: A common missense variant of the *CDKN2A* gene (A148T) predisposes to malignant melanoma in Poland. An association between malignant melanoma and breast cancer has been reported in several families with *CDKN2A* mutations,
Objective: To determine whether this variant also predisposes to breast cancer.
Methods: Genotyping was undertaken in 4209 cases of breast cancer, unselected for family history, from 18 hospitals throughout Poland and in 3000 controls.
Results: The odds ratio (OR) associated with the *CDKN2A* allele for women diagnosed with breast cancer before the age of 50 was 1.5 (p = 0.002) and after age 50 it was 1.3 (p = 0.2). The effect was particularly strong for patients diagnosed at or before the age of 30 (OR = 3.8; p = 0.0002).
Conclusions: *CDKN2A* appears to be a low penetrance breast cancer susceptibility gene in Poland. The association should be confirmed in other populations.

There is continuing interest in identifying low penetrance genes that are associated with increased susceptibility to common types of cancer. There are several approaches to this problem, including the use of chip based single nucleotide polymorphism (SNP) arrays to interrogate a large number of genes simultaneously, and preselecting candidate genes of interest. Candidate genes for cancers at a particular site may be selected because they are known to predispose to malignancies in other organs, or because they are mutated somatically in the cells from the cancer of the interest. It is possible that missense variants of genes for which truncating mutations are clearly pathogenic may also be deleterious, but with reduced penetrance. In this situation the association may be overlooked unless large numbers of cancers are studied. The *CDKN2A* (OMIM 600160) gene is a tumour suppressor gene that is involved in susceptibility to malignant melanoma[1] and has also been implicated in familial pancreatic cancer.[2] The p16 protein is a cyclin dependent kinase inhibitor that suppresses cell proliferation[3] and is expressed in a wide range of tissues, including the breast, and in breast cancers.[4] Somatic mutations of *CDKN2A* are present in tumours of various sites,[5] including head and neck tumours,[6] squamous cell carcinoma of the larynx,[7] colon cancer,[8] clear cell sarcoma,[9] and respiratory tract tumours.[10]

In Poland, a single missense variant of *CDKN2A* (an alanine to threonine substitution at codon 148: A148T) is present in approximately 3% of the general population.[11 12] We have recently shown that this variant predisposes to malignant melanoma (odds ratio (OR) = 2.5; p = 0.0003).[13] It has been suggested by Borg and colleagues that protein truncating *CDKN2A* mutations predispose women to breast cancer in the context of a syndrome of melanoma, pancreatic cancer, and breast cancer.[14] They observed eight cases of breast cancer in the Swedish families, compared with 2.1 expected (p = 0.002). There are few other data on this topic. Ghiorzo *et al* observed a non-significant excess of breast cancers in seven melanoma families with CDKN2A mutations (OR = 1.9; 95% confidence interval (CI), 0.4 to 5.6).[15]

Somatic *CDKN2A* mutations have not been well studied in breast cancers, but silencing of *CDKN2A* through methylation appears to be a relatively common way of inactivating this tumour suppressor gene in the breast.[16] Furthermore, deletion or loss of heterozygosity at the *CDKN2A* locus (9p21) is relatively common in breast cancers.[17] To establish whether or not this common missense variant of *CDKN2A* predisposes to breast cancer we undertook an association study on 4209 cases of breast cancer and 3000 ethnically matched controls from Poland.

