

Lymphoid tumours and breast cancer in ataxia telangiectasia; substantial protective effect of residual ATM kinase activity against childhood tumours

A Reiman¹, V Srinivasan¹, G Barone¹, JI Last¹, LL Wootton¹, EG Davies², MM Verhagen³, MA Willemsen³, CM Weemaes⁴, PJ Byrd¹, L Izatt⁵, DF Easton⁶, DJ Thompson⁶ and AM Taylor^{*,1}

¹School of Cancer Sciences, College of Medical and Dental Sciences, University of Birmingham, Vincent Drive, Edgbaston, Birmingham, B15 2TT, UK; ²Department of Immunology, Great Ormond Street Hospital & Institute of Child Health, Guilford Street, London WC1N 3JH, UK; ³Department of Paediatric Neurology, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; ⁴Department of Paediatrics, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands; ⁵Clinical Genetics Department, Guy's and St Thomas' NHS Foundation Trust, 7th floor Borough Wing, Great Maze Pond, London SE1 9RT, UK; ⁶Cancer Research UK Genetic Epidemiology Unit, Department of Public Health and Primary Care, University of Cambridge, Strangeways Research Laboratory, Worts Causeway, Cambridge CB1 8RN, UK

BACKGROUND: Immunodeficiency in ataxia telangiectasia (A-T) is less severe in patients expressing some mutant or normal ATM kinase activity. We, therefore, determined whether expression of residual ATM kinase activity also protected against tumour development in A-T.

METHODS: From a total of 296 consecutive genetically confirmed A-T patients from the British Isles and the Netherlands, we identified 66 patients who developed a malignant tumour; 47 lymphoid tumours and 19 non-lymphoid tumours were diagnosed. We determined their ATM mutations, and whether cells from these patients expressed any ATM with residual ATM kinase activity.

RESULTS: In childhood, total absence of ATM kinase activity was associated, almost exclusively, with development of lymphoid tumours. There was an overwhelming preponderance of tumours in patients < 16 years without kinase activity compared with those with some residual activity, consistent with a substantial protective effect of residual ATM kinase activity against tumour development in childhood. In addition, the presence of eight breast cancers in A-T patients, a 30-fold increased risk, establishes breast cancer as part of the A-T phenotype.

CONCLUSION: Overall, a spectrum of tumour types is associated with A-T, consistent with involvement of ATM in different mechanisms of tumour formation. Tumour type was influenced by ATM allelic heterogeneity, residual ATM kinase activity and age. *British Journal of Cancer* (2011) **105**, 586–591. doi:10.1038/bjc.2011.266 www.bjancer.com

Published online 26 July 2011

© 2011 Cancer Research UK

Keywords: ataxia telangiectasia; ATM; ATM kinase; lymphoma; breast cancer

Ataxia-telangiectasia (A-T) is a rare autosomal recessive neurological disorder, typically diagnosed in early childhood and characterised by progressive cerebellar degeneration and oculocutaneous telangiectasia. Almost all cases of A-T are caused by homozygous or (more commonly) compound heterozygous mutations in the *ATM* gene (MIM 607585) (Savitsky *et al*, 1995; Taylor and Byrd, 2005). Ataxia-telangiectasia patients show a predisposition to the development of different types of lymphoid tumours as children or as young adults and, less frequently, they may also develop brain and other tumours (Spector *et al*, 1982; Morrell *et al*, 1986; Taylor *et al*, 1996). The spectrum of leukaemias in children with A-T is different to that in the general population in which infant and childhood leukaemias are mostly pre-B and B-precursor ALL, involving a stage before V(D)J recombination. About half of them involve Bcr-Abl, Tel-AML, MLL and other rearrangements (Greaves and Wiemels, 2003). There appears to be no role of ATM in

protecting against B-precursor ALL, as there is no observed increase in these tumours in A-T patients. The proposed role of ATM in V(D)J recombination (Bredemeyer *et al*, 2006, 2008; Huang *et al*, 2007; Vacchio *et al*, 2007; Hewitt *et al*, 2009) is consistent with the predisposition to lymphoid tumour development that involves chromosome translocations, derived through errors in immune system gene rearrangement, occurring in A-T patients often at an earlier age than the same tumour in the normal population (Taylor *et al*, 1996).

In contrast, heterozygous female *ATM* mutation carriers in A-T families have an increased risk of developing breast cancer, without any evidence for an increased risk of lymphoid tumours (Swift *et al*, 1991; Geoffroy-Perez *et al*, 2001; Olsen *et al*, 2001; Thompson *et al*, 2005). Ataxia-telangiectasia shows substantial phenotypic heterogeneity, both neurological (Hiel *et al*, 2006; Verhagen *et al*, 2009) and immunological (Staples *et al*, 2008). There is also substantial allelic heterogeneity, and a consideration of possible mechanisms for tumour development in A-T patients must take into account the expression of either different mutant ATM proteins, or combinations of two ATM proteins with or

*Correspondence: Dr AM Taylor; E-mail: A.M.R.Taylor@bham.ac.uk
Received 27 April 2011; revised 20 June 2011; accepted 22 June 2011;
published online 26 July 2011

without kinase activity. We sought to establish the relationship between absence or presence of ATM kinase activity and cancer risk, based on a large cohort of A-T patients from the UK and the Netherlands.

