



Molecular Genotyping Shows That Ataxia-Telangiectasia Heterozygotes Are Predisposed to Breast Cancer

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ABSTRACT: About 1.4% of the general population are heterozygous carriers of the gene for ataxia-telangiectasia (A-T), an autosomal recessive progressive neurologic syndrome in which cancer incidence of homozygotes is approximately 100-fold greater than the general population's rates. The hypothesis that A-T heterozygotes are predisposed to breast cancer was tested by the unbiased statistically powerful index-test method based on molecular genotyping. The A-T gene carrier status of 775 blood relatives in 99 A-T families was determined by tracing the A-T gene in each family through tightly linked flanking DNA markers. There were 33 women with breast cancer who could be genotyped; 25 of these were A-T heterozygotes, compared to an expected 14.9 (odds ratio 3.8, 95% confidence limits 1.7–8.4, one-sided $p = .0001$). This demonstrates that the A-T gene predisposes heterozygotes to breast cancer. For the 21 breast cancers with onset before age 60, the odds ratio was 2.9 (1.1–7.6, $p = .009$) and for the 12 cases with onset at age 60 or older, the odds ratio was 6.4 (1.4–28.8, $p = .002$). Thus the breast cancer risk for A-T heterozygous women is not limited to young women but appears even higher at older ages. Of all breast cancers in the United States, 6.6% may occur in women who are A-T heterozygotes. This proportion is several fold greater than the estimated proportion of carriers of BRCA1 mutations in breast cancer cases with onset at any age. © Elsevier Science Inc., 1996

INTRODUCTION

Ataxia-telangiectasia (A-T) is an autosomal recessive syndrome characterized by progressive cerebellar ataxia and oculocutaneous telangiectasia [1]. A-T homozygotes develop new incident cancers at a rate approximately 100 times the age-specific population rate [2]. Lymphoid cancers predominate in childhood, and epithelial cancers, including breast cancer, are seen in adolescent and young adult A-T patients [3].

These observations gave rise to the hypothesis that A-T heterozygotes might also have an excess risk of cancer [4]. This hypothesis, particularly for female breast cancer, has been supported by retrospective and prospective studies of A-T families in the United States and Europe [5–9]. However, it is still regarded by some as “just a hypothesis” [10], a “controversial suggestion” [11], or a “possibility” [12, 13]. Thus, it is important to confirm this hypothesis using the best available genetic methods.

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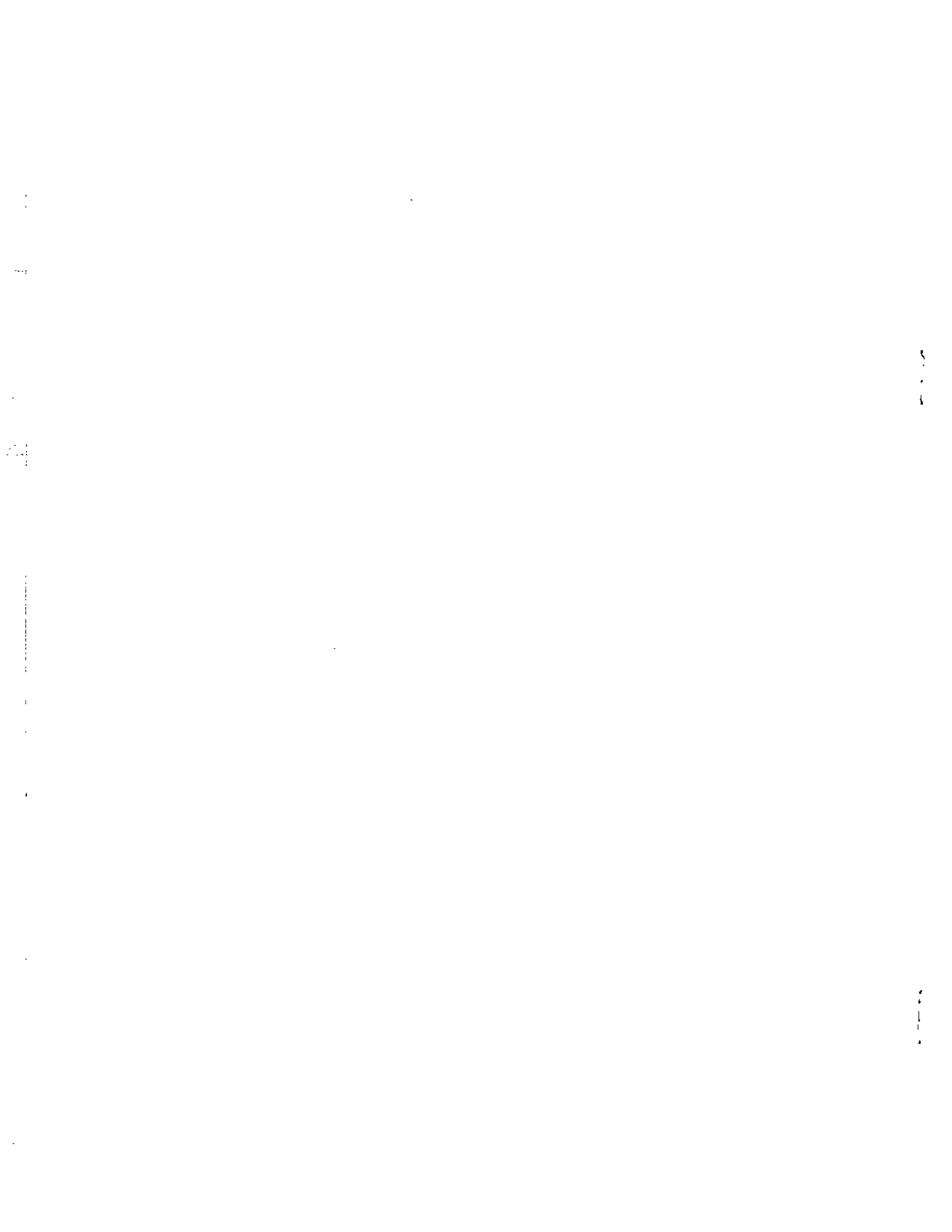
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Highly polymorphic tightly linked flanking markers [14] make it possible to accurately genotype blood relatives in families of A-T homozygotes. Such molecular genotyping of blood relatives with breast cancer in A-T families, analyzed by the index-test method [15], now demonstrates unequivocally that female A-T heterozygotes are predisposed to breast cancer.

