ORIGINAL ARTICLE

Genotype–phenotype correlations in ataxia telangiectasia patients with *ATM* c.3576G>A and c.8147T>C mutations

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ABSTRACT

Background Ataxia telangiectasia (A-T) is a neurodegenerative disorder. While patients with classic A-T generally die in their 20s, some patients with variant A-T, who have residual ataxia-telangiectasia mutated (ATM) kinase activity, have a milder phenotype. We noticed two commonly occurring *ATM* mutations that appeared to be associated with prolonged survival and decided to study patients carrying one of these mutations.

Methods Data were retrospectively collected from the Dutch, Italian, German and French A-T cohorts. To supplement these data, we searched the literature for patients with identical genotypes.

Results This study included 35 patients who were homozygous or compound heterozygous for the *ATM* c.3576G>A; p.(Ser1135_Lys1192del58) mutation and 24 patients who were compound heterozygous for the *ATM* c.8147T>C; p.(Val2716Ala) mutation. Compared with 51 patients with classic A-T from the Dutch cohort, patients with *ATM* c.3576G>A had a longer survival and were less likely to develop cancer, respiratory disease or immunodeficiency. This was also true for patients with *ATM* c.8147T>C, who additionally became wheelchair users later in life and had fewer telangiectasias. The oldest patient with A-T reported so far was a 78-year-old patient who was compound heterozygous for *ATM* c.8147T>C. ATM kinase activity was demonstrated in cells from all patients tested with the *ATM* c.8147T>C mutant protein and only at a low level in some patients with *ATM* c.3576G>A.

Conclusion Compared with classic A-T, the presence of *ATM* c.3576G>A results in a milder classic phenotype. Patients with *ATM* c.8147T>C have a variant phenotype with prolonged survival, which in exceptional cases may approach a near-normal lifespan.

INTRODUCTION

Ataxia telangiectasia (A-T; OMIM 208900) is an autosomal recessive neurodegenerative disease caused by biallelic mutations in the ataxia-telangiectasia mutated (*ATM*) gene (OMIM 607585), which encodes the ATM kinase enzyme.[1] ATM kinase plays a role in numerous cellular processes such as cell cycle control and DNA repair and is activated by DNA double strand breaks.[2] The classic phenotype includes childhood-onset cerebellar ataxia with extrapyramidal movement disorders, oculocutaneous telangiectasias, immunodeficiency with recurrent infections, pulmonary dysfunction, increased sensitivity to ionizing radiation, increased serum alpha-fetoprotein (AFP) levels and high risk of malignancies. Patients with classic A-T usually become wheelchair users around the age of 10 years and die in the second or third decade of life due to a malignancy or respiratory failure.[3 4]

Besides this classic phenotype, milder so-called variant phenotypes exist. The disease course in such patients is characterised by a later-onset and slower progression of milder and predominantly extrapyramidal (instead of cerebellar) motor abnormalities.[5] Respiratory disease and immunodeficiency are not evident in variant A-T, although these patients still have an increased cancer risk. Their lifespans are much longer compared to patients with the classic phenotype.[4 5] Variant A-T is frequently associated either with missense or leaky splice site mutations that allow for some ATM protein with residual ATM kinase activity to be formed.[6–9] Apart from residual ATM kinase activity, other factors, such as modifying genes and environmental factors, are suggested to play a role in the mechanisms that lead to milder phenotypes of A-T.[10]

A-T is a rare disorder with an estimated incidence of circa 3:1.000.000.[11 12] Hundreds of different mutations of the *ATM* gene have been recorded that lead to A-T.[13] Although founder mutations have been reported, true mutational hotspots have never been recognised in the *ATM* gene, and many patients seem to have 'private' mutations. All of these factors together hamper the recognition of potential genotype–phenotype correlations. Phenotype prediction based on patient genotype, however, would be very welcome given the wide disease spectrum of A-T and may become even more important since whole exome sequencing and neonatal screening for primary immunodeficiencies may increase the number of—preclinical—patients that will be diagnosed.[14]

Here we compare the phenotypes of patients with A-T who are compound heterozygous or homozygous for one of two pathogenic *ATM* mutations, c.3576G>A and c.8147T>C, instigated by

the clinical impression that both groups of patients appeared to have a milder disease course.