MATERIALS AND METHODS

Patient cohort

Ethical approval was given by the National Research Ethics Committee (REC reference 07/H1210/155). Ataxia-telangiectasia patients from the UK ($n = 244$) and the Netherlands ($n = 52$) were included in the analysis (Table 1). Participants were recruited on the basis of a clinical diagnosis of A-T and without reference to their cancer status. The follow-up period was defined as beginning at birth and continuing until the earliest of death, cancer diagnosis, loss of contact or database completion. Time period of follow-up ranged from 10 months to 66 years (median = 18 years, IQR = 11–28 years), with a total of 6239 person-years of follow-up.

Identification of A-T patients whose cells expressed ATM kinase activity

As many as possible *ATM* mutations were identified mainly by genomic sequencing (primer data available on request), in all UK and Dutch A-T patients, both with tumours (*ATM* mutations categorised in Supplementary Tables S1–S5) and without tumours (mutations not shown). Cell lines were available from all UK A-T patients and most Dutch A-T patients. In conjunction with sequencing, we also used western blotting on all cell lines to determine whether or not there was expression of ATM protein. As our hypothesis was that expression of residual ATM kinase protected against tumour development in A-T patients, we also determined whether cells showed ATM kinase activity as assessed by detection of phosphorylated substrates of ATM, using phospho-specific antibodies (see Materials And Methods below). Using a combination of *ATM* mutation identification and western blotting for ATM protein detection, the following were used as criteria by which cells were deemed not to have any ATM kinase activity.

Criteria by which cells were deemed not to have any ATM kinase activity

- (1) The presence of two truncating *ATM* mutations. Truncating *ATM* mutations result in instability and loss of the ATM protein from the cell. Therefore, those A-T patients with two truncating *ATM* mutations were assumed not to express ATM protein (and therefore no ATM kinase activity). Nevertheless, western blotting was used to confirm, in most cases, whether or not cell lines expressed any ATM protein. We could show that where there were two truncating *ATM* mutations there was always absence of ATM protein (Supplementary Table S1).
- (2) Where ATM was found to be expressed, the mutational cause was always investigated. Where ATM protein was expressed, ATM kinase activity was measured. Absence of ATM kinase activity was associated with following types of mutation

producing ATM protein (Supplementary Table S2, Supplementary Figure S1).

- (i) Some founder mutations (e.g., c.7638_7646del9; p.Arg2547_Ser2549del) resulted in loss of three in-frame amino acids (Stankovic *et al*, 1998); previous work showed expression of a low level of mutant protein and absence of ATM kinase activity (Stewart *et al*, 2001).
- (ii) Splice site mutations, resulting in exon skipping, that allowed expression of a very low level of ATM protein, were tested for ATM kinase activity. In all the cases, measurable kinase activity was absent. These occurred, for example, in 25 out of 66 A-T patients with tumours (Supplementary Table S2).
- (iii) A small group of A-T patients carried missense mutations that did not result in expression of any ATM protein (for A-T patients with tumours, see Supplementary Table S1).
- (iv) A small group of A-T patients carried missense mutations that resulted in expression of low-level mutant protein but without any activity (for A-T patients with tumours, see Supplementary Table S2).
- (v) Mutation in the initiating methionine of the coding sequence leading to likely use of the next downstream methionine, as judged by the slightly truncated mutant protein (for A-T patients with tumours, see Supplementary Table S2).
- (vi) A small group of A-T patients carried mutations that resulted in expression of an almost normal level of mutant ATM protein but without any kinase activity. In the non-tumour patients, these included c.9022C>T (p.Arg3008Cys) (Angele *et al*, 2003) and in 3 out of 66 tumour patients, two with c.8293G>A (p.Gly2765Ser) (Supplementary Table S3, Supplementary Figure S2).

In total, 135 out of 185 A-T patients without tumours showed absence of ATM kinase activity and out of 66 patients with tumours 51 did not show kinase activity.

Criteria by which cells were deemed to have ATM kinase activity With respect to the presence of ATM kinase activity, this was always observed in cells from A-T patients with or without tumours who carried:

- (i) The c.5763-1050A>G (p.Pro1922fs) leaky splice site mutation (all from the UK and Ireland). Where not tested, cells with this mutation were assumed to be expressing a low level of normal ATM with kinase activity as previously shown (McConville *et al*, 1996; Stewart *et al*, 2001), (Supplementary Table S4, Supplementary Figure S4).
- (ii) The c.1066-6T>G p.Val356fs mutation. This is assumed to be a leaky splice site mutation (Austen *et al*, 2008) (Supplementary Table S4, Supplementary Figure S4).
- (iii) Some, but not all, missense mutations. These could express either a relatively high level of mutant ATM protein (e.g., c.7271T>G; p.Val2424Gly) (Stankovic *et al*, 1998; Stewart *et al*, 2001) or a low level (c.8494C>T) mutant ATM protein (Barone *et al*, 2009), but with clear ATM kinase activity (Supplementary Table S4, Supplementary Figure S4); therefore, the mutant ATM could have either low (e.g., p.Val2424Gly) or high (e.g., p.Arg2832Cys) specific activity.

