Genetic variation in five genes important in telomere biology and risk for breast cancer

SA Savage*1,2, SJ Chanock2,3, J Lissowska4, LA Brinton5, D Richesson5, B Peplonska6, A Bardin-Mikolajczak4, W Zatonski4, N Szeszenia-Dąbrowska5 and M Garcia-Closas5

1 Division of Cancer Epidemiology and Genetics, Clinical Genetics Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA; 2 Pediatric Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA; 3 Division of Cancer Epidemiology and Genetics, Core Genotyping Facility, National Cancer Institute, National Institutes of Health, Gaithersburg, MD 20877, USA; 4 Department of Epidemiology and Prevention, Cancer Center and M Sklodowska-Curie Institute of Oncology, Warsaw, Poland; 5 Division of Cancer Epidemiology and Genetics, Hormonal and Reproductive Epidemiology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA; 6 Department of Epidemiology, Nofer Institute of Occupational Medicine, Łódź, Poland

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Telomeres, located at the ends of chromosomes, consist of long TTAGGG nucleotide repeats and an associated protein complex. Chromosome ends are protected from end-to-end fusion and degradation by this telomere complex, termed shelterin (de Lange, 2005). The TTAGGG repeats shorten with each cell division, and the shelterin complex, composed of telomeric repeat-binding factor 1 (TRF1, TERF1, TERF2 and TERT) is involved. Genetic variation in these genes could affect cancer risk. In this study, we investigated genetic variation in five genes that captured most of the common genetic variation in five genes important in telomere biology. The studied genes included telomerase (TERT (protein name), TERF1, TERF2) and telomerase-associated protein (TP1, TEP1, 14q11.2) (Poderycki et al., 2005), telomeric repeat-binding factor 1 (TRF1, Ex2-659G>A) may be associated with reduced risk of breast cancer among individuals with a family history of breast cancer (odds ratios 0.73, 0.66, and 0.57, 95% confidence intervals 0.53–1.00, 0.46–0.95 and 0.39–0.84, respectively). In conclusion, our data do not support substantial overall associations between SNPs in TERT pathway and breast cancer risk. Intriguing associations with variants in TERT among women with a family history of breast cancer warrant follow-up in independent studies.

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Materials and Methods

Study population

The design of this population-based breast cancer case–control study has been described (Garcia-Closas et al, 2006a). Eligible

Telomere shortening is associated with cancer risk.
cases included women aged 20–74 years who were Polish residents of either Warsaw or Łódź with pathologically or cytologically confirmed in situ or invasive breast cancer, newly diagnosed in 2000–2003. An estimated 90% of eligible cases were identified through a rapid identification system at five participating hospitals. Information from Cancer Registries was used to identify the remaining 10% of eligible breast cancer cases. Eligible control subjects were residents of Warsaw and Łódź who did not have a history of breast cancer at enrollment. Controls were randomly selected from population lists, and frequency-matched to breast cancer cases by city and 5-year age groups. Women provided a personal interview on known and suspected risk factors. Venous blood samples were collected by a trained nurse. The study protocol was reviewed and approved by local and National Cancer Institute (NCI) Institutional Review Boards. All participants provided written informed consent. Of the 3037 eligible cases and 3639 eligible controls identified, 2386 (79%) cases and 2502 (69%) controls agreed to participate in the personal interview. The present study is limited to women with blood DNA samples: 1995 cases (6% in situ) and 2296 controls, which represented 84 and 94%, respectively, of the study population.

**Laboratory methods**

Genomic DNA for genotype analyses was isolated from buffy coat or whole blood samples using the Autopure LS® DNA Purification System (Gentra Systems Inc., Minneapolis, MN, USA). Twenty-four SNPs in POT1, TEP1, TERFI, TERF2, and TERT were genotyped by investigators blinded to case–control status, using TaqMan or MGB Eclipse platforms at the Core Genotyping Facility of the Division of Cancer Epidemiology and Genetics, NCI (Table 1). Assay conditions are available at http://snp500cancer.nci.nih.gov. If an rs number has not yet been assigned, an E number was used also to determine the global score -value, haplotype frequencies, ORs and 95% CIs.

**Single nucleotide polymorphism selection**

Initial SNP selection criteria included MAF greater than 5% in Caucasians from SNP500 Cancer (n = 31), even spacing across the gene, SNPs with potential functional implications and/or patterns of nucleotide diversity and linkage disequilibrium (LD) previously determined through extensive re-sequence analysis (Savage et al., 2005; Packer et al., 2006) and assay availability at the time of SNP selection. The SNPs selected using these criteria were evaluated as haplotype-tagging SNPs compared with all common SNPs identified in the prior re-sequence analysis using tagSNPs (Stram, 2004) and TagZilla (http://tagzilla.ncbi.nlm.nih.gov). was the pairwise correlation coefficient between SNPs determined by these programs. SNPs with 0.8 were considered highly correlated.