METHODS

Ascertainment of Dutch cohort

Two *ATM* mutations that are prevalent in the Dutch A-T cohort were studied: the c.3576G>A; p.(Ser1135_Lys1192del58) splice site mutation in exon 24 and the c.8147T>C; p.(Val2716Ala) missense mutation in exon 55. This study arose from the observation of an unexpected clinically milder phenotype in A-T patients with these mutations.

First, Dutch patients who were compound heterozygous or homozygous for one of these two *ATM* mutations were included. Clinical, laboratory and genetic data were retrospectively collected from our database and from patient medical records at Radboud University Medical Center, Nijmegen, the Netherlands. Second, we collected corresponding data on patients with classic A-T (who did not have any of the two mutations mentioned above) from the Dutch cohort. The classic A-T phenotype was defined as described in the introduction part of this paper, with absence of ATM kinase activity. We finally compared the clinical and laboratory characteristics of patients with the *ATM* c.3576G>A or c.8147T>C mutations to the patients with classic A-T.

ATM gene mutation analysis and measurement of ATM protein expression and ATM kinase activity in lymphoblastoid cell lines were performed using methods described previously.[15 16] In families in which only one patient was studied at the protein level, we assumed the same results for siblings.[6] All patients have been reported in previous papers,[4–6 17 18] except for one with *ATM* c.3576G>A, and 10 patients who were included in the classic A-T reference group. In this study, the follow-up period was extended until 1 April 2018.

Literature search and ascertainment of Italian, German and French patients

To expand the data from the Dutch cohort, we searched the literature (PubMed) and the *ATM* database of the Leiden Open Variation Database (LOVD)[13] for patients with A-T who were compound heterozygous or homozygous for one of the two *ATM* mutations mentioned above. In PubMed, we combined the search terms 'ataxia telangiectasia', '*ATM* gene' and 'mutations'. Additional articles were retrieved by hand-searching through reference lists.

For patients of whom clinical data were incomplete or missing,[19–24] authors were contacted for additional information. This led to a collaboration with our Italian (LC), German (TD, CS and DS) and French (AF and NM) coauthors, enabling a substantial extension of data on previously described patients[19–29] and inclusion of novel data on hitherto unpublished patients.

ATM gene mutation analysis and measurement of ATM protein expression and ATM kinase activity had been performed using methods previously described in the Italian,[25 30] German[28 31] and French[29] cohorts. In patients 55.F2, 55.F3a, and 55.F6a, 1 hour treatment with 1 µM camptothecin (Sigma-Aldrich) was performed instead of ionising radiation for phospho-KAP1 activation by western blot. The latter was revealed using Odyssey blocking buffer (LI-COR #927–40000), appropriate fluorescent secondary antibody and LI-COR-Odyssey infrared scanner (LI-COR).

Data extraction and analysis

In addition to the genotype, the following data were collected for all patients: gender, age at 1 April 2018 or age of death, cause of death, age of onset, presenting feature, age of being wheelchair users or age of loss of independent walking, presence of respiratory disease (ie, recurrent infections or pulmonary dysfunction measured with spirometry), presence of telangiectasias, serum AFP and immunoglobulin levels (IgA, IgM, IgG and subclasses), expression of ATM protein and presence of residual ATM kinase activity. *ATM* mutations were designated according to the LOVD,[13] and the revised *ATM* exon numbering system was used. Serum AFP levels were considered elevated if the level exceeded the age-related reference values of our laboratory (ie, >10 µg/L) or in case this was stated in the original paper. Serum immunoglobulin levels were compared with age-related reference values[32] and were classified as deficient, low, normal or high. Deficiency was defined as serum IgA levels below 7 mg/dL, IgG levels below 250 mg/dL, IgG_2 levels below 50 mg/dL and IgM levels below 10–15 mg/dL (children) and 20–30 mg/dL (adults).[32]

