Breast Cancer Incidence and the Effect of Cigarette Smoking in Heterozygous Carriers of Mutations in the Ataxia-Telangiectasia Gene

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Abstract

Background: **Mutations in the ataxia-telangiectasia (A-T) gene cause an autosomal recessive syndrome in homozygotes and compound heterozygotes and predispose female heterozygous carriers to breast cancer. No environmental agent has been previously shown to increase the risk of cancer for women who carry a mutated gene that predisposes to breast cancer. This study assesses the effect of cigarette smoking on the risk of breast cancer in A-T mutation carriers and determines age-specific and cumulative incidence rates for breast cancer among such carriers.**

Methods: **Clinical data were collected between 1971 and 1999 from blood relatives from 274 families of patients with A-T. The A-T mutation carrier status of 973 females was determined by molecular analysis of blood and tissue samples. The breast cancer rates in carrier smokers and nonsmokers were compared. Age-specific and cumulative breast cancer rates were also compared between carriers and noncarriers using Kaplan-Meier survival curves.**

Results: **The cumulative incidence through age 80 years was 80% for carriers who smoked and 21% for carriers who never smoked ($P = 0.01$). Six cases of breast cancer were diagnosed between ages 70 and 79 years among carriers who smoked. The cumulative breast cancer incidence among A-T mutation carriers was 43% by age 80 years, compared with 17% for noncarriers ($P = 0.002$). Carriers had new incident breast cancers at an annual rate of 1.4% from ages 65 through 79 years; for noncarriers the rate was 0.20%.**

Conclusions: **A-T carrier females had an elevated risk of breast cancer, most pronounced at older ages, compared with noncarriers, and smoking increased this risk substantially.** (Cancer Epidemiol Biomarkers Prev 2008;17(11):3188–92)

Introduction

The gene for ataxia-telangiectasia (A-T) was recognized because children with two mutated alleles have a distinctive autosomal recessive syndrome with progressive neurologic disability beginning with gait ataxia; oculocutaneous telangiectasia appears later (1). A-T patients develop cancers in childhood and early adult life at a ~100-fold greater rate than the general population (2).

Carriers of a single A-T mutation show no distinctive signs or symptoms. These mutations predispose female carriers to an excess risk of breast cancer that has been estimated to be between 2- and 8-fold greater than that of noncarriers (3-16).

Families of A-T patients have been recruited into a continuing study of cancer incidence since 1971 (17); an excess of breast cancers was first reported among the blood relatives in 1987 (3). After it became possible, in the mid-1990s, to identify which blood relatives carried the family-specific A-T mutation, the genotypes of 25 women with breast cancer confirmed definitively that such mutations predisposed women to breast cancer (5). The index-test method (18) found the odds ratio to be 3.8 based on this molecular genotyping.

Now, genotyping of blood relatives has made it possible to compare the cumulative incidence rate during that continuing study of breast cancer for carrier females in these families who smoked to those who never smoked. We also compared the annual and cumulative breast cancer incidence of carriers and noncarriers. Noncarriers within A-T families closely match the carriers in genetic make-up and ethnic origin.

Materials and Methods

Study Sample. From 1971 to 1999, families of patients with A-T were recruited from the United States and Canada into a continuing study of A-T patients and their families. All families were referred to us because at least one family member was an A-T patient. The methods and selection criteria have been described previously (3, 4, 5, 17). All first-, second-, and third-degree blood relatives alive on January 1, 1930 or later were eligible for inclusion in the study. Initial contact was sought through the parents of patients with A-T, and a four-generation pedigree was constructed.

Relatives were excluded if they had lost contact with the family and could not be located, declined participation in the study, did not reside in the United States or Canada, or if adequate health or death information could not be obtained.

Vital Information. At the beginning of enrollment and annually or semiannually thereafter (for those who were

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living), health questionnaires were sent to study participants or, for those participants who were deceased, to their closest living relatives. The questionnaires asked about current health problems and past medical history, including a list of all major hospitalizations. They also asked about height, weight, occupation, parity, and whether the subject had ever smoked cigarettes, was a current smoker, or had smoked at one time and quit. They did not ask about the duration or intensity of smoking or about how long ago a subject had quit.

Death certificates and records of all hospitalizations were obtained as described previously. Underlying causes of death were coded according to the International Classification of Diseases, Eighth Revision (ICD-8).

For each deceased subject, the age at death and the underlying cause were entered in a computer file. For all cancers and other major illnesses, the age at diagnosis and the ICD code were entered in the same file, provided the applicable medical records were available. Breast cancer diagnosis dates ranged from 1953 to 1997.