MATERIALS AND METHODS

For genotyping we obtained DNA samples (from blood or fixed tissue) from A-T homozygotes, who are the index individuals, their nuclear families, and all available blood relatives in their extended families. Informed consent was obtained from each study participant under a protocol approved by the New York Medical College Committee for the Protection of the Rights of Human Subjects. When DNA from a grandmother was not available, but DNA from her husband was, we genotyped the grandmother as a carrier when her husband was not, and vice versa. The requisite DNA samples for determining A-T gene carrier status were available for 99 out of the 261 A-T families participating in our continuing prospective study of mortality and cancer incidence.

Using standard procedures DNA was extracted from the blood lymphocytes of the A-T family members. From par-



affin-embedded tissue we directly amplified without a xylene deparaffinization step or DNA extraction [16]. Genotyping was done through CA repeat markers D11S1778 and D11S1819 [14] closely flanking the A-T gene locus. In 15 families in which haplotypes based on these two marker loci were not fully informative, D11S1818 [14] and D11S384 [17] also were determined. The CA strand primer was end-labeled with (γ - 32 P)ATP and polymerase chain reaction (PCR) was performed using standard conditions. The PCR products were analyzed on a 6% polyacrylamide sequencing gel, and autoradiographs were read after 2 to 4 hours of exposure.

In two families there was a single recombination between D11S1778 and D11S1819; in both families the haplotype of the A-T chromosome was specified uniquely by D11S384, which shows zero recombination with the A-T locus [17] and D11S1778. Neither of these recombinations affected the genotyping of breast cancer cases. Marker determinations, haplotypes, and carrier status were reviewed independently three different times. Of the 775 A-T blood relatives (not obligate heterozygotes or homozygotes) who were genotyped in 99 A-T families, 390 were found to be carriers and 385 noncarriers. The expected numbers were 397.275 and 377.725, respectively.

In the 99 genotyped families, 43 female relatives with breast cancer were identified from our previously published studies [5-7], from the retrospective data for A-T families incorporated into this study subsequent to those studies, and from our ongoing prospective observation of A-T families. Hospital records from each presumed case were reviewed, prior to knowing the carrier status of the case, to confirm the diagnosis of breast cancer. Ten cases were excluded: 2 with lobular in situ carcinoma, 6 for which the marker loci did not amplify cleanly from their tissue sample, and 2 whose carrier status depended directly on that of another test subject with breast cancer.

Testing the association of A-T heterozygosity with breast cancer through the index-test method requires, in A-T families, determining the A-T gene carrier status of blood relatives with previously identified breast cancers. These test relatives cannot be homozygotes or obligate heterozygotes, whose genotypes are fixed. Both the A-T gene carrier status and relationship of each breast cancer case to the proband in each family were entered into a spreadsheet that calculated the odds ratio, 95% confidence limits, and *t*-statistic, as previously described [15]. These calculations were based on comparing the observed number of carriers to the number expected on the basis of Mendelian inheritance and the population frequency of the A-T gene. The prior probability of heterozygosity for the A-T gene is approximately 0.67 for siblings, 0.5 for aunts and grandmothers, and 0.25 for first cousins of the homozygotes.