Table 1 Details of the study cohorts

| Cohort | No. of A-T patients (male/female) | No. of families | No. of cancers | No. of person-years | Median age at end of follow-up* (IQR) | ATM kinase activity present/absent/unknown |
|-----------------|-----------------------------------|-----------------|----------------|---------------------|---------------------------------------|--|
| UK | 244 (130/114) | 208 | 47 | 5044 | 18 (11–28) | 52/151/41 |
| The Netherlands | 52 (25/27) | 38 | 19 | 1195 | 16.5 (11–34) | 12/36/4 |
| All | 296 (155/141) | 246 | 66 | 6239 | 18 (11–28) | 64/187/45 |

Abbreviations: A-T = ataxia telangiectasia; ATM = ataxia telangiectasia mutated; IQR = inter-quartile range; UK = United Kingdom. *Follow-up period began at birth and continued until the earliest of death, first cancer diagnosis, loss of contact or database completion.

In total, 50 out of 185 A-T patients without tumours showed the presence of some ATM kinase activity and 14 out of 66 A-T patients with tumours (in one tumour patient the ATM kinase activity could not be determined – Supplementary Table S5). The ATM kinase activity in these individuals, therefore, came either from normal ATM as a result of a leaky splice site mutation or from mutant ATM expressed by an allele with a missense mutation.

The smaller proportion of tumours in UK compared with Dutch A-T patients was due in part to the presence of the IVS40 splice site mutation in the British and Irish population and also the higher proportion of older Dutch patients with more years at risk.

Cell culture

We derived a lymphoblastoid cell line from each A-T patient's blood by separating the lymphocytes, infecting the peripheral blood mononuclear cells with Epstein–Barr virus by adsorption for 4 h, followed by addition of cyclosporin A ($0.1 \mu\text{g ml}^{-1}$) to kill any remaining T cells and allowing 14 days for the transformed B lymphocytes to emerge. The lymphoblastoid cell line was subsequently grown in RPMI 1640 medium (Sigma-Aldrich, Irvine, UK) supplemented with 10% fetal calf serum (PAA Laboratories, Pasching, Austria). We derived lymphoblastoid cell lines from normal controls in the same manner.

Immunoblotting for ATM expression and ATM activity assays

For ATM protein expression assays, patient-derived lymphoblastoid cells were harvested and cell pellets were resuspended in UTB buffer (9 M urea, 50 mM Tris, pH 7.5, 150 mM β -mercaptoethanol) and lysed on ice by sonication. For ATM activity assays, patient-derived lymphoblastoid cells were either mock-irradiated or exposed to 5 Gy ionising radiation and harvested after 30 min before the cell pellets were resuspended in UTB buffer and lysed on ice by sonication. Whole-cell lysate (50 μg) was separated by SDS–polyacrylamide gel electrophoresis, and the proteins transferred to nitrocellulose membrane (Pierce, Rockford, IL, USA). Nitrocellulose strips were subjected to immunoblotting and the protein bands visualised using the enhanced chemiluminescence (ECL) system (Amersham, Little Chalfont, UK) and exposing to Hyperfilm MP autoradiography film (GE Healthcare, Little Chalfont, UK). Antibodies used for immunoblotting were ATM (custom-made mouse monoclonal antibody clone 11G12 previously described), pATM Ser1981 #AF1655 (R&D Systems, Abingdon, UK), Smc1 #A300-055A, pSmc1 Ser966 #A300-050A, Nbs1 #Ab398, pNbs1 Ser343 #Ab47272 (Abcam, Cambridge, UK), p53 Ser15 #9284, pChk2 Thr68 #2661, Chk2 and p53 (gift from Dr G Stewart).

Statistical methods

The statistical analyses were based on data from 296 A-T patients from the UK ($n=244$) and the Netherlands ($n=52$), having excluded 13 A-T patients for whom date of birth was unknown. For the main analysis, the at-risk person-years were defined as beginning at birth and continuing until the earliest of death, first cancer diagnosis, loss of contact or database completion (end of 2009 for the Netherlands; March 2010 for UK). Cancer incidence rate ratios for patients with ATM kinase activity relative to a baseline of no kinase activity were estimated using Poisson regression. Survival until cancer diagnosis for patients with and without kinase activity was compared using a Kaplan–Meier plot and the log-rank test. Differences in the proportions of lymphoid and non-lymphoid tumours between age-groups and between kinase-absent and -present patients were compared using Fisher's exact test. We were not able to obtain kinase activity information

from all patients, but this did not introduce bias, as all comparisons were between patients with known status.

The breast-cancer-specific analysis, differed in that follow-up, continued until the earliest of breast cancer diagnosis, death, loss of contact or database completion, and was left-truncated at 1960, as population rates are unreliable before this date.

RESULTS

Distribution of tumours in the patient cohort

Overall, 66 first cancer diagnoses were made including several rare types of tumour; 47 tumours were lymphoid and 19 non-lymphoid; 42 were diagnosed ≤ 16 years of age and 24 were diagnosed > 16 years (Table 2). The proportion of tumours, which were lymphoid, was significantly greater in the younger age-group ($P=0.002$).