Statistical analysis

Because of non normal distribution, medians with ranges are presented in table 1. All comparisons were made for the group with the *ATM* c.3576G>A mutation versus classic A-T, or the group with the *ATM* c.8147T>C mutation versus classic A-T. Differences in the categorical variables (ie, sex, specific cause of death and presence of malignancy, telangiectasias, respiratory disease, immunodeficiency, increased serum AFP level, ATM protein expression and ATM kinase activity) were assessed using the Fisher's exact test. For differences in the follow-up duration and median (age-related) serum AFP levels between groups, the Mann-Whitney-Wilcoxon test was used. Kaplan-Meier survival curves were constructed and differences in the time dependent variables (ie, survival, median age at time of death, age at time of onset, age at time of wheelchair users/loss of autonomous walking and age at time of malignancy) were established using the log-rank rest, including only patients who had experienced the event by the end of follow-up or censoring patients who were alive at the end of follow-up in case of survival. Cox's proportional hazards analyses were performed to calculate HRs with 95% CIs for these variables, censoring patients without the event at the end of follow-up. The statistical analysis was done using SPSS V.22 for Windows. A p value below 0.05 was considered statistically significant.

RESULTS

Details of the patients in the study cohort are described in online supplementary table 1 for the *ATM* c.3576G>A mutation and in online supplementary table 2 for the *ATM* c.8147T>C mutation. The Dutch A-T cohort included four patients with the *ATM* c.3576G>A mutation and five patients with the *ATM* c.8147T>C mutation. In the Italian cohort, 20 patients with the *ATM* c.3576G>A mutation were available.[21–23 25 26] The German cohort comprised five patients with the *ATM* c.3576G>A mutation[28] and two patients with the *ATM* c.8147T>C mutation.[19 27] The French cohort included two patients with the *ATM* c.3576G>A mutation[24 29] and 11 patients with the *ATM* c.8147T>C mutation.[24 33] The literature contained four additional patients with the *ATM* c.3576G>A mutation[27] and six patients with the *ATM* c.8147T>C mutation.[27 34 35] Altogether 59 patients with A-T from 41 families were included in this study (24 men, 35 women, age range: 2–78 years). Follow-up

Table 1 Characteristics of patients with the *ATM* c.3576G>A and c.8147T>C mutations, compared with patients with classic A-T

	Classic A-T (n=51)	c.3576G>A (n=35)		c.8147T>C (n-=24)	
	Frequency	Frequency	P value or 95% CI	Frequency	P value or 95% CI
Characteristics					
Number of patients					
Dutch cohort	51	4		5	
Italian cohort	0	20		0	
German cohort	0	5		2	
French cohort	0	2		11	
Literature		4		6	
Mutations*					
Homozygous	13	18	0.011†	0	0.007†
Compound heterozygous	38	15		24	
Sex					
Male	27	17	0.827†	7	0.081†
Female	24	18		17	
Status					
Deceased	30	11	0.016†	2	<0.001†
Alive	21	24		22	
Median follow-up duration *(years)*	14	27	0.001‡	36	0.001‡
	Range 4–54	Range 2–56		Range 2–78	
Estimated survival *(years)*	18	46	<0.001§	¶	<0.001§
Death					
Number of deaths*	30/51	9/33	0.007†	2/ 24	<0.001†
Cause of death known*	27	9		2	
Malignancy	12	3	0.705†	2	0.224†
Respiratory failure	9	5	0.267†	0	1.000†
Both	3	0	0.558†	0	1.000†
Other	3	1	1.000†	0	1.000†
Median age at time of death *(years)*	15	33	0.098§	¶	0.870§
	Range 4–54	Range 13– 50		Range 7–47	
HR for death	Ref	HR 0.25	0.12 0.52**	HR 0.07	0.02- 0.29**
Onset of first neurological symptoms					
Number of patients*	50/51	31/32	0.153†	21/24	0.094†
Median age at time of onset of neurological symptoms *(months)*	17 Range 4–60	24 Range 8–156	0.001§	24 Range 6–384	<0.001§
HR for onset	ref	HR 0.48	0.30–0.78**	HR 0.32	0.17- 0.61**
Wheelchair use / loss of autonomous walking					
Number of patients*	31/40	18/18	0.045†	7/17	0.013†
Median age at time of wheelchair use/loss of autonomous walking *(years)*	10 Range 7–18	10 Range 4–23	0.223§	35 Range 9–63	<0.001§
HR for wheelchair use/loss of autonomous walking	ref	HR 0.75	0.39–1.46**	HR 0.03	0.00- 0.21**
Malignancy					
Number of patients	20/51	7/35	0.097†	6/24	0.301†
Median age at diagnosis of malignancy *(years)*	12 Range 4–52	16 Range 6–33	§0.807§	32 Range 7–42	0.120§
HR for malignancy	Ref	HR 0.33	0.14–0.78**	HR 0.29	0.11- 0.73**
Telangiectasias*					
Number of patients	46/49	32/34	1.000†	8/22	<0.001†
Respiratory disease* (including recurrent infections)					
Number of patients	42/49	10/27	<0.001†	1/21	<0.001†
Immunodeficiency *					
Number of patients	41/50	6/32	<0.001†	3/21	<0.001†