RESULTS

In the 99 genotyped families we were able to determine the A-T gene carrier status for 26 women with breast cancer from blood samples, 5 from fixed tissues, and 2 others using DNA from the husbands. Thus, 33 breast cancer cases in 28 families were genotyped. Seventeen of these

Table 1 Age at diagnosis for genotyped breast cancers in A-T blood relatives

Age at diagnosis	A-T heterozygotes	Noncarriers
30-34	2	0
35-39	0	0
40-44	1	2
45-49	5	0
50-54	3	3
55-59	4	1
60-64	1	0
65-69	6	1
70-74	2	0
75-79	1	1
80+	0	0
Total	25	8

cases had been reported previously [5-7]. Only 1 of the 6 tissue samples for which PCR was unsuccessful came from an operation in 1980 or later, and 4 of the 5 successfully amplified specimens came from operations in 1980 or later ($p = .07$).

Of the 33 women with invasive breast cancer, 25 were found to be A-T heterozygotes (Table 1). Ages at the onset of the cancers ranged from 31 to 77. These cancers were diagnosed between 1953 and 1995. Twelve of the breast cancers had occurred in aunts, 13 in grandmothers, 5 in great-aunts, and 1 in a sibling, a cousin, and a great-grandmother of an A-T proband. All eight of the noncarrier women, and 17 of the 25 A-T heterozygotes with breast cancer, were living as of October 1, 1995. Five of the carrier women (20%) and 2 of the noncarriers (25%) had bilateral breast cancer.

The expected number of A-T heterozygotes in the 33 breast cancer cases was 14.9. If the cancer incidence of carriers and that of noncarriers within the same families were equal, the odds ratio would be approximately 1, indicating no association. Instead, for all breast cancers in this sample the odds ratio, which estimates the relative risk of carriers compared to noncarriers, was 3.8 (95% confidence limits 1.7-8.4, one-sided $p = .0001$). For the 21 breast cancers with onset before age 60, the odds ratio was 2.9 (1.1-7.6, $p = .009$) and for the 12 cases with onset at age 60 or older, the odds ratio was 6.4 (1.4-28.8, $p = .002$).

DISCUSSION

The finding of 25 A-T gene carriers among 33 breast cancer cases in A-T families is compelling evidence that A-T heterozygotes are predisposed to breast cancer. There is no other explanation for this highly significant excess over the 14.9 expected on the basis of Mendelian inheritance and the A-T gene frequency. Because these data come from molecular genotyping, they are fully independent of all previous analyses of breast cancer incidence that compared blood relatives to spouse controls. Whereas the comparison of blood relatives to spouse controls relies on how well these two groups are matched and on the fact that a high proportion of blood relatives are heterozygotes,

gene carriers are identified directly with molecular genotyping in the index-test method.

The estimated relative risk, 3.8, of invasive breast cancer for A-T gene carriers compared to noncarriers is close to the most recent previous estimates from the prospective comparison of spouse controls with all blood relatives, 5.1, or with obligate heterozygotes, 3.8 [7]. The large number of observed breast cancer cases in A-T blood relatives in their thirties, forties, and fifties suggested that the excess breast cancer risk might be especially high for A-T heterozygotes in that age range [7]. In contrast, molecular genotyping now shows that the relative risk above age 60 may be more than twice that for younger women.

The frequency of A-T heterozygotes in the U.S. population was estimated by maximum likelihood to be 1.4%, based on the number of families in which A-T homozygotes appeared in more than one sibship in an extended family [18]. Based on this estimated heterozygote frequency and the estimated relative risks of 2.9 for breast cancers before age 60 and 6.4 for cases with onset after age 60, approximately 6.6% of all breast cancers in the United States occur in A-T heterozygotes (see Appendix). If the risk of female A-T heterozygotes for breast cancer with onset from age 60 through age 79 is 6.4, then approximately 8.3% of all breast cancers arising in this age group occur in A-T heterozygotes.

Two genes, BRCA1 and BRCA2, that also predispose to breast cancer have been identified through molecular studies of families in which the risk of this cancer is very high [19]. The gene frequency of BRCA1 is much greater than that of BRCA2. In contrast to the A-T gene, the risk of breast cancer for BRCA1 gene carriers is highest at young ages; it has been estimated that the proportion of breast cancer cases in the general population due to BRCA1 is 5.3% for onset before age 40 years [20]. For cases with onset ages 20–69, the estimated proportion is 1.7%. Because about 35% of all breast cancers occur after age 70 [21], the proportion of BRCA1 carriers in all breast cancer cases is likely to be 1% or less, several fold lower than the estimated proportion of A-T gene carriers among all cases. A direct comparison of the impact of BRCA1, BRCA2, and the A-T gene on breast cancer incidence will be possible when population screening for mutations at these loci becomes practical.