Of the 44 families with multiple cases of A-T, 7 families contained two A-T patients with a cancer diagnosis. For five of the families, the two cases were of the same tumour type (breast cancer, mixed-cellularity Hodgkin's lymphoma, Hodgkin's lymphoma, T-cell lymphoma and T-ALL, respectively). The other two families contained a T-PLL and a pancreatic cancer, and a myeloma and a myeloid leukaemia, respectively.

Relationship of the presence of ATM kinase to tumour

Our hypothesis was that the majority of tumours at ≤ 16 years were related to absence of ATM kinase activity. We sought to identify the ATM mutations in as many patients as possible, and to determine whether their cells expressed any ATM protein with residual ATM kinase activity (Supplementary Tables S1–S5 and Supplementary Figures S1–S4). We were able to determine whether there was any ATM kinase activity for 251 patients; 187 showed no kinase activity and 64 did. Overall survival until first cancer diagnosis was significantly better among A-T patients with some ATM kinase activity (log-rank test $P=0.0001$) (Figure 1). The proportion of tumours that were lymphoid as opposed to non-lymphoid was significantly lower in those with some ATM kinase activity ($P=0.018$) (Table 2).

Table 3 shows the incidence rates of lymphoid and non-lymphoid tumours, according to the absence or presence of ATM kinase activity. The incidence rate for lymphoid tumours was significantly lower in those with kinase activity than in those without (incidence rate ratio (IRR) = 0.22, 95% confidence interval (CI) = 0.10–0.49, $P<0.001$), but there was no difference in the rates of non-lymphoid tumours between the two groups ($P=0.91$). The presence of kinase activity was also associated with a lower risk of lymphoid tumours when restricted to tumours diagnosed ≤ 16 years of age (IRR = 0.15, 95% CI = 0.03–0.63, $P=0.01$ for lymphoid tumours) (Table 4). All six of the non-lymphoid tumours diagnosed at ≤ 16 years, including three brain tumours, were in patients with no kinase activity, hence it was not possible to estimate a non-lymphoid IRR. In fact, only two cancers of any type were diagnosed before the age of 16 years in A-T patients with kinase activity; a T-cell lymphoma at an age of 2 years in a patient with two missense mutations and T-ALL at 9 years in a patient with both a frameshift and leaky splice site mutation (Supplementary Table S4).

The IRRs associated with kinase activity were less extreme in the over-16 age-group, and none of the differences were significant, possibly due to the smaller numbers of tumours. Importantly, the proportion of A-T patients with kinase activity was significantly higher in the older age-group; of patients with known kinase status, 64 out of 251 = 25% of patients in the ≤ 16 age-group had kinase activity, compared with 57 out of 138 = 41% of patients who survived beyond the age of 16 years ($P=0.002$). This likely reflects

Table 2 Tumour types observed in ataxia telangiectasia patients by age and presence/absence of ATM kinase activity

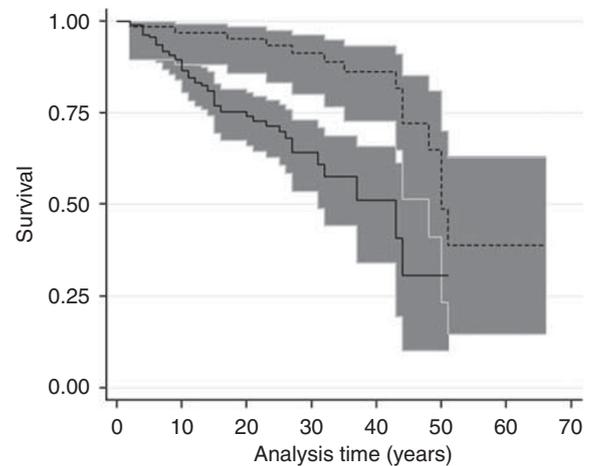
| Lymphoid tumours | n | Non-lymphoid tumours | n |
|--------------------------------|----|--------------------------|---|
| <i>Diagnosed ≤ 16 years</i> | | | |
| ATM kinase absent | 33 | | 6 |
| AILD | | Astrocytoma | |
| ALL | | DFSP | |
| B cell lymphoma (5) | | Ganglioglioma | |
| B cell NHL (2) | | Hepatocellular carcinoma | |
| Burkitt-like lymphoma | | Medulloblastoma | |
| Hodgkin's lymphoma (3) | | Myeloid leukaemia | |
| Hodgkin's-mixed cellularity | | | |
| Hodgkin's lymphoma, T-ALL | | | |
| Lymphoma (6) | | | |
| Lymphosarcoma | | | |
| NHL | | | |
| T cell lymphoma (3) | | | |
| T-ALL (7) | | | |
| ATM kinase present | 2 | | 0 |
| T cell lymphoma | | | |
| T-ALL | | | |
| ATM kinase unknown | 1 | | 0 |
| B cell NHL | | | |
| <i>Diagnosed > 16 years</i> | | | |
| ATM kinase absent | 7 | | 4 |
| B cell lymphoma | | Breast cancer (2) | |
| Burkitt lymphoma | | Pancreatic cancer | |
| Hodgkin-mixed cellularity | | Testicular seminoma | |
| T-ALL | | | |
| T-PLL (3) | | | |
| ATM kinase present | 4 | | 9 |
| ALL | | Breast cancer (5) | |
| Myeloma | | Ectopic pituitary tumour | |
| T-ALL | | Myeloid leukaemia | |
| T-PLL | | Thyroid carcinoma (2) | |