Continued

Table 1 Continued

	Classic A-T (n=51)	c.3576G>A (n=35)		c.8147T>C (n-=24)	
	Frequency	Frequency	P value or 95% CI	Frequency	P value or 95% CI
Increased serum AFP level *					
Number of patients	45/46	23/24	1.000†	19/19	1.000†
Median AFP level (µg/L)	240	52	<0.001‡	172	0.029‡
	Range 3–660	Range 10–145		Range 29–400	
Expression of ATM protein *					
Number of patients	12/30	13/19	0.079†	11/11	0.001†
ATM kinase activity *					
Number of patients	0/30	3/9	0.009†	11/11	<0.001†

*For some patients, values were missing: those patients were excluded from statistical analysis.
†Fisher's exact test.
‡Mann-Whitney-Wilcoxon.
§Log-rank test.
¶Could not be calculated because of small numbers of deceased patients.
**Cox regression analysis.
A-T, ataxia telangiectasia; AFP, alpha-fetoprotein; ATM, ataxia-telangiectasia mutated.

started at birth for all patients, despite the diagnosis being established several months or years later. Forty-six patients were alive at the end of follow-up or at the time of publication in the literature. A general summary of the characteristics of the patients per mutation, compared with 51 Dutch patients with classic A-T, are shown in figures 1, 2; table 2, figure 3 and table 1.

c.3576G>A; p.(Ser1135_Lys1192del58) splice site mutation (deletion of exon 24)

A total of 35 patients with this *ATM* mutation were included. Gender distribution was equal. Eighteen patients were homozygous for the *ATM* c.3576G>A mutation and 15 were compound heterozygous. The majority of patients were of Mediterranean origin: 21 were Italian, 8 Turkish, 2 Georgian and 2 Bulgarian. The remaining two patients were of German and Belgian origin. The median follow-up period of this group was 27 (range 2–56) years, with the oldest patient being 56 years at the end of follow-up (24.It12). During the follow-up period, 11 patients died (of which two with an unknown age of death) with a median age at time of death of 33 (range 13–50) years. Only five patients died before age 30 years, while 15 patients survived beyond this age. The other patients were under 30 years and still alive at the end of follow-up. The estimated survival was 46 years. Compared with classic A-T, patients with the *ATM* c.3576G>A mutation had a better survival (HR 0.25 [95% CI 0.12 to 0.52]) (figure 2) (see online supplementary table 1).

