Breast cancer risk in ataxia telangiectasia (AT) heterozygotes: haplotype study in French AT families

N Janin1*, N Andrieu2*, K Ossian1, A Laugé3, M-F Croquette4, C Griscelli5, M Debré5, B Bressac-de-Paillerets4, A Aurias3 and D Stoppa-Lyonnet3

1Institut Gustave Roussy, 39 rue Camille Desmoulins, 94805 Villejuif Cedex, France; 2U351 Inserm, Institut Gustave Roussy, 39 rue Camille Desmoulins, 94805 Villejuif Cedex, France; 3Institut Curie, Unité de Génétique Oncologique, Laboratoire de pathologie moléculaire des cancers, 26 rue d’Ulm 75231 Paris Cedex 5, France; 4Centre Hospitalier Feron-Vrau, 219 & 329 Boulevard Victor Hugo, BP 255, 59019 Lille, Cedex, France; 5Hôpital des Enfants Malades, Service d’Immuno-Hematologie pédiatrique, 149 rue de Sèvres, 75743 Paris, Cedex 15, France

Summary
Epidemiological studies in ataxia telangiectasia (AT) families have suggested that AT heterozygotes could have an increased cancer risk, especially breast cancer (BC) in women. It has also been suggested that an increased sensibility of AT heterozygotes to the effect of ionizing radiation could be responsible for the increased BC risk. BC relative risk (RR) estimation in AT heterozygotes within families ascertained through AT children is presented here. Family data collected included demographic characteristics, occurrence of cancers, past radiation exposures and blood samples. DNA samples were studied using seven ATM linked microsatellites markers allowing AT haplotypes reconstitution. The relative risk of BC was assessed using French estimated incidence rates. A significant increase risk of BC is found among obligate ATM heterozygote female relatives with an age ≤ 44 years (RR = 4.55, P = 0.005). The BC relative risk is statistically borderline among the obligate ATM heterozygote female relatives with an age ≥ 45 years (RR = 2.48, P = 0.08). The estimated BC relative risk among ATM heterozygotes is consistent with previously published data. However, the increased risk is only a little higher than classical reproductive risk factors and similar to the risk associated with a first-degree relative affected by BC.

Keywords: ataxia telangiectasia heterozygosis; breast cancer risk; family study

Epidemiological studies of ataxia telangiectasia (AT) families have suggested that AT heterozygotes could have an increased cancer risk, especially breast cancer (BC) in women (Swift et al, 1987, 1991; Pippard et al, 1988; Børresen et al, 1990; Morrell et al, 1990; Athma et al, 1996; Stankovic et al, 1998). The estimation of this increased BC risk assessed from the combined analysis of available data in 1994 was 3.9 (Easton, 1994). In Europe, two out of three studies (Pippard et al, 1998; Børresen et al, 1990; Stankovic et al, 1998) have shown a significant increased risk of BC but with wide confidence intervals (Stankovic et al, 1988; Børresen et al, 1990). Moreover, it has been suggested that an interaction between AT heterozygosis and ionizing radiation exposures could be involved in the increase in BC risk (Swift et al, 1991). However, no data have been published that sustain this hypothesis. The gene for AT (ATM) was identified in 1995 (Savitsky et al, 1995), allowing the identification of ATM heterozygotes in families with an AT-affected child, through segregation of AT-linked haplotypes. Thus an epidemiological study of cancer risks associated with AT heterozygosis collecting information on ionizing radiation exposures has been performed in France. In the present paper, BC relative risk estimation in AT heterozygotes within AT children families is presented.

DATA COLLECTION AND METHODS
A family study of the AT children population was carried out in France from June 1994 to February 1997. AT children were recruited by paediatricians who have been surveying this population since early childhood and cytogeneticians who have contributed to their disease diagnosis. AT children were eligible if their family was living in France at the time of the study. For each participant who signed a consent form, a blood sample was taken; and a questionnaire was administrated by a physician to all adult relatives. A blood or a buccal cell sample was taken from the AT children and their siblings with parental agreement.

Demographic characteristics (gender, date of birth and, if deceased, age at death and cause of death) and the occurrence of BC and any other cancer, including age at diagnosis and places of medical care were collected from first-degree (parents and siblings of AT child), second-degree (uncles, aunts and grandparents) and third-degree (granduncles and -aunts, and great-grandparents, cousins of AT child) relatives. Epidemiological data on first- and second-degree relatives aged 18 years or over concerned medical history, exposure to medical and professional radiation and detailed reproductive factors for females.

