Recent reports suggest that two ATM gene mutations, 7271T>G and IVS10-6T>G, are associated with a high risk of breast cancer among multiple-case families. To assess the importance of these two mutations in another ‘high-risk’ group, young women (under age 51) with multiple primaries, we screened a large population-based series of young women with bilateral breast cancer and compared the frequency of these mutations among similar women diagnosed with unilateral breast cancer. The 1149 women included were enrolled in an ongoing population-based case-control study of the genetic factors that contribute to bilateral breast cancer; they were not selected on the basis of family history of cancer. Screening for 7271T>G and IVS10-6T>G ATM gene mutations was conducted using DHPLC followed by direct sequencing. The 7271T>G mutation was detected in one out of 638 (0.2%) women with unilateral breast cancer and in none of the bilateral cases, and the IVS10-6T>G mutation in one out of 511 (0.2%) bilateral and in eight out of 638 (1.3%) unilateral breast cancer cases. Carriers of either mutation were not limited to women with a family history. Given the likelihood that young women with bilateral breast cancer have a genetic predisposition, the observed mutation distribution is contrary to that expected if these two mutations were to play an important role in breast carcinogenesis among individuals at high risk.

Keywords: ATM gene screening; 7271T>G mutation; IVS10-6T>G mutation; breast cancer; bilateral breast cancer
cancer (Thompson, 1986; Begg and Berwick, 1997). So, examining the frequency of a genetic mutation among women with bilateral breast cancer and comparing it with the frequency among similar women with unilateral breast cancer may provide important clues as to its role in breast carcinogenesis.

Given the extremely high risk of breast cancer associated with two ATM gene mutations, 7271T>G and IVS10-6T>G, observed by Chenevix-Trench et al. (2002) among multiple-case families, the question arises concerning the role they play in other genetically susceptible high-risk groups, including women with multiple primary cancers. In this study, we investigated the prevalence of these two ATM gene mutations in a large population-based series of women with bilateral breast cancer compared with the frequency among women with unilateral breast cancer.

MATERIALS AND METHODS

The 1149 women included in this analysis were available from the ongoing multicentre, population-based case–control study of second primary breast cancer and gene–environment interactions, the Women’s Environmental, Cancer, and Radiation Epidemiology (WECARE) Study. The study population currently includes 511 women with asynchronous bilateral breast cancer, who serve as cases, and 638 women with unilateral breast cancer, who serve as controls. All participants are identified through five population-based tumour registries (Los Angeles County Cancer Surveillance Program, Cancer Surveillance System of the Fred Hutchinson Cancer Research Center, State Health Registry of Iowa, Cancer Surveillance Program of Orange County/San Diego-Imperial Organization for Cancer Control, and the Danish Cancer Registry). Selection is independent of a woman’s family history of cancer. Women eligible for inclusion were under age 55 at diagnosis of the first primary breast cancer (invasive, with localised or regional, but not metastatic disease) and were first diagnosed between 1/1/1985 and 12/31/2000. All epidemiologic information is ascertainment using a structured telephone-administered questionnaire, and blood samples are drawn by a study phlebotomist. The questionnaire contains items addressing known and suspected risk factors for breast cancer, including the family history of breast cancer and personal demographics. Each participant provided informed consent in accordance with the Institutional Review Board at each study site that approved the study.

The bilateral breast cancer cases were diagnosed with contralateral breast cancer (in situ or invasive, any stage) at least 1 year after their first primary diagnosis. The second primary can be an in situ carcinoma as women with a history of breast cancer are more likely to be closely monitored for a second primary cancer. All women were alive at the time of contact and had no history of other cancers (except nonmelanoma skin cancer). For this analysis, we selected all WECARE Study participants who had completed their interview and donated a blood sample as of 11/10/2002.

The ATM gene-mutation analyses were conducted using a staged approach of initial screening by denaturing high-performance liquid chromatography (DHPLC) followed by nucleotide sequencing of exons yielding variant DHPLC profiles. DHPLC conditions were optimised using positive controls with known mutation status. Two independent readers scored all DHPLC and sequencing output traces. For quality-control purposes, we randomly selected 11% of the 1149 samples (69 bilaterals and 55 unilateral) to be rescreened, and obtained 100% agreement between the duplicate samples. All laboratory screening was performed blinded to sample characteristics (Bernstein et al., 2003). In all, 10 samples with IVS10-6T>G were haplotyped with microsatellite markers S1819 (Rotman et al., 1994), NS22 (Udat et al., 1999), S2179 (Vanagaitė et al., 1995) and S1818 (Rotman et al., 1994), using standardised alleles (Mitui et al., 2003).