Abbreviations: AILD = angioimmunoblastic T-cell lymphoma; ALL = acute lymphoblastic leukaemia; ATM = ataxia telangiectasia mutated; DFSP = dermatofibrosarcoma protuberans; NHL = non-Hodgkin's lymphoma; T-ALL = T-cell acute lymphoblastic leukaemia; T-PLL = T-cell-prolymphocytic leukaemia. Numbers in brackets indicate number of cases when this is > 1. In addition, the patient with a testicular seminoma at an age of 27 years went on to develop a cerebral diffuse large B-cell lymphoma (DLBL) at an age of 34 years. One of the patients with a T-ALL at age of 15 years (kinase absent) also went on to develop a breast cancer at an age of 29 years. Of the 44 families with multiple cases of A-T, 7 families contained two A-T patients with a cancer diagnosis. For five of the families, the two cases were of the same tumour type (breast cancer, mixed-cellularity Hodgkin's lymphoma, Hodgkin's lymphoma, T-cell lymphoma and T-ALL, respectively). The other two families contained a T-PLL and a pancreatic cancer, and a myeloma and a myeloid leukaemia, respectively.

the generally milder phenotype typically associated with kinase-active mutations, as well as the lower cancer risk.

In the 24 A-T adults with a tumour, 11 were of lymphoid origin, 7 with the same spectrum as the paediatric lymphoid tumours (Table 2). The remaining five included four T-PLLs (diagnosed at ages 27, 32, 35 and 43 years) and a myeloma (48 years), all normally associated with old age in the general population. The 13 non-lymphoid adult tumours consisted of one pancreatic, two thyroid, one ectopic pituitary, a testicular seminoma, a myeloid tumour and seven breast cancers (Table 2). The breast cancers were diagnosed at ages 27, 32, 37, 43, 44, 44 and 50 years and were all diagnosed in women.

Given the existing evidence for an increased risk of breast cancer in female heterozygous ATM mutation carriers (Swift *et al*, 1991; Olsen *et al*, 2001; Thompson *et al*, 2005; Geoffroy-Perez *et al*, 2001; Renwick *et al*, 2006), we performed a separate analysis of



| Number at risk | 0 | 10 | 20 | 30 | 40 | 50 | 60 | 70 |
|--------------------|-----|----|----|----|----|----|----|----|
| No kinase activity | 187 | 63 | 6 | 0 | | | | |
| Kinase activity | 64 | 54 | 23 | 1 | | | | |

Figure 1 Kaplan–Meier survival to cancer diagnosis by ATM kinase activity. Shaded areas are 95% CIs. The solid line is for A-T patients with no ATM kinase activity, and the dashed line is for patients with some kinase activity. The values under the graph refer to the number of patients in each group at risk at the corresponding analysis time.

breast cancer risk in A-T patients (i.e., with biallelic ATM mutations) Using breast cancer diagnosis as the exit criteria, rather than diagnosis with any cancer, the cohort contained an eighth eligible breast cancer, diagnosed at the age of 29 years following a T-ALL at the age of 15 years. Overall, 0.26 breast cancers were expected in these 141 women (2963 person years) according to population rates (UK and the Netherlands, as appropriate, by 5-year age-group and ~5-year calendar period) (see Supplementary references for cancer in five continents). This gives a standardised mortality ratio of 31.1 (with a wide 95% CI = 14.4–74.7) equivalent to a cumulative risk of breast cancer diagnosis by the age of 50 years of 45% (95% CI = 24–76%), or 10-year risks of 13% (95% CI = 6–28%) between ages 30–39 years and 36% (95% CI = 19–66%) between ages 40–49 years.

DISCUSSION

We show here that development of childhood tumours (lymphoid and brain) in A-T patients is associated almost exclusively with absence of ATM kinase activity. Conversely our findings suggest that expression of some residual ATM kinase activity has a strongly protective effect against tumour development in A-T in childhood. The source of the residual ATM kinase activity was a low level of either normal ATM or mutant ATM. The origin of the retained kinase activity in 25 patients (median age, 29 years) was the presence of the IVS40-1050A>G splice site mutation (McConville *et al*, 1996; Stewart *et al*, 2001; Sutton *et al*, 2004) expressing a low level (~5%) of normal ATM. Despite the protection against tumour development provided by retained ATM kinase activity, two developed tumours, although significantly only in adulthood (one patient had a T-ALL at 17 years of age; the other patient had a testicular seminoma at an age of 27 years and a cerebral diffuse large B-cell lymphoma at an age of 34 years). This apparent contradiction may be reconciled if the level of ATM kinase activity in these IVS40-1050A>G-carrying patients is close to a threshold of ATM kinase activity that would normally be tumour suppressive.