All contacted families, with the exception of one, consented to participate. Thirty-four French families were recruited. AT children were aged from 3 to 32 years. Eighteen of 29 breast malignancies reported in families could be confirmed by pathological
Breast cancer risk and the ATM gene

British Journal of Cancer (1999) 80(7), 1042–1045

© 1999 Cancer Research Campaign

Table 1 ATM heterozygosis (ATM het) status repartition among female relatives of the 34 families

| ATM het status | A priori probability approach | Mixed approach |
|----------------|-----------------------------|----------------|
|                | No. of females (%)          | No. of females (%) | Mean age (s.d.) | Person-years |
| Obligate       | 40 (5.6)                    | 115 (16.2)       | 44.2 (19.5)    | 5079         |
| 50%            | 195 (27.3)                  | 201 (28.3)       | 45.2 (26.6)    | 9085         |
| 25%            | 344 (48.2)                  | 107 (15.1)       | 48.5 (23.5)    | 5192         |
| 12.5%          | 117 (16.4)                  | –               | –             | –            |
| Obligate non   | 18 (2.5)                    | 288 (40.5)       | 46.8 (25.4)    | 13468        |
| All females    | 711                         | 711             | 46.2 (24.6)    | 32823        |

s.d. = standard deviation.

Table 2 BC risk according to ATM het status among female relatives of the 34 families

| ATM het status | Mixed approach |
|----------------|----------------|
|                | O E O/E 95% CI |
| Obligate       | 9 2.71 3.32 (1.75–6.38) |
| 50%            | 5 6.24 0.80 (0.33–1.92) |
| 25%            | 3 3.42 0.88 (0.28–2.73) |
| Obligate non   | 11 9.26 1.19 (0.66–2.15) |

O = observed number of BC cases; E = expected number of BC cases.

Table 3 BC risk according to ATM het status and age among female relatives of the 34 families

| Age of female relatives ≤ 44 years | Age of female relatives ≥ 45 years |
|-----------------------------------|-----------------------------------|
| ATM Het status | O E O/E 95% CI | O E O/E 95% CI |
|----------------|----------------|----------------|
| Obligate       | 5 1.10 4.55 (1.89–10.9) | 4 4.61 2.48 (0.93–6.61) |
| 50%            | 0 1.64 0.00 – | 5 4.60 1.09 (0.45–2.62) |
| 25%            | 2 0.99 2.02 (0.51–8.08) | 1 2.44 0.41 (0.06–2.91) |
| Obligate non   | 2 2.60 0.77 (0.19–3.08) | 9 6.66 1.35 (0.70–2.59) |

O = observed number of BC cases; E = expected number of BC cases.

RESULTS

The 34 families included 1429 persons with a mean number of 42 persons per family (s.d. = 19). DNA samples from 401 individuals were studied. In addition, classification of an extra 412 individuals as ATM obligate heterozygotes or ATM obligate non-heterozygotes was made possible by the mixed approach. The AT children siblings for whom there was no DNA sample were very few (12
out of 44 with a mean age of 17 years), and they were included in the 0.5 ATM heterozygote category. Among the 1429 persons, 29 BC cases had been diagnosed between 1943 and 1996. All cases with the exception of one female, that is 28 female cases. Ten BC cases were ATM heterozygotes, 11 were not ATM heterozygotes. For the remaining eight BC cases, the ATM status was uncertain with an a priori probability of 0.5 for five and 0.25 for three. Among the 34 families, at least one BC occurred in 20 families. In 14 families only one BC case occurred, in four families two BC cases occurred, in one family three cases, and in another family four cases of BC occurred. The male case was ATM heterozygote with a BC diagnosed in 1972 at 55 years of age.

The age-range of female BC cases at diagnosis was 35–97 years. Among obligate ATM heterozygote female BC cases, diagnoses occurred between 1969 and 1995. Among the others, ATM het status female BC cases, 16 were diagnosed between 1943 and 1995, three had an unknown date of diagnosis, and thus year of their death was used. The three BC cases with an unknown date of diagnosis died at 35, 55 and 70 years respectively, their respective ATM het status is 0.25, 0 and 0.5.