METHODS

Study subjects

This study population includes prospectively ascertained cases of invasive breast cancer diagnosed at 18 treatment centres throughout Poland from 1997 to 2003. The study was approved by the ethics committee of the Pomeranian University. We are conducting a national study of unselected breast cancer patients diagnosed at or before the age of 50. Patients diagnosed before 2003 at the affiliated hospitals and who were aged 50 or less were eligible. Patients with pure intraductal or intralobular cancer (DCIS or LCIS) were excluded but those with DCIS with microinvasion were included. The patient was invited to participate in person during her hospital stay or through a mailed invitation. During the interview the goals of the study were explained, informed consent was obtained, genetic counselling was given, and a blood sample was taken for DNA analysis. A detailed family history of cancer was taken (first and second degree relatives included) and a risk factor questionnaire was completed. In all, 4778 incident cases of early onset invasive breast cancer were identified at the 18 different centres during the study period. Of these, 3627 women (76%) accepted the invitation to participate in the genetic study. The medical record and pathology report were reviewed. In a companion study, seven of these centres also provided data on unselected breast cancer cases diagnosed above the age of 50 between 2000 and 2002. In the present study we provide data on patients diagnosed before the age of 50 and on a smaller number of patients diagnosed at 51 and above. The results of the two study groups are presented separately.

We excluded 309 patients from the analyses: 114 patients died before providing a blood sample and 16 refused to participate at a later date. We were unable to carry out molecular tests in 164 cases because of poor DNA quality. In 15 cases we had no data on age of onset. This brought the total number of breast cancer cases studied to 4209, including 3318 cases diagnosed at 50 or below and 891 diagnosed at age 51 and above.

Controls

Two control groups were combined. The first group consisted of 2000 newborn male and female children from 10 hospitals situated throughout Poland in 2003 and 2004. Samples of cord blood from unselected infants were forwarded to the study centre in Szczecin. The second control group consisted of 1000 adults from the region of Szczecin unselected for cancer family history, sex, or age. These were patients randomly selected from the patient roles of participating family physicians. They were unaffected by cancer and were not selected for family history. To ensure comparability of the control groups, the frequency of the A148T allele was computed separately for the adult and neonatal control groups, and by geographical origin, and the groups compared. Because the two controls groups were comparable (see below) they were combined.

Laboratory methods

DNA samples were obtained from peripheral blood or from umbilical chord blood in newborns. The A148T variant was analysed by restriction fragment length polymorphism polymerase chain reaction (PCR), using np16ex2f (AGGGGTAATTAGACACCTGG) and np16ex2r (TTTGGAAGCTCTCAGGGTAC) primers. PCR products were digested with the SacII enzyme and separated in 2–3% agarose gels. The presence of the A148T change was confirmed by direct DNA sequencing.

Statistical analysis

Statistical analysis included a comparison of the prevalence of the A148T allele in cases and controls. Odds ratios were generated from 2×2 tables and statistical significance was assessed using a χ^2 test.

RESULTS

The A148T variant was detected in 5.1% of breast cancer patients diagnosed at age 50 or below, in 4.5% of cases diagnosed at age 51 and above, and in 3.5% of Polish controls (table 1). For women diagnosed below age 50 the odds ratio was 1.5 (p = 0.002). For women diagnosed over age 50 the odds ratio was modest and non-significant (OR = 1.3; p = 0.2). In the small group of patients diagnosed with breast cancer at age 30 and under, the prevalence of the *CDKN2A* variant was 12.1% and the association was much stronger (OR = 3.8; p = 0.0002).

No A418T homozygotes were seen in the either cases and controls. There were 0.9 homozygotes expected among controls (p = 0.3) and 2.4 expected among breast cancer cases (p = 0.1).

Among the 3318 women diagnosed with breast cancer under age 50, 692 had a family history of a first or second degree relative with breast cancer. For the familial cases the odds ratio observed with the A148T allele was 1.4 (95% CI, 0.9 to 2.1) and among the non-familial cases the odds ratio was 1.5 (1.1 to 1.9). For 732 cases data on family history were not available.

Our control group was drawn both from the newborns of 10 Polish cities and from the adult population from the region of Szczecin. However, the frequency of the alleles was similar in the newborn population (3.5%) and the adult population (3.6%). The allele was equally frequent among males (3.5%) and females (3.6%) and among controls recruited from Szczecin (3.5%) and from elsewhere in Poland (3.6%).