The onset of neurological symptoms was a little later in life than in classic A-T (with a median of 24 [range 8–156] months vs

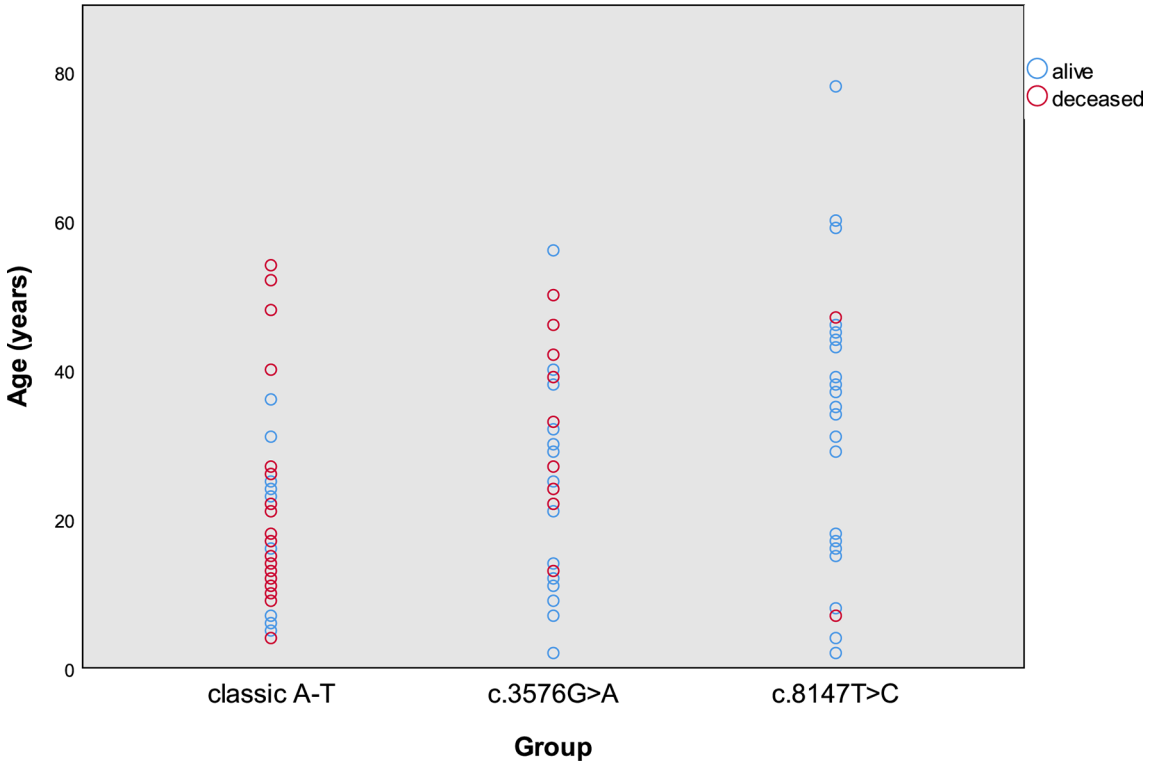

Figure 1 Scatter plot of patients with classic A-T and A-T patients with *ATM* c.3576G>A and c.8147T>C mutations. A-T, ataxia telangiectasia.