TEP1 (54 exons, 40.7 kilobase pairs (kbp)) has minimal LD and eight common SNPs in the 31 SNP500 Caucasians. The five TEP1 SNPs genotyped (Table 1) gave an of 0.84, indicating representative coverage of common genetic variation across TEP1. TERFI (10 exons, 15.3 kbp) has very limited nucleotide diversity with only four common SNPs in SNP500 Caucasians between introns 7 and 9 (Savage et al., 2005). Three of these SNPs were genotyped and very good correlation for the fourth SNP was noted, . The 10 SNPs genotyped in our study spanned 43 kbp from –1644A>G to Ex16+203C>T and were representative of common genetic variation, . We were unable to genotype TERT Ex14 + 7C>T (E3661_301, H1001H) due to lack of assay availability, which would have increased the to 0.83; however, we did genotype Ex16 +203C>T (rs2853690), which was only 1776bp 3’ of TERT Ex14 + 7C>T. The four SNPs genotyped in POT1 (17 exons, 74.7 kbp) spanned 73.1 kbp (–1386G>A through IVS13–98T>G), a region with strong LD and 11 common SNPs in SNP500 Caucasians (Savage et al., 2005). These SNPs (Table 1) were good representatives of common genetic variation across POT1, .

**Statistical analyses**

Odds ratios (OR) and 95% confidence intervals (CI) from logistic regression models with dummy variables for matching factors (age in 5-year categories and study site (Warsaw or Łódź)) were used to estimate relative risks for the genotypes examined. The association between genotypes and breast cancer risk was tested using a 2 degrees of freedom (df) likelihood ratio test and a trend test. Heterogeneity of genotype ORs among groups of women defined by age categories and family history of breast cancer in first-degree relatives was evaluated by introducing interaction terms in logistic regression models. A positive family history was defined for women reporting one or more first-degree relatives diagnosed with breast cancer in the study questionnaire. An additive genetic model was assumed in interaction analyses. Age was considered as a continuous variable in tests for genotype–age interactions. Haplotypes were constructed for cases and controls using PHASE v2.1 (Stephens et al., 2001; Stephens and Donnelly, 2003) and HaploStats (Lake et al., 2003). The global case–control permutation test was performed using PHASE v2.1 (Stephens et al., 2001; Stephens and Donnelly, 2003). HaploStats (Lake et al., 2003) was also used to determine the global score P-value, haplotype frequencies, ORs and 95% CIs.

**RESULTS**

Most cases (74%) and controls (69%) in the study were postmenopausal, and cases were diagnosed at an average age (standard deviation) of 56 (±10) years. The established risk factors were associated with breast cancer risk in comparable direction with similar estimates of magnitude reported by others (Garcia-Closas et al., 2006b). Case–control analyses showed no statistically significant associations between the 24 SNPs in TEP1, TERFI, TERT and POT1 and risk of breast cancer (Table 1). Specific haplotypes derived from the evaluated SNPs were also not associated with increased risk of breast cancer in this study (data not shown). There were no statistically significant associations among age, SNP and breast cancer risk (Supplementary Table 1).

Case–control analyses suggested inverse associations between homozygous variants of TERT and breast cancer risk at two SNP sites, TERT-1654A>G (OR 0.85, 95% CI 0.72–1.02) and TERT Ex2-659G>A (A305A) (OR 0.76, 95% CI 0.58–1.00) (Table 1). The inverse association of TERT Ex2-659G>A (A305A) and two other linked TERT SNPs appeared to be limited to individuals with a
Table 1  Association between 24 single nucleotide polymorphisms in five genes important in telomere biology and breast cancer risk among cases and controls