Ataxia telangiectasia patients also clearly show a very substantially increased risk of breast cancer at young ages; the estimated

Table 3 Cancer incidence rates according to presence or absence of ATM kinase activity, for lymphoid and non-lymphoid tumours

| | No. of families | Person-years | Cancers | Lymphoid/ non-lymphoid | Incidence rate per 1000 person-years (95% CI) | | | Incidence rate ratio versus kinase absent patients (95% CI) | | |
|----------------|------------------------|--------------|---------|---------------------------|---|-----------------|---------------|---|------------------------------|------------------------------|
| | | | | | All cancer | Lymphoid | Non-lymphoid | All cancer | Lymphoid | Non-lymphoid |
| Kinase absent | 187 (156) | 3258 | 51 | 40/11 | 15.7 (11.7–20.6) | 12.3 (8.8–16.7) | 3.4 (1.7–6.0) | 0.40 (0.23–0.68) P = 0.001 | 0.22 (0.1–0.49) P < 0.001 | 1.05 (0.44–2.51) P = 0.91 |
| Kinase present | 64 (50) | 2165 | 14 | 6/8 | 6.5 (3.5–10.8) | 2.8 (1.0–6.0) | 3.7 (1.6–7.3) | | | |
| Kinase unknown | 45 (41) | 816 | 1 | 1/0 | 1.2 (0–6.8) | 1.2 (0–6.8) | n/a | | | |
| Total | 296 (246) ^a | 6239 | 66 | 47/19 | 10.6 (8.2–13.5) | 7.5 (5.5–10.0) | 3.0 (1.8–4.8) | | | |

Abbreviations: ATM = ataxia telangiectasia mutated; CI = confidence interval. ^aNumbers of families do not sum to 246 because 1 family contains a pair of siblings with ATM kinase activity and a second cousin with no activity. Incidence rate ratios are adjusted for country of residence.

Table 4 Cancer incidence rates according to presence or absence of ATM kinase activity, for lymphoid and non-lymphoid tumours, by age at diagnosis

| Age-group | ATM kinase status | Person-years | Cancers | Lymphoid/ non-lymphoid | Incidence rate per 1000 person-years (95% CI) | | | Incidence rate ratio vs kinase absent patients (95% CI) | | |
|------------|-------------------|--------------|---------|---------------------------|---|-----------------|----------------|---|------------------------------|------------------------------|
| | | | | | All cancer | Lymphoid | Non-lymphoid | All cancer | Lymphoid | Non-lymphoid |
| ≤ 16 years | Kinase absent | 2398 | 39 | 33/6 | 16.3 (11.6–22.2) | 13.8 (9.5–19.3) | 2.5 (0.9–5.4) | 0.12 (0.03–0.53) P = 0.005 | 0.15 (0.03–0.63) P = 0.01 | n/a |
| | Kinase present | 990 | 2 | 2/0 | 2.0 (0.2–7.3) | 2.0 (0.2–7.3) | n/a | | | |
| | Kinase unknown | 595 | 1 | 1/0 | 1.7 (0–9.4) | 1.7 (0–9.4) | n/a | | | |
| > 16 years | Total | 3983 | 42 | 36/6 | 10.5 (7.6–14.3) | 9.0 (6.3–12.5) | 1.5 (0.6–3.3) | 0.68 (0.32–1.47) P = 0.33 | 0.41 (0.12–1.34) P = 0.14 | 1.05 (0.36–3.09) P = 0.93 |
| | Kinase absent | 860 | 12 | 7/5 | 14.0 (7.2–24.4) | 8.1 (3.3–16.8) | 5.8 (1.9–13.6) | | | |
| | Kinase present | 1175 | 12 | 4/8 | 10.2 (5.3–17.8) | 3.4 (0.9–8.7) | 6.8 (2.9–13.4) | | | |
| | Total | 2256 | 24 | 11/13 | 10.6 (6.8–15.8) | 4.9 (2.4–8.7) | 5.8 (3.1–9.9) | | | |

Abbreviations: ATM = ataxia telangiectasia mutated; CI = confidence interval. Incidence rate ratios are adjusted for country of residence.

risk by the age of 50 years is 45%, which is higher than the equivalent risks, in unselected carriers of *BRCA1* or *BRCA2* mutations (31% and 20%, respectively) (Antoniou *et al*, 2008). In contrast, the breast cancer risk, by the age of 50 years, associated with a heterozygous *ATM* mutation, is ~9% (Thompson *et al*, 2005). Given the small number of adult female A-T patients, the 95% CI for this estimate is wide (24–76%), but even the lower bound would represent a very important level of risk. The eight cases included a pair of sisters who are homozygous for the c.7271T>G (p.Val2424Gly) mutation, and who have previously been reported as having breast cancer (Stankovic *et al*, 1998). This mutation has also been reported as being a breast cancer risk allele in its heterozygous form (Chenevix-Trench *et al*, 2002). Excluding these two sisters (the only 7271T>G carriers in this study), the estimated breast cancer risk by the age of 50 years was 39% (95% CI = 18–75%), hence, the high risk among A-T patients is not restricted to this mutation. This observation has important implications for the clinical management of women with AT.