Beginning in 1987, blood and tissue samples were requested from all study participants for determination of A-T mutation carrier status. Tissue samples from any operation were used when they could be obtained. All relatives who could be genotyped by haplotype or mutation analysis, as well as all obligate carriers of an A-T gene mutation, were included in this study. Informed consent was obtained from each subject or subject's guardian.

Of the genotyped subjects, 1,148 were female; 729 of those were carriers and 419 were noncarriers. Two hundred twenty-nine genotyped female subjects (139 carriers and 90 noncarriers) were excluded from the study because of inadequate health data or missing death certificates. In total, 919 eligible females (590 carriers and 329 noncarriers) representing 241 families were genotyped and were eligible for participation in the study. Of these 919 women, data about smoking habits were available for 859 (539 carriers and 320 noncarriers); the remaining 60 women did not provide this information in their questionnaires.

Statistical Analyses. The starting date for each subject was the date we received the first completed questionnaire. The closing date for each subject was the date of breast cancer diagnosis for each case and its matched controls, date of death, or date of last contact (via questionnaire or telephone contact with the subject or close relative), whichever came first. The ages at the closing dates were entered in the MedCalc program (MedCalc Software, Belgium) to obtain the cumulative probabilities, for carriers and noncarriers, of being diagnosed with breast cancer, based on the Kaplan-Meier method. The same approach compared the cumulative probabilities of breast cancer for subjects who smoked to those who did not. The significance of the difference between the two curves in each Kaplan-Meier plot was tested with the exact log-rank test. Breast cancer incidence for women ages 20 to 79 years was analyzed because there were no cases diagnosed before this interval and only four after.

Data about the duration and intensity of smoking habits for those who smoked and those who had quit smoking and information about the length of time since the subjects last smoked were not available.

All P values were two-sided. The exact log-rank tests were done using StatXact (Cytel Software Corp.). The data were analyzed according to a Cox model using SPSS by MiniMax Consulting. All other statistical analyses were done using MedCalc.

This study was approved by the Committees on the Protection of the Rights of Human Subjects at the New York University School of Medicine from 1970 through 1972, at the University of North Carolina School of Medicine from 1972 through 1992, and at New York Medical College from 1992 through 2001, when data collection stopped. Informed consent forms were signed and received from each subject at the time they entered the study.

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Results

Carrier and Noncarrier Cases. Breast cancer was diagnosed in carriers between 28 and 78 years of age (median, 51) and in noncarriers between ages 38 and 79 years (median, 53). In total, 77 breast cancer cases were distributed among 66 families in total; 60 carrier cases occurred in 49 families, whereas the 17 noncarrier cases each came from a different family.

For 10 of the carrier cases, the mutation type was unknown. The mutations detected in the remaining 50 carrier cases fell into the following categories: truncating, 30; splicing, 8; nonsense, 5; missense, 4; deletion, 2; and initiation, 1. Six individual mutations were found in more than one family: four mutations were found in two families each, one was found in three families, and one was found in four families.

Incidence of Breast Cancer. The annual incidence among carriers exceeded that among noncarriers at all ages, although the excess was modest between ages 50 and 69 years (Table 1).

Over all ages 20 to 79 years, the incidence for carriers was 296.3 (95% confidence interval, 224-384) and for noncarriers 123.6 (95% confidence interval, 69.2-204). This corresponds to a relative risk for carriers of 2.4 (95% confidence interval, 1.3-4.3) compared with noncarriers, over ages 20 through 79 years. The incidence rate for the noncarriers is comparable to that reported by the Surveillance Epidemiology and End Results registry (19) for White females of 134.0.

The cumulative breast cancer risk (Fig. 1) for female carriers was significantly higher than the risk for noncarriers ($P = 0.002$), with the difference between the groups appearing after age 40 years and becoming most pronounced after age 65 years. About 9% of noncarriers had a breast cancer by age 65 years; the cumulative risk of 17% to age 80 years is based on only two new incident cases. In contrast, carriers had a cumulative risk of ~12% by age 65 years and 43% by age 80 years.

Smoking and Breast Cancer. The smoking status was known for 539 A-T carriers included in this study; 126 were current smokers, 288 had never smoked, and 125 subjects had smoked at one time and quit. Among the carriers who smoked, 18 breast cancer cases were observed in 15 different families, 3 of which contained two cases each. Among the nonsmokers, there were

Table 1. Breast cancer incidence among A-T mutation carriers and noncarriers

Age group (y)	Carriers			Noncarriers		
	No. breast cancer cases	Person-years	Annual incidence*	No. breast cancer cases	Person-years	Annual incidence*
20-29	1	5,754	17.4	0	3,184	0.0
30-39	6	5,173	116.0	0	2,898	0.0
40-49	18	3,740	481.3	3	2,394	125.3
50-59	13	2,540	511.8	7	1,873	373.7
60-69	9	1,455	618.6	4	1,218	328.4
70-79	10	573	1,745.2	1	571	175.1

*Incidence per 100,000 population.