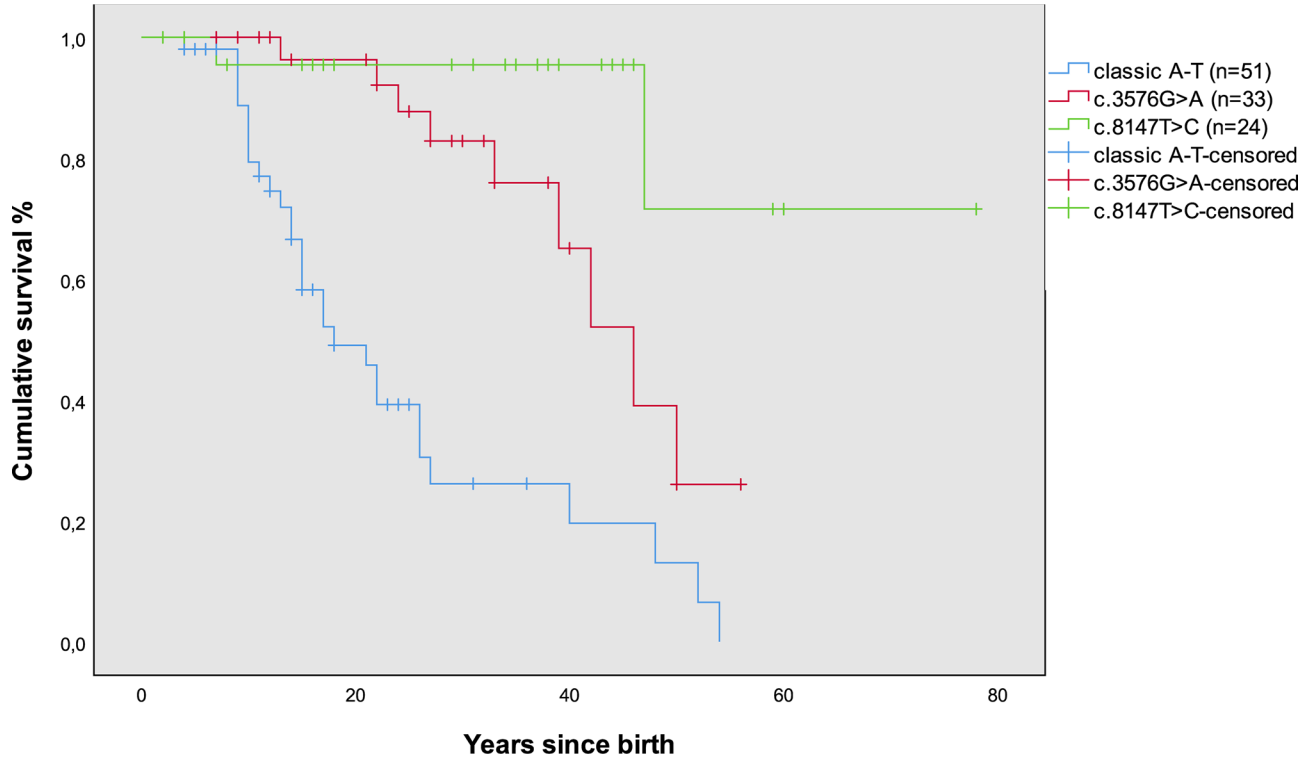

Figure 2 Survival of patients with classic A-T and A-T patients with *ATM* c.3576G>A and c.8147T>C mutations. A-T, ataxia telangiectasia.

Table 2 Additional table to figure 2

	Years	0	10	20	30	40	50	60	70	80
Classic	At risk	51	34	15	6	3	2	0		
	Deceased	0	9	20	26	27	28	30		
	Censored†	0	8	16	19	21	21	21		
c.3576G>A*	At risk	33	29	25	13	6	1	0		
	Deceased	0	0	1	4	5	9	9		
	Censored†	0	4	7	16	22	23	24		
c.8147T>C	At risk	24	20	16	15	8	3	1	1	0
	Deceased	0	1	1	1	1	2	2	2	2
	Censored†	0	3	7	8	15	19	21	21	22

*Two patients with the ATM c.3576G>A mutation (24.It6c-d) were excluded from this analysis.
†Censored patients are patients that were alive in this age category at the end of follow-up. Differences in number of patients at risk are a result of deceased and censored patients. Deceased and censored numbers are cumulative.

17 [range 4–60] months [p=0.001] and an HR for onset of 0.48 [95% CI 0.30 to 0.78]). Loss of independent walking occurred at the same time as patients with classic A-T (median 10 years for both groups, HR for wheelchair 0.75, 95% CI 0.39 to 1.46). Malignancies occurred less frequently in patients with the *ATM* c.3576G>A mutation compared with patients with classic A-T (7/35 vs 20/51 patients, HR for malignancy 0.33, 95% CI 0.14 to 0.78), but no difference was observed in age of diagnosis of malignancy between these two groups (median 16 years vs 12 years). Respiratory disease and immunodeficiency were less common among patients with the *ATM* c.3576G>A mutation (both p=<0.001). Serum AFP levels were lower in patients with *ATM* c.3576G>A mutations compared with patients with classic A-T (p=<0.001) (figure 3).