Two unrelated breast cancer A-T patients were compound heterozygous for the p.Val2716Ala protein, which retains some ATM kinase activity, although greatly reduced compared with

normal (Verhagen *et al*, 2009). Interestingly, development of breast cancer, in A-T patients, may occur either in the total absence of ATM kinase activity (Supplementary Tables S1–S3) or in the presence of a low level of retained ATM activity (Supplementary Table S4), and it is the longer survival of A-T patients with some residual ATM kinase that allows this predisposition for breast cancer to manifest.

Overall, the protective effect of residual ATM kinase does not appear to extend to non-lymphoid tumours. The difference may be because the mechanism for development of these solid tumours is different to the translocation-driven lymphoid tumour.

ACKNOWLEDGEMENTS

We thank Cancer Research-UK (C1016/A7395) for continued support and also the Ataxia telangiectasia Society of the UK.

Supplementary Information accompanies the paper on British Journal of Cancer website (<http://www.nature.com/bjc>)

REFERENCES

- Angele S, Lauge A, Fernet M, Moullan N, Beauvais P, Couturier J, Stoppa-Lyonnet D, Hall J (2003) Phenotypic cellular characterization of an ataxia telangiectasia patient carrying a causal homozygous missense mutation. *Hum Mutat* **21**(2): 169–170
- Antoniou AC, Cunningham AP, Peto J, Evans DG, Lalloo F, Narod SA, Risch HA, Eyfjord JE, Hopper JL, Southey MC, Olsson H, Johannsson O,

- Borg A, Pasini B, Radice P, Manoukian S, Eccles DM, Tang N, Olah E, Anton-Culver H, Warner E, Lubinski J, Gronwald J, Gorski B, Tryggvadottir L, Syrjakoski K, Kallioniemi OP, Eerola H, Nevanlinna H, Pharoah PD, Easton DF (2008) The BOADICEA model of genetic susceptibility to breast and ovarian cancers: updates and extensions. *Br J Cancer* **98**(8): 1457–1466