25 cases in 21 families, 4 of which contained 2 cases each. Note that, although a total of 60 breast cancer cases were observed among the carriers in this study, smoking status was unknown for 6 of those cases and another 11 of those cases were among those who had quit smoking. Breast cancer incidence among carriers who smoked exceeded modestly that among those who had never smoked for ages 40 through 59 years, but the difference was substantial for cancer incidence between ages 70 and 79 years (Table 2). The cumulative breast cancer risk for female A-T carriers who were current smokers significantly ($P = 0.01$) exceeded the risk for carriers who never smoked (Fig. 2). Through age 79 years, the cumulative breast cancer risk for the carriers who never smoked was ~21%; the cumulative risk for current smokers was nearly 80%. Cox analysis confirmed the significant associations of both carrier status ($P = 0.013$) and smoking ($P = 0.013$) with breast cancer, but found no evidence ($P = 0.754$) for interaction between being a smoker and being a carrier.

When the subjects who smoked at one time and quit were included in the analysis and grouped with the current smokers for a comparison of those who had never smoked with those who had ever smoked, the results were nearly identical (the cumulative breast cancer risk was 80% for ever-smokers).

The cumulative breast cancer risk through age 79 years was also examined in noncarriers ($n = 243$; 195 nonsmokers and 47 smokers). The cumulative risk for the noncarriers who smoked (20%) was slightly higher than that for the nonsmokers (16%), but the difference was not significant ($P > 0.1$) and was based on only 12 total cases (9 among nonsmokers and 3 among smokers). When the 79 additional subjects who quit smoking (3 additional breast cancer cases) were included in the analysis as smokers, the cumulative risk was 10%. The relative risk of breast cancer between noncarrier smokers and nonsmokers was 1.5 (95% confidence interval, 0.36-6.6), compared with a relative risk between carrier smokers and nonsmokers of 2.1 (95% confidence interval, 1.2-5.1).

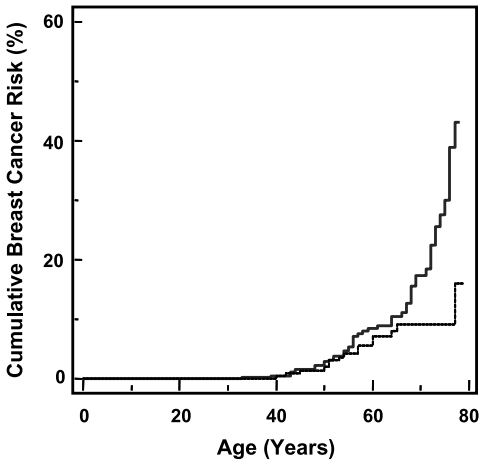

Figure 1. Cumulative breast cancer incidence in female carriers and noncarriers ages 20 to 79 years. Cumulative risk of breast cancer for carriers (*solid line*) and noncarriers (*dotted line*) of an A-T mutation ($P = 0.002$).

Discussion

Some previous studies of links between cigarette smoking and breast cancer in both the general population or in any genetically defined subpopulation such as BRCA1/2 mutation carriers have found modest evidence for an association whereas others have found little or no such evidence (20-27). Terry et al. (21) reviewed reports through 2002, with most studies finding little or no increase in breast cancer risk associated with smoking, whereas others reported relative risks ranging from 0.8 to 2.9 in studies with varying numbers of subjects and statistical power. Later studies were equally conflicting, with some finding no association of smoking with increased breast cancer risk (20, 22-24) whereas others reported relative risks of breast cancer between smokers and nonsmokers ranging from 1.1 to 1.5 (25, 26). Among BRCA1/2 carriers, previous studies are also conflicting; one study estimated the relative risk of breast cancer at 1.3 (27), whereas another determined that smoking did not increase the breast cancer risk among carriers (28).

The finding that smoking increased the risk of breast cancer for A-T mutation carriers from age 40 years on may influence the smoking habits of women who know they are carriers because they are mothers of A-T children or have been genotyped as a carrier by DNA testing as a member of an A-T patient's family.