RESULTS

Table 1 summarises the characteristics of the 1149 breast cancer cases. The percent of women with a relative with breast cancer was greater among women with two primaries compared to women with only one (any first-degree relative: $\chi^2 = 19.74$ ($P < 0.0001$); any second-degree relative: $\chi^2 = 4.66$ ($P < 0.03$)). This observation is consistent with previous studies suggesting that women with bilateral breast cancer have a genetic predisposition towards breast cancer, and supports our hypothesis that this population may be enriched for breast cancer susceptibility genes. Among the 1149 women in this study, the 7271T>G mutation was found in one out of 638 (0.16%) woman with unilateral breast cancer (Table 2). The IVS10-6T>G mutation was found in one out of 511 (0.19%) bilateral and in eight out of 638 (1.25%) unilateral breast cancer. Compared to women with unilateral breast cancer, the chance of being a carrier of either mutation among the bilateral breast cancer cases was $0.137$ (95% confidence interval: 0.003–0.996). Full screening and quality control of the other 64 coding exons of the ATM gene was complete for seven of the 10 women. No other mutations were detected in these samples, although two silent polymorphisms were observed. All women with a mutation were aged 50 or younger at diagnosis of their first primary, all had invasive primary breast cancer, and all were Caucasian. Their family histories were diverse and moderate; one woman reported two affected first-degree relatives with breast cancer, three women had one affected first-degree relative, and four subjects reported at least one affected second-degree relative. The samples with IVS10-6T>G were haplotyped with microsatellite markers. Although phase was not determined, the data were compatible with the interpretation that each sample shares a haplotype with the homozygous IVS10-6T>G A-T patient described by Broeks et al. (2003).

Table 1 Characteristics of 1149 young women with unilateral and bilateral breast cancer included in this study

| Characteristic | Bilateral (N = 511) | Unilateral (N = 638) |
|---------------|-------------------|---------------------|
| Age at diagnosis (mean and range) | | |
| First primary | 46 (25, 54) | 46 (25, 54) |
| Second primary | 50 (28, 68) | NA | NA |
| Follow-up (years) after diagnosis (mean and range) | 10.8 (2.4, 17.1) | 10.5 (3.6, 16.9) |
| Race (N and %) | | |
| White | 486 | 95.1% | 603 | 94.5% |
| Black | 10 | 2.0 | 13 | 2.0 |
| Asian or Pacific Islander | 2 | 0.4 | 8 | 1.3 |
| Native American, Aleut, or Eskimo | 3 | 0.6 | 3 | 0.5 |
| Other | 10 | 2.0 | 11 | 1.7 |
| Family history of breast cancer (N and %) | | |
| Any first-degree relative | 161 | 31.5% | 128 | 20.1% |
| Mother | 108 | 21.1 | 86 | 13.5 |
| ≥ One sister | 76 | 14.9 | 53 | 8.3 |
| ≥ One daughter | 1 | 0.2 | 1 | 0.2 |
| Any second-degree relative | 148 | 29.0% | 149 | 23.4% |
| Maternal grandmother | 37 | 5.3 | 50 | 7.9 |
| Paternal grandmother | 25 | 4.9 | 21 | 3.3 |
| ≥ One maternal aunt | 63 | 12.3 | 70 | 11.0 |
| ≥ One paternal aunt | 54 | 10.6 | 56 | 8.8 |

*Time between first primary diagnosis and interview date.
Table 2  Detailed characteristics of the 10 women with breast cancer, who were found to be heterozygotes for one of the two ATM gene mutations 7271T>G or IVS10-6T>G.