Female relatives of the 34 families are described in Table 1 according to ATM het status comparing the a priori probability approach to the mixed approach (i.e. haplotype study or a priori probability for those whose het status was undetermined by molecular approach). The obvious interest of the mixed approach is an increase by three of the obligate ATM heterozygote number, but also an increase by 16 of the non-obligate ATP-heterozygote number. The mean age of the women across ATM het status is similar.

In Table 2, the BC relative risks according to ATM het status among the female relatives are shown. A significant increased risk of BC is found among obligate ATM heterozygotes with a point estimate of 3.32 and a 95% CI of 1.75–6.38 (P = 0.002). A slight non-significantly increased BC risk is found among obligate non-ATM heterozygotes (O/E = 1.19, IC95% = 0.66–2.15). The BC risk among other ATM het status groups are not significant with similar point estimates (0.5 class: O/E = 0.80, IC95% = 0.33–1.92; 0.25 class: O/E = 0.88, IC95% = 0.28–2.73).

In Table 3, the BC relative risks are calculated according to ATM het status and age of the female relatives. Among female relatives with an age equal to or less than 44 years, a significant increase risk of BC is found among obligate ATM heterozygotes with a point estimate of 4.55 and a 95% CI of 1.89–10.9 (P = 0.005). A non-significant decrease BC risk is found among obligate non-ATM heterozygotes (O/E = 0.77, IC95% = 0.19–3.08). Among female relatives with an age equal to or more than 45 years, a statistically borderline increase risk of BC is found among obligate ATM heterozygotes with a point estimate of 2.48 and a 95% CI of 0.93–6.61 (P = 0.08). A non-significant increase BC risk is found among obligate non-ATM heterozygotes (O/E = 1.35, IC95% = 0.70–2.59). The BC risk among the other ATM het status groups remains non-significant when taking age into account with variable point estimates and wide confidence intervals (0.5 class: among females ≤ 44 years, zero observed BC case, among females ≥ 45 years O/E = 1.09, IC95% = 0.45–2.62; 0.25 class: among females ≤ 44 years, O/E = 2.02, IC95% = 0.51–8.08; among females ≥ 45 years O/E = 0.41, IC95% = 0.06–2.91).

**DISCUSSION**

The finding of this study is in agreement with the increased BC risk previously detected among ATM heterozygotes (Swift et al, 1987, 1991; Pippard et al, 1988; Børresen et al, 1990; Athma et al, 1996). The estimated BC relative risk of 3.32 is consistent with Athma et al’s (1996) estimate (RR = 3.8) and Easton’s (1994) combined estimate (RR = 3.9). The point estimate of BC risk appears higher among female relatives less than 44 years of age than among those more than 45 years of age. This result cannot be affected by a possible misclassification of the three BC cases with an unknown age at diagnosis, none of them being ATM heterozygotes. This result is the opposite to that of Athma et al’s (1996) and FitzGerald et al’s findings (1997). Indeed, FitzGerald et al (1997) did not find evidence for an increase frequency of ATM heterozygotes among women with early onset of BC. However, let us note that given the low estimated frequency of ATM carriers, they had little chance of detecting an increase risk (40% chance for a fourfold increase in risk and 6% for a twofold increase) (Bebb et al, 1997; Bishop and Hopper, 1997). In the same way, Athma et al (1996) found an increased risk of BC for ATM heterozygotes and even higher at older ages (60 or older).

A possible bias in family recruitment due to a higher participation of families with a BC may be suggested. Indeed, the slight non-significant increase of BC risk among obligate non-ATM heterozygotes may reflect a possible ascertainment bias. However, the BC higher risk among the obligate ATM heterozygotes remains, even when adjusted (i.e. adjusted RR = 2.8).

The slight non-significantly decreased BC risk found among the groups at 50% or 25% risk to be ATM carriers could reflect a bias towards a higher proportion of known heterozygosis status for BC cases than for the non-BC cases leading to an overestimation of obligate ATM carriers BC risk. Indeed, 29% (eight out of 28) of BC cases have an uncertain ATM carrier status against 44% of non-BC cases (300 out of 683). However, the observed decreased BC risk is not statistically significant and the obligate ATM carriers BC risk may not be dramatically overestimated.