The index-test method definitively confirmed that A-T mutations predispose women to breast cancer. This method, because it relies solely on probabilities of carrying the familial mutation according to Mendelian principles, cannot be influenced by other risk factors, selection bias, or genetic heterogeneity. The present study shares with the index-test method its indifference to genetic heterogeneity and undetected stratification because the carrier and noncarrier blood relatives share,

Table 2. Breast cancer incidence among A-T mutation carriers who smoke and those who never smoked

Age group (y)	Smokers			Nonsmokers		
	No. breast cancer cases	Person-years	Annual incidence*	No. breast cancer cases	Person-years	Annual incidence*
20-29	1	1,233	81.1	0	2,959	0.0
30-39	2	1,066	187.6	4	2,638	151.6
40-49	5	704	710.2	6	1,906	314.8
50-59	3	445	674.2	7	1,302	537.6
60-69	2	196	1,020.4	4	804	497.5
70-79	5	47	10,638.3	4	319	1,253.9

*Incidence per 100,000 population.

randomly, a high proportion of other genes and other risk factors such as ethnic origins, familial environment, and socioeconomic status.

The elevated risk for smoking carriers cannot be explained by the well-known familial aggregation of smokers because only three families contained more than one breast cancer case who smoked. Further, the Cox analysis found no evidence for an interaction between being a smoker and being a carrier.

The proportion of missense mutation carriers among the breast cancer patients (4 of 50, 8.0%) was lower, although not significantly ($P > 0.3$), than that observed overall in a prior study of A-T families (9 of 71, 12.6%; ref. 29). These data do not support the conjecture that A-T missense mutations predispose more strongly to breast cancer than do truncating mutations (16, 30).

The high incidence of breast cancer cases after age 70 years among A-T carriers who smoked suggests that long exposure to cigarette smoke may account for the substantial breast cancer incidence in carriers at older ages. The excess incidence among carriers cannot be explained by confounders, undetected stratification, or bias, conscious or unconscious. However, the elevated incidence among carrier nonsmokers ages 70 through 79 years also suggests that cumulative exposure to other environmental factors may lead to some cancers in carriers. It is also possible that there is a long latency between time of exposure and clinical cancer. Identifying all environmental exposures that increase the incidence of breast cancer among A-T mutation carriers could lead to effective preventive measures.

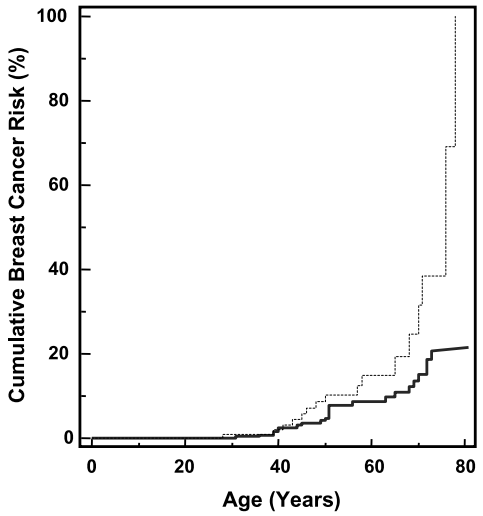

Figure 2. Cumulative breast cancer incidence in smoking and nonsmoking carriers ages 20 to 79 years. Cumulative risk of breast cancer in A-T mutation carriers who smoked (*dotted line*) and carriers who never smoked (*solid line*; $P = 0.01$).

Cells carrying deleterious A-T mutations have a decreased ability to repair DNA double-strand breaks, which is thought, but not proved, to explain why such cells are more likely to undergo carcinogenic transformation. Although the carcinogens in tobacco smoke cause primarily bulky DNA adducts, not double-strand breaks, the repair of adduct-damaged DNA does require repair of double-strand breaks. Adducts are frequently found in breast tissue obtained from smokers, which may explain why smoking increases the risk of breast cancer in mutation carriers.

Although the lifetime cumulative incidence of breast cancer among A-T mutation carriers was estimated as 43% compared with the 43% to 74% estimated for BRCA1/2 mutation carriers (31-33), the effect of A-T mutations could be much greater because they are estimated to occur at a frequency between 1% and 5% (9, 10, 12-15), compared with 0.1% to 0.2% for BRCA1/2 mutations (32, 33). When the risks of breast cancer associated with the most frequently occurring A-T mutations (9-15) are established, genetic screening can be offered to men and women who wish to know more about their disease risks, without a high probability of obtaining an ''indeterminate'' result. Whereas all individuals should be cautioned to avoid or stop smoking because of myriad health concerns, A-T mutation carriers can benefit further by a reduction in breast cancer risk by doing so.

Conclusions. Carrying an A-T mutation increases the risk of female breast cancer, and smoking increases this risk substantially. More research is needed to develop a population screening test so that carriers can be identified and told of these risks.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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