| ATM mutation | Laterality | Age at dx | Follow-up after dx | First-degree relative | Second-degree relative |
|--------------|------------|-----------|-------------------|-----------------------|------------------------|
|              |            |           |                   | Any | Mother | Sister | Daughter | Any | Maternal grandmother | Paternal grandmother | Maternal aunt | Paternal aunt |
| 7271T>G      | Unilateral | 41-45     | ≤10                | 0   | 0      | 0      | 0        | 0   | 0                   | 0               | 0           | 0           |
| IVS10-6T>G   | Bilateral  | ≤40       | >10                | 1   | 1      | 0      | 0        | 1   | 0                   | 0               | 0           | 0           |
|              | Unilateral | ≤40       | >10                | 0   | 0      | 0      | 0        | 1   | 0                   | 0               | 0           | 0           |
|              | Unilateral | 41-45     | ≤10                | 0   | 0      | 0      | 0        | 0   | 0                   | 0               | 0           | 0           |
|              | Unilateral | 41-45     | >10                | 2   | 1      | 1      | 0        | 0   | 0                   | 0               | 0           | 0           |
|              | Unilateral | 46-50     | ≤10                | 1   | 1      | 0      | 0        | 0   | 0                   | 0               | 0           | 0           |
|              | Unilateral | 46-50     | >10                | 0   | 0      | 0      | 0        | 2   | 0                   | 1               | 0           | 1           |
|              | Unilateral | 46-50     | ≤10                | 0   | 0      | 0      | 0        | 1   | 0                   | 0               | 1           | 0           |
|              | Unilateral | 46-50     | >10                | 1   | 0      | 1      | 0        | 2   | 0                   | 1               | 0           | 1           |
|              | Unilateral | 46-50     | >10                | 0   | 0      | 0      | 0        | 0   | 0                   | 0               | 0           | 0           |

*Age at diagnosis. For a subject with bilateral breast cancer; this is the age at first diagnosis; the second diagnosis was between 46 and 50 years of age. bTime between first primary diagnosis and interview date, range 5.1 – 16.7 years.

DISCUSSION

In the present study, we focused on the frequency of the 7271T>G and IVS10-6T>G ATM gene mutations, because prior studies of multiple-case families found these two mutations to be associated with excess breast cancer. The 1149 women in our study were population-based, and all diagnosed with a first breast cancer at a young age (under age 51). We observed a higher prevalence of IVS10-6T>G among unilateral breast cancer cases than bilateral breast cancers. This frequency distribution among bilateral and unilateral breast cancer cases is consistent with the study by Broeks et al (2003), which also focused on early-onset breast cancer; two out of 49 unilateral breast cancer cases and one out of 33 bilateral breast cancer cases were found to carry the IVS10-6T>G mutation. However, in our study, the prevalence of this mutation was more than 20-fold lower than that observed in the Broeks study. In contrast, the family study conducted by Chenevix-Trench et al (2002) found no IVS10-6T>G mutations in the 262 breast cancer patients who were unselected for family history; two were observed in the 76 multiple-case breast cancer families. Similar to our study’s 7271T>G mutation prevalence of 0.08% (one out of 1149), Chenevix-Trench et al (2002) observed one mutation among 525 cases and 381 controls (0.11%); all women included in the study were under age 40. The prevalence of this mutation was greater among the high-risk families where one out of 76 (1.3%) carried the mutation. The 7271T>G mutation, originally detected in a Scottish family (Stankovic et al, 1998), to our knowledge, has only been observed in countries with a heavy British population origin. Thus, the low prevalence of this mutation in our study may reflect population heterogeneity.

Among the bilateral breast cancer cases, 23% were diagnosed with an in situ second primary breast cancer; however, the single carrier of the IVS10-6T>G with bilateral breast cancer was diagnosed with an invasive secondary primary, 9.3 years after her first breast cancer was diagnosed. Further of note, from the haplotyping data, it appears that the IVS10-6T>G variant is carried on an ancestral haplotype that is common to patients with both A-T and breast cancer. This concurs with the findings of Broeks et al (2000), who also reported that this haplotype is observed in normal individuals carrying IVS10-6T>G and may confer incomplete penetrance. Ancient founder ATM mutations have been associated with other ancestral haplotypes as well (Campbell et al, 2003).

Given the likelihood that young women with bilateral breast cancer may be carriers of inherited susceptibility alleles at loci such as ATM, and that they are more genetically predisposed than women with unilateral breast cancer, in our series, we would have expected a greater frequency of germline mutations in the bilateral group. However, the observed mutation distribution, a higher prevalence in unilateral breast cancers than in bilateral breast cancers, is contrary to this and to what we would have expected if these two mutations were to play an important role in breast carcinogenesis among such individuals at high risk. Nevertheless, in the absence of information on the presence of any other ATM mutations associated with breast cancer in these patients, these results need to be interpreted cautiously.

CONCLUSION

We examined the prevalence of two ATM gene mutations associated with breast cancer in women with unilateral and bilateral breast cancer; one out of 638 unilateral cases carried the 7271T>G mutation, while one out of 511 bilateral and eight out of 638 unilateral breast cancer cases harboured the IVS10-6T>G mutation. Neither mutation was associated with family history of breast cancer, age, or laterality.

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Appendix

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