DISCUSSION

We have shown that the A148T allele of the *CDKN2A* gene is overrepresented in a population of unselected patients with breast cancer in Poland, compared with controls. It will be of interest to see if this mutation or other founder *CDKN2A* alleles are found to be associated with breast cancer susceptibility in other ethnic groups. Our study was notable because of the large sizes of the case and control groups. Our cases and controls were both drawn from the Polish population and the great majority of the residents are ethnic Poles. This level of genetic homogeneity has enabled us to find founder alleles of several other breast cancer genes, including BRCA1 and CHEK2.[18 19] Our cases and control groups differed in terms of age, sex, and geographical distribution, but none of these factors was associated with the frequency of the p16 A148T allele.

Large well controlled studies will be required to estimate with precision the risk of breast cancer associated with CDKN2A founder alleles in different populations. Given the magnitude of the relative risk observed here (1.5) and the low prevalence of the variant allele in the population (3.5%), studies of a few hundred cases would have insufficient power to detect similar risks. For example, the A148T variant was overrepresented in melanoma kindreds from Australia (3%) in comparison with the general population (1.8%), but the study was relatively small (200 controls) and the finding was not significant (p = 0.73).[20] In England, Bertram and colleagues found the A148T in 4.9% of adults from 179 melanoma-prone families and in 5.2% of controls.[21] Ghiorzo *et al* found the A148T variant in five of 14 melanoma families in Italy.[15] Because the variant did not segregate completely with the melanoma phenotype in these families, the investigators concluded that the allele was a polymorphic variant. However, the allele frequency was not measured in controls and the data are consistent with that of a low penetrance melanoma gene.

Table 1 Frequencies of CDKN2A variant alleles in breast cancer patients and controls

	Number tested	Mutation positive	Prevalence	Odds ratio	p Value
Controls	3000	105	3.5%		
Breast cancer cases					
Under 50 y					
20–30 y	66	8	12.1%	3.8	0.0002
31–40 y	582	24	4.1%	1.2	0.46
41–50 y	2670	136	5.1%	1.5	0.003
Total	3318	168	5.1%	1.5	0.002
Over age 50 y					
51+ y	891	40	4.5%	1.3	0.17

Given that the relative risk observed for carriers of this allele is 1.5 for breast cancer, we do not expect this to generate familial cancer clusters. In our study we did not observe a greater risk with familial versus non-familial cancers.

We provide epidemiological evidence to support the deleterious nature of the A148 allele. Previous functional studies suggested that this variant is a polymorphism and appears not to have a major effect on p16 function.[22 23] It is possible that the A148T allele subtly affects p16 function or reduces its level of expression, or that the A148T variant is in linkage disequilibrium with another genetic alteration that does affect protein function. We have previously shown that the A148T variant is in strong linkage disequilibrium with a second alteration in the *CDKN2A* promoter (the P493 variant).[13] We have now genotyped 100 individuals (50 with and 50 without the A148T variant) for the P493 variant and found complete concordance between the presence of the two variants.

Three previous studies have reported that breast cancers appeared more commonly than expected in melanoma kindreds with truncating *CDKN2A* mutations[14 15 24] but there have been no previous *CDKN2A* mutation surveys among unselected cases of breast cancer, and no reports of breast cancer associated with a missense mutation.

In summary, these data provide compelling evidence that *CDKN2A* should be considered to be a breast cancer susceptibility gene. We found that the association between the *CDKN2A* variant and breast cancer risk was particularly strong for cases diagnosed before age 30. It is of interest that in the study of Borg *et al* one of the breast cancer cases identified in a family with the *CDKNA* 113insArg mutation in Sweden was only 23 years old. This situation is reminiscent to that of p53 and Li-Fraumeni syndrome, in which very early onset cases of breast cancer are characteristic.[25]

Identification of breast cancer susceptibility genes that are associated with modest penetrance requires very large association studies. Not all populations harbour carriers at the same frequency, and different mutations may be associated with different cancer risks. Large well controlled studies are needed to establish the full range of risks associated with *CDKN2A* founder alleles in different populations and to estimate the corresponding risks associated with various mutations for various cancer sites.

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