The declared BCs among families have been verified for more than the majority. Moreover, BC has been found to be reported with great accuracy in numerous studies. In particular, Theis et al (1994) found concordance of 99% between case report and the pathological report. However, the non-affected-by-cancer individuals in families have not been checked because of lack of a national French registry, although it has been estimated that 98% of negative families’ history were correct (Aitken et al, 1995). A poor sensitivity of self-reported family history of BC (i.e. under reporting) may lead to an under-estimate of the BC relative risk across heterozygosis status classes.

The calculations of expected numbers of BC are known to be sensitive to the reference population used. Because national incidence data are not available in France, estimated incidences of BC between 1978 and 1987 were used as the reference population in this study (Benhamou et al, 1990; De Vathaire et al, 1996). This might induce an over-estimate of the expected numbers of BC among exposed women before 1978 (mostly grandmothers, grand-aunts and great-grandmothers of AT children) and an underestimate of this number among exposed women after 1987 (mostly mothers and aunts) since the BC incidence rate has been increasing in West European countries for a number of decades (Parkin et al, 1993). This may not explain the increased risk of BC among all of obligate AT heterozygotes but might partially explain the observed difference in BC relative risk between younger and older female relatives. For this reason, comparison between the BC relative risks of young and old ATM obligate heterozygote females is more...
suitable, adjusted on respective obligate non-ATM heterozygotes BC relative risks. Thus, an observed difference in the point estimates remains with a BC risk of 5.9 among the younger ATM obligate heterozygote female relatives and 1.8 among the older. Since neither shows convincing difference in RR estimated by age bands given the RR confidence intervals, other studies are needed to clarify this point.

Although still imprecise, the detected increased risk may have a point estimate of about 3, which is only a little higher than classical reproductive risk factors (i.e. a young age at menarche, a nulliparity or a late age at first childbirth etc) and similar to the risk associated with a family history of BC among first-degree relatives (Kelsey and Horm-Ross, 1993). However, the previous suggestion that the ATM heterozygotes would be highly ionizing-radiation sensitive (Swift et al, 1987) is still unconfirmed. Indeed, studies on highly clinical-radiotherapeutic BC patients did not show evidence for an elevated ATM heterozygote rate (Appleby et al, 1997; Shayeghi et al, 1998). Thus it does not appear necessary, so far, to subject women with ATM het status to any different screening program which is not already available to women with a first-degree relative affected by BC. Nevertheless, further studies are needed for assessing the BC risk among ATM heterozygotes according to their past ionizing-radiation exposures. Indeed, such studies may allow us to improve the understanding of the underlying mechanisms involved in the observed increased BC risk among ATM heterozygotes.

ACKNOWLEDGEMENTS

This project was supported by the Institutes Gustave Roussy, Villejuif and Curie, Paris, the Ligue Nationale Contre le Cancer, the Comité des Hauts-de-Seine de la Ligue Contre le Cancer, the Service de Radioprotection d’Electricité de France, INSERM. We are very indebted to the physicians who helped us contact families with AT children: C Billard, M-T Bogaufs, J-P Gout, B Leheup, N Philip and J-P Pollet. We are very grateful to all the participating families. We would like to thank Josyane Le Calvez for technical assistance and Diane Mathewson for linguistic revision of the manuscript.

REFERENCES

Aitken J, Bain C, Ward M, Siskind V and MacLennan R (1995) How accurate is self-reported family history of colorectal cancer? Am J Epidemiol 141: 863–871

Appleby JM, Barber JBP, Levine E, Varley JM, Taylor AMR, Stankovic T, Heighway J, Warren C and Scott D (1997) Absence of mutations in the ATM gene in breast cancer patients with severe responses to radiotherapy. Br J Cancer 72: 1546–1549

Athma P, Rappaport R and Swift M (1996) Molecular genotyping shows that ataxia-telangiectasia heterozygotes are predisposed to breast cancer. Cancer Genet Cytogenet 92: 130–134

Bebb G, Glickman B, Gelmon K and Gatti R (1997) ‘AT risk’ for breast cancer. Lancet 349: 1784–1785

Benthamou E, Laplanche A, Wartelle M, Faivre J, Gignoux M, Menegoz F, Robillard J, Schaffer P, Schraub S and Flamant R (1990) Incidence des cancers en France 1978–1982. In Statistiques de santé, INSERM (ed). INSERM: Paris