- Austen B, Barone G, Reiman A, Byrd PJ, Baker C, Starczynski J, Nobbs MC, Murphy RP, Enright H, Chaila E, Quinn J, Stankovic T, Pratt G, Taylor AM (2008) Pathogenic ATM mutations occur rarely in a subset of multiple myeloma patients. *Br J Haematol* 142(6): 925–933
- Barone G, Groom A, Reiman A, Srinivasan V, Byrd PJ, Taylor AM (2009) Modeling ATM mutant proteins from missense changes confirms retained kinase activity. *Hum Mutat* 30(8): 1222–1230
- Bredemeyer AL, Huang CY, Walker LM, Bassing CH, Sleckman BP (2008) Aberrant V(D)J recombination in ataxia telangiectasia mutated-deficient lymphocytes is dependent on nonhomologous DNA end joining. *J Immunol* 181(4): 2620–2625
- Bredemeyer AL, Sharma GG, Huang CY, Helmink BA, Walker LM, Khor KC, Nuskey B, Sullivan KE, Pandita TK, Bassing CH, Sleckman BP (2006) ATM stabilizes DNA double-strand-break complexes during V(D)J recombination. *Nature* 442(7101): 466–470
- Chenevix-Trench G, Spurdle AB, Gatei M, Kelly H, Marsh A, Chen X, Donn K, Cummings M, Nyholt D, Jenkins MA, Scott C, Pupo GM, Dörk T, Bendix R, Kirk J, Tucker K, McCredie MR, Hopper JL, Sambrook J, Mann GJ, Khanna KK (2002) Dominant negative ATM mutations in breast cancer families. *J Natl Cancer Inst* 94(3): 205–215
- Geoffroy-Perez B, Janin N, Ossian K, Laugel A, Croquette MF, Griscelli C, Debre M, Bressac-de-Paillerets B, Aurias A, Stoppa-Lyonnet D, Andrieu N (2001) Cancer risk in heterozygotes for ataxia-telangiectasia. *Int J Cancer* 93(2): 288–293
- Greaves MF, Wiemels J (2003) Origins of chromosome translocations in childhood leukaemia. *Nat Rev Cancer* 3(9): 639–649
- Hewitt SL, Yin B, Ji Y, Chaumeil J, Marszalek K, Tenthorey J, Salvaggio G, Steinel N, Ramsey LB, Ghysdael J, Farrar MA, Sleckman BP, Schatz DG, Busslinger M, Bassing CH, Skok JA (2009) RAG-1 and ATM coordinate monoallelic recombination and nuclear positioning of immunoglobulin loci. *Nat Immunol* 10(6): 655–664
- Hiel JA, van Engelen BG, Weemaes CM, Broeks A, Verrips A, ter LH, Vingerhoets HM, van den Heuvel LP, Lammens M, Gabreels FJ, Last JJ, Taylor AM (2006) Distal spinal muscular atrophy as a major feature in adult-onset ataxia telangiectasia. *Neurology* 67(2): 346–349
- Huang CY, Sharma GG, Walker LM, Bassing CH, Pandita TK, Sleckman BP (2007) Defects in coding joint formation *in vivo* in developing ATM-deficient B and T lymphocytes. *J Exp Med* 204(6): 1371–1381
- McConville CM, Stankovic T, Byrd PJ, McGuire GM, Yao QY, Lennox GG, Taylor MR (1996) Mutations associated with variant phenotypes in ataxia-telangiectasia. *Am J Hum Genet* 59(2): 320–330
- Morrell D, Cromartie E, Swift M (1986) Mortality and cancer incidence in 263 patients with ataxia-telangiectasia. *J Natl Cancer Inst* 77(1): 89–92
- Olsen JH, Hahnemann JM, Borresen-Dale AL, Brondum-Nielsen K, Hammarstrom L, Kleinerman R, Kaariainen H, Lonnqvist T, Sankila R, Seersholm N, Tretli S, Yuen J, Boice Jr JD, Tucker M (2001) Cancer in patients with ataxia-telangiectasia and in their relatives in the Nordic countries. *J Natl Cancer Inst* 93(2): 121–127
- Renwick A, Thompson D, Seal S, Kelly P, Chagtai T, Ahmed M, North B, Jayatilake H, Barfoot R, Spanova K, McGuffog L, Evans DG, Eccles D, Easton DF, Stratton MR, Rahman N (2006) ATM mutations that cause ataxia-telangiectasia are breast cancer susceptibility alleles. *Nat Genet* 38(8): 873–875
- Savitsky K, Bar-Shira A, Gilad S, Rotman G, Ziv Y, Vanagaite L, Tagle DA, Smith S, Uziel T, Sfez S, Ashkenazi M, Pecker I, Frydman M, Harnik R, Patanjali SR, Simmons A, Clines GA, Sartiel A, Gatti RA, Chessa L, Sanal O, Lavin MF, Jaspers NG, Taylor AM, ARLETT CF, Miki T, Weissman SM, Lovett M, Collins FS, Shiloh Y (1995) A single ataxia telangiectasia gene with a product similar to PI-3 kinase. *Science* 268(5218): 1749–1753
- Spector BD, Filipovich AH, Perry GS, Kersey JH (1982) Epidemiology of cancer in ataxia telangiectasia. In *Ataxia Telangiectasia. A Cellular And Molecular Link Between Cancer, Neuropathology And Immune Deficiency* Bridges BA, Harnden DG (eds), pp 103–138. John Wiley and Sons: Chichester
- Stankovic T, Kidd AM, Sutcliffe A, McGuire GM, Robinson P, Weber P, Bedenham T, Bradwell AR, Easton DF, Lennox GG, Haites N, Byrd PJ, 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(2): 334–345
- Staples ER, McDermott EM, Reiman A, Byrd PJ, Ritchie S, Taylor AM, Davies EG (2008) Immunodeficiency in ataxia telangiectasia is correlated strongly with the presence of two null mutations in the ataxia telangiectasia mutated gene. *Clin Exp Immunol* 153(2): 214–220
- Stewart GS, Last JJ, Stankovic T, Haites N, Kidd AM, Byrd PJ, Taylor AM (2001) Residual ataxia telangiectasia mutated protein function in cells from ataxia telangiectasia patients, with 5762ins137 and 7271T->G mutations, showing a less severe phenotype. *J Biol Chem* 276(32): 30133–30141
- Sutton IJ, Last JJ, Ritchie SJ, Harrington HJ, Byrd PJ, Taylor AM (2004) Adult-onset ataxia telangiectasia due to ATM 5762ins137 mutation homozygosity. *Ann Neurol* 55(6): 891–895
- Swift M, Morrell D, Massey RB, Chase CL (1991) Incidence of cancer in 161 families affected by ataxia-telangiectasia. *N Engl J Med* 325(26): 1831–1836
- Taylor AM, Byrd PJ (2005) Molecular pathology of ataxia telangiectasia. *J Clin Pathol* 58(10): 1009–1015
- Taylor AM, Metcalfe JA, Thick J, Mak YF (1996) Leukemia and lymphoma in ataxia telangiectasia. *Blood* 87(2): 423–438
- Thompson D, Duedal S, Kirner J, McGuffog L, Last J, Reiman A, Byrd P, Taylor M, Easton DF (2005) Cancer risks and mortality in heterozygous ATM mutation carriers. *J Natl Cancer Inst* 97(11): 813–822
- Vacchio MS, Olaru A, Livak F, Hodes RJ (2007) ATM deficiency impairs thymocyte maturation because of defective resolution of T cell receptor alpha locus coding end breaks. *Proc Natl Acad Sci USA* 104(15): 6323–6328
- Verhagen MM, Abdo WF, Willemsen MA, Hogervorst FB, Smeets DF, Hiel JA, Brunt ER, van Rijn MA, Majoer KD, Oldenburg RA, Broeks A, Last JJ, van't Veer LJ, Tijssen MA, Dubois AM, Kremer HP, Weemaes CM, Taylor AM, van DM (2009) Clinical spectrum of ataxia-telangiectasia in adulthood. *Neurology* 73(6): 430–437



This work is licensed under the Creative Commons Attribution-NonCommercial-Share Alike 3.0 Unported License. To view a copy of this license, visit <http://creativecommons.org/licenses/by-nc-sa/3.0/>