| Gene   | SNP*       | Genotype | Controls |          | Cases |          | OR   | 95% CI | P-value | P trend |
|--------|------------|----------|----------|----------|-------|----------|------|--------|---------|---------|
| TEP1   | Ex1-222 T>C | TT       | 1089     | 48       | 959   | 49       | 1.00 |        |         |         |
|        |            | TC       | 972      | 43       | 831   | 42       | 0.97 | 0.86   | 1.11    | 0.68    |
|        |            | CC       | 203      | 9        | 183   | 9        | 1.02 | 0.82   | 1.27    | 0.84    |
|        | Rs1760897  | AA       | 89       | 4        | 75    | 4        | 0.96 | 0.70   | 1.32    | 0.80    |
|        | Ex4+51 C>A  | TT       | 795      | 35       | 712   | 36       | 1.00 |        |         |         |
|        | rs872072    | TC       | 1078     | 47       | 928   | 47       | 0.97 | 0.84   | 1.10    | 0.61    |
|        |            | CC       | 413      | 18       | 337   | 17       | 0.91 | 0.77   | 1.09    | 0.32    |
|        | Ex24+49 T>C | TT       | 625      | 28       | 503   | 26       | 1.00 |        |         |         |
|        | Si195P     | TC       | 1096     | 48       | 967   | 49       | 1.10 | 0.95   | 1.27    | 0.22    |
|        | rs1760904  | CC       | 540      | 24       | 495   | 25       | 1.14 | 0.97   | 1.35    | 0.12    |
|        | Ex45+36 G>A | GG       | 1433     | 63       | 1279  | 64       | 1.00 |        |         |         |
|        | V2214I     | GA       | 760      | 33       | 616   | 31       | 0.91 | 0.79   | 1.03    | 0.14    |
|        | rs1713449  | AA       | 88       | 4        | 92    | 5        | 1.18 | 0.87   | 1.60    | 0.28    |
|        | TERF1      | IVS7+82C | CC       | 1360     | 60     | 1146    | 58   | 1.00   |         |         |
|        |            | CT       | 812      | 36       | 731   | 37       | 1.07 | 0.94   | 1.21    | 0.31    |
|        |            | TT       | 106      | 5        | 106   | 5        | 1.19 | 0.89   | 1.57    | 0.24    |
|        | IVS8-124G  | CC       | 983      | 44       | 836   | 43       | 1.00 |        |         |         |
|        |            | AA       | 254      | 11       | 225   | 12       | 1.05 | 0.86   | 1.28    | 0.65    |
|        | IVS9-163T  | CC       | 1152     | 49       | 1060  | 48       | 0.93 | 0.82   | 1.06    | 0.30    |
|        | TERT       | IVS6+27G | CC       | 1603     | 70     | 1389    | 70   | 1.00   |         |         |
|        |            | CT       | 612      | 27       | 535   | 27       | 1.01 | 0.88   | 1.16    | 0.88    |
|        |            | AA       | 63       | 3        | 50    | 3        | 0.92 | 0.63   | 1.34    | 0.66    |
|        | IVS7-42T   | CC       | 1081     | 47       | 894   | 45       | 1.00 |        |         |         |
|        |            | AA       | 112      | 5        | 112   | 5        | 1.19 | 0.89   | 1.57    | 0.24    |
|        | IVS10+269C | CC       | 242      | 11       | 218   | 11       | 1.09 | 0.89   | 1.34    | 0.39    |
|        | TERT       | –1654A>G | AA       | 702      | 31     | 644     | 33   | 1.00   |         |         |
|        |            | AG       | 1132     | 50       | 963   | 49       | 0.90 | 0.78   | 1.03    | 0.13    |
|        |            | GG       | 443      | 19       | 357   | 18       | 0.85 | 0.72   | 1.02    | 0.08    |
|        | –1381C>T   | CC       | 695      | 29       | 634   | 29       | 1.00 |        |         |         |
|        |            | CT       | 1167     | 49       | 1121  | 51       | 1.05 | 0.92   | 1.20    | 0.46    |
|        | –967T>C    | TT       | 498      | 21       | 447   | 20       | 0.98 | 0.83   | 1.15    | 0.78    |
|        | rs7712562  | TC       | 1671     | 73       | 1409  | 72       | 1.00 |        |         |         |
|        |            | CC       | 128      | 6        | 122   | 6        | 0.87 | 0.68   | 1.11    | 0.27    |
|        | –244C>T    | CC       | 1224     | 54       | 1095  | 55       | 1.00 |        |         |         |
|        | rs2853669  | CT       | 900      | 39       | 766   | 39       | 0.95 | 0.84   | 1.08    | 0.42    |
|        |            | TT       | 158      | 7        | 124   | 6        | 0.87 | 0.68   | 1.11    | 0.27    |
|        | Ex2-659G>A | AA       | 1313     | 58       | 1171  | 60       | 1.00 |        |         |         |
|        |            | AG       | 811      | 36       | 699   | 36       | 0.97 | 0.85   | 1.10    | 0.59    |
| POT1   | –1386G>A   | CC       | 966      | 42       | 851   | 43       | 1.00 |        |         |         |
|        |            | GA       | 1055     | 46       | 913   | 46       | 0.98 | 0.86   | 1.11    | 0.74    |
|        |            | AA       | 256      | 11       | 221   | 11       | 0.98 | 0.80   | 1.20    | 0.84    |
family history of breast cancer in first-degree female relatives, 