The estimated relative risk of 3.8 is based on breast cancers that occurred in the United States between 1953 and 1995. However, the risk for A-T heterozygotes may vary with different environmental conditions or the genetic composition of a population. Because it will be valuable to attempt to replicate our findings as soon as possible, the risk estimate from our sample of persons of European origin could be compared to an independent estimate readily available through the A-T family registries already established in Europe [8, 9, 22, 23]. It also will be of great interest to collect family medical data and DNA samples in Africa and Asia to measure this risk in non-European populations.

Previously we found evidence that exposure to certain medical diagnostic X-ray procedures increased the risk of breast cancer for blood relatives in A-T families [5, 6]. If

breast cancer with onset above age 60 is more closely associated with A-T heterozygosity than earlier onset breast cancer, as our present data demonstrate, the difference may be explained by increasing exposure to medical diagnostic X-ray procedures with advancing age. Because some of the blood relatives with breast cancer in previous studies may have been noncarriers, we plan to reexamine this issue through a case-control analysis in which the X-ray exposures of identified carriers with breast cancer will be compared to that in matched carrier controls. We also plan to compare the histopathology or survival of A-T gene carriers with breast cancer to that of noncarriers in the same families. There are no data showing that bilateral breast cancer is more frequent among A-T heterozygotes than among noncarriers.

Unlike previous comparisons of blood relatives to spouse controls, these molecular findings cannot be explained by undetected confounders or unintended bias. No confounder can affect the result of the index-test method because each individual's genotype is fixed at the time of conception. Unintended bias is implausible because the cases were selected, before genotyping, by the single criterion of having hospital record confirmation of breast cancer. The blood relatives did not know their own carrier status at the time they contributed blood samples. If carriers were more likely, because of some behavioral effect of the A-T gene, to contribute blood samples for genotyping than noncarriers, the proportion of carriers and noncarriers among all genotyped blood relatives in these 99 A-T families would have deviated significantly from the expected proportion. Further, it is not possible that more samples were available from breast cancer cases who are carriers because they survive longer because the proportion of living cases was higher among the noncarriers. If there were an undetected breast cancer risk factor in the genotyped families, it would have affected breast cancer incidence in carriers and noncarriers equally and randomly. It is unlikely that the ability to PCR from stored fixed tissue influenced our result, as this factor appeared to be a function simply of the length of time specimens were stored.

Carrier determination through highly informative flanking haplotypes was reliable, as we detected no recombination between the closest markers and the A-T locus. Currently available methods for screening for mutations at this locus are tedious and costly and seem to detect only 50%–60% of all such mutations [12, 24–26]. When population screening for A-T heterozygosity becomes practical, A-T heterozygotes found through such screening could be the index individuals for population-based assessments, using the index-test method, of the risk of breast cancer for female A-T heterozygotes.

The A-T alleles segregating in the study families are a random sample of A-T alleles in the general population, as the only distinctive feature of the study families is that two heterozygotes met and had an offspring affected by A-T. Families in which this gene is segregating are ideal for testing hypothesized gene–disease associations, because there is natural matching for important risk factors between carriers and noncarriers in these families. It will be

of considerable interest to measure the proportion of A-T heterozygotes among breast cancer patients in different populations when population screening is possible. However, comparing these proportions to the general population heterozygote frequency will be less reliable than the index-test method as a test of A-T heterozygote cancer predisposition because of the well-known difficulty in matching populations for confounders, such as ethnicity or social class, that affect both A-T heterozygote frequency and breast cancer incidence.

Now that the excess risk of breast cancer for A-T heterozygotes has been measured by molecular genotyping and the index-test method, it is imperative to understand the molecular actions of the A-T gene and to identify the environmental and other genetic factors that interact with it to produce cancer.

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APPENDIX

If the relative risk of breast cancer is 2.9 cancers with onset before age 60 and the proportion of A-T heterozygotes in the general population is 0.014, then the ratio of breast cancer cases in A-T heterozygotes to the total number of cases with onset before age 60 is given by

$$\frac{2.9 \times 0.014}{2.9 \times 0.014 + (1 \times 98.6)} = 0.0395$$

For breast cancers with the onset at age 60 or older, the relative risk is 6.4, and the resulting proportion is 0.0833. If 40% of all breast cancers occur before age 60 [21], then

$$\begin{aligned}0.4 \times 0.0395 &= 0.01580 \\0.6 \times 0.0833 &= 0.04998 \\0.01580 + 0.04998 &= 0.06578\end{aligned}$$

which is the basis for the estimate that 6.6% of all breast cancer cases occur in A-T heterozygotes.