Bishope DT and Hopper J (1997) AT-troubled risk? Nat Genet 15: 226

Bartres AL, Andersen TI, Trefet S, Heiberg A and Moller P (1990) Breast cancer and other cancers in Norwegian families with ataxia-telangiectasia. Genes Chromosomes Cancer 2: 339–403

Breslow NE and Day NE (1987) The design and analysis of cohort studies. In Statistical Methods in Cancer Research, Davis W (ed). IARC: Lyon

Coleman M, Douglas A, Hermon C and Peto J (1986) Cohort study analysis with a Fortran computer program. Int J Epidemiol 15: 134–137

De Vathaire F, Koscielny S, Rezvani A, Laplanche A, Estève J and Ferlay J (1996) Estimation of the incidence of the cancers in France 1983–1987. In Statistiques de santé, INSERM (ed). INSERM: Paris

Easton DF (1994) Cancer risks in A-T heterozygotes. Int J Radiat Biol 6: S171–82

FitzGerald MG, Bean JM, Hegle SR, Unsal H, MacDonald DJ, Harkin DP, Finkelstein DM, Isselbacher KJ and Haber DA (1997) Heterozygous ATM mutations do not contribute to early onset of breast cancer. Nat Genet 15: 307–310

Kelsey JL and Horm-Ross PL (1993) Breast cancer: magnitude of the problem and descriptive epidemiology. Epidemiol Rev 15: 7–16

Laube K, Odérgard A, Andersen TI, Biukholm IK, Kaeresen R, Nesland JM, Ottested L, Shiloh Y and Bartres-Dale AL (1997) Loss of heterozygosity at 11q23.1 in breast carcinomas: indication for involvement of a gene distal and close to ATM. Genes Chromosomes Cancer 18: 175

Morrell D, Chase CL and Swift M (1990) Cancers in 44 families with ataxia-telangiectasia. Cancer Genet Cytogenet 50: 119–123

Parkin DM, Muir CS, Whelan SL, Gao YT, Ferlay J and Powell J (1992) Cancer Incidence in Five Continents, Vol 5. IARC: Lyon

Pippard EC, Hall AJ, Barker DJ and Bridges BA (1988) Cancer in homozygotes and heterozygotes of ataxia-telangiectasia and xeroderma pigmentosum in Britain. Cancer Res 48: 2929–2932

Savekty K, Bar-Shira A, Gilad S, Rotman G, Ziv Y, Vanagaitë L, Tagle DM, Smith S, Uziel T, Sze1 S, Ashkenazi M, Pecker I, Frydman M, Harmik R, Pantanajli SR, Simmons A, Clines GA, Sartiel A, Gatti RA, Chessa L, Sanal O, Lavin MF, Jasberg, N, Taylor AMR, Arlett CF, Miki T, Weissman SH, Lovett M, Collins FS and Shiloh Y (1995) A single ataxia-telangiectasia gene with a product similar to PI-3 kinase. Science 268: 1749–1753

Shayeghi M, Seal S, Regan J, Collins N, Barfoot R, Rahman N, Ashton A, Moohan M, Wooster R, Owen R, Bliss JM, Stratton ME and Yarnold J (1998) Heterozygosity for mutations in the ataxia telangiectasia gene is not a major cause of radiotherapy complications in breast cancer patients. Br J Cancer 78: 922–927

Stankovic T, Kidd AMJ, Sutcliffe A, McGuire GM, Robinson P, Weber P, Bedenham T, Bradwell AR, Easton DF, Lennog GX, Haïtes N, Byrd PJ and Taylor AM (1998) ATM mutations and phenotypes in ataxia-telangiectasia families in the British Isles: expression of mutant ATM and the risk of leukemia, lymphoma, and breast cancer. Am J Hum Genet 62: 334–345

Swift M, Reitnauer PJ, Morrell D and Chase CL (1997) Breast and other cancers in families with ataxia-telangiectasia. N Engl J Med 336: 1289–1294

Swift M, Morrell D, Massey RB and Chase CL (1991) Incidence of cancer in 161 families affected by ataxia-telangiectasia. N Engl J Med 325: 1831–1836

Thibis B, Boyd N, Lockwood G and Trickler D (1994) Accuracy of family history in breast cancer patients. Eur J Cancer Prev 3: 321–327