-1381C>T (OR 0.73, 95% CI 0.53–1.00), –244C>T (OR 0.66, 95% CI 0.46–0.95), and Ex2-659G>A (A305A) (OR 0.57, 95% CI 0.39–0.84) (Table 2 and Supplementary Table 2). These SNPs were not significantly related to family history of cancer among the control population, and analyses of breast cancer cases with a family history of breast cancer in first-degree female relatives, produced similar results (data not shown). These three SNPs appeared to be in LD by D’ regardless of family history, produced similar results (data not shown). These SNPs were not significantly related to family history of cancer among the control population, and analyses of breast cancer cases with a family history of breast cancer in first-degree female relatives, produced similar results (data not shown).

**Table 2** Association between selected single nucleotide polymorphisms in TERT and TERF and breast cancer risk among cases and controls, stratified by family history of breast cancer in first-degree female relatives

| Gene SNP | Homozygous common | Heterozygous | Homozygous variant | Per minor allele relative risk |
|----------|-------------------|-------------|-------------------|------------------------------|
|          | Controls          | Cases       | Controls          | Cases                        | OR   | 95% CI | P-value | P trend |
| TERT     |                   |             |                   |                              |      |        |         |         |
| IVS6-33G>A | 1496             | 1243        | 592               | 482                          | 60   | 46    | 0.97    | 0.86    | 1.10    | 0.67   | 0.06   |
| rs7784168  | 107              | 146         | 20                | 53                           | 3    | 4     | 1.57    | 0.97    | 2.55    | 0.07   | 0.06   |
| rs10263573 | 40               | 66          | 65                | 106                          | 28   | 33    | 0.86    | 0.63    | 1.18    | 0.35   | 0.04   |
| rs10250202 | 1243             | 1037        | 761               | 634                          | 130  | 93    | 0.96    | 0.86    | 1.07    | 0.05   | 0.06   |
| rs10250202 | 70               | 134         | 50                | 65                           | 11   | 4     | 0.57    | 0.39    | 0.84    | 0.004  | 0.01   |
| rs10250202 | 1023             | 810         | 898               | 768                          | 227  | 201   | 1.06    | 0.97    | 1.17    | 0.22   | 0.006  |
| rs10250202 | 59               | 105         | 59                | 89                           | 14   | 11    | 0.75    | 0.53    | 1.06    | 0.06   | 0.006  |

Disputes between total number of cases and controls shown in table are due to missing genotype information.

**DISCUSSION**

To our knowledge, this is the first study to investigate genetic variation within genes important in telomere biology (POT1, TEP1, TERF1, TERF2 and TERT) and breast cancer risk. The SNPs genotyped were representative of common genetic variation across the genomic region of interest, and showed no significant overall associations with breast cancer risk. However, data suggested association between variants in TERT among women with a positive family history of breast cancer.

TERT Ex2-659G>A showed a borderline statistically significant association with a reduced risk of breast cancer in analysis of all cases and controls, which appeared to be stronger for individuals with a family history of breast cancer. Similar associations of two other SNPs, –1381C>T and –244C>T, in individuals with a
family history of breast cancer were also noted. TERT –244T > C was noted to have increased telomerase activity related to the T allele in a recent study of non-small cell lung cancer (Hsu et al., 2006). TERT –1381C>T also appears to be a functional SNP. Studies of promoter function at this site (noted at –1327 by the authors, but with the same rs number, rs2735940) suggested longer telomere length in with TT homozygotes compared with CC (Matsubara et al., 2006). Our findings suggested that variants in TERT could have an effect in individuals already at increased genetic risk of breast cancer, although the number of individuals with a family history of breast cancer was small.

TERF2 IVS6 +27G > A (E673_301) was also associated with a reduced risk of breast cancer in individuals with a family history of breast cancer, however, the functional significance of the SNP is unknown. It does not appear to affect an intron–exon splice site (Conde et al., 2004).

The SNPs evaluated in this study were chosen based on previous knowledge of common genetic variation resulting from re-sequencing analysis, captured most of the common variation in the five studied genes (i.e. POT1, TEP1, TERF1, TERF2 and TERT), and could be related to breast cancer risk based on the role suggested for telomere biology in this disease (Baykal et al., 2004; Wacholder et al., 2004; Savage et al., 2005). Although associations with less common SNPs are possible, our data indicate that common variation in these genes is unlikely to substantially affect overall breast cancer risk. The associations of TERT –1381C>T, –244G > T, Ex2-659G > A and the corresponding haplotype in individuals with a family history of breast cancer are intriguing and warrant follow-up in independent studies.

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