Expression of ATM protein was assayed in 19 patients with the *ATM* c.3576G>A mutation and identified in 13 of them slightly more that in the group of patients with classic A-T (p=0.079).

Some very low ATM kinase activity was described for three homozygous patients from the literature,[27] but we could not find measurable ATM kinase activity in three homozygous patients from the Dutch cohort (analysed before 2013).[6] For patient 24.D1, ATM kinase activity assays were repeated in February 2018, again showing absence of ATM activity (figure 4).

c.8147T>C; p.(Val2716Ala) missense mutation (exon 55)

The *ATM* c.8147T>C mutation was detected in 24 patients who were all compound heterozygous for this mutation. The majority of patients were women (70.1%, p=0.081) and of Western European origin; the median follow-up period was 36 (range 7–78) years. Only two patients were deceased at the end of follow-up (at 7 years and 47 years, HR for death 0.07, 95% CI 0.02 to 0.29) (figure 2), so median survival and age at time of death could not be calculated. Both died from a malignancy, one

Figure 3 Scatter plot of serum AFP levels of patients with classic A-T and A-T patients with *ATM* c.3576G>A and c.8147T>C mutations (every patient with known serum AFP level is displayed only once). A-T, ataxia telangiectasia; AFP, alpha-fetoprotein.

of them at the age of 7 years. Fifteen patients survived beyond 30 years of age. The oldest patient was 78 years old at last visit (55.G1) (see online supplementary table 2).

The median age at time of onset of first neurological symptoms was much later compared with patients with classic A-T (24 [range 6–384] months; p=<0.001), while the HR for onset was 0.32 (95% CI 0.17 to 0.61). Only seven patients were wheelchair users at a median age of 35 years, whereas 10 patients were still able to walk at the end of follow-up. Six patients developed a malignancy (HR for malignancy 0.29, 95% CI 0.11 to 0.73); five of them had breast cancer. The median age at time of onset of the malignancy was 32 (range 7–42) years compared with 12 (range 4–52) years among patients with classic A-T (p=0.120). Patients with the *ATM* c.8147T>C mutation less often had telangiectasias, respiratory disease or immunodeficiency, compared with patients with classic A-T (all p=<0.001). Serum AFP levels were increased in all patients with this mutation and lower compared with patients with classic A-T (p=0.029).

ATM protein expression and ATM kinase activity were studied in 11 patients and found to be present in all, in contrast to the results in patients with classic A-T (p=0.001 and p=<0.001, respectively).

DISCUSSION

Compared with patients with classic A-T, patients with one or two *ATM* c.3576G>A mutations in our study generally have a longer survival and are less likely to develop a malignancy, respiratory disease and immunodeficiency. Presence of an *ATM* c.8147T>C mutation in variant A-T patients appears to be correlated with longer survival, a much later median age at time of onset and wheelchair and with a reduced likelihood to develop a malignancy, telangiectasias, respiratory disease and immunodeficiency.

c.3576G>A; p.(Ser1135_Lys1192del58) splice site mutation

Although A-T patients with the *ATM* c.3576G>A mutation had a statistically significantly later age of onset of first neurological symptoms compared with patients with classic A-T, the difference of 7 months is unlikely to be clinically relevant. Since there is no difference in age at becoming wheelchair users either, we believe the neurological phenotype of patients with this particular mutation does not differ from the classic A-T phenotype. However, the non-neurological characteristics in this group are milder compared with patients with classic A-T. This would imply that in the clinical spectrum of A-T, patients with *ATM* c.3576G>A mutations are phenotypically between 'classic' and 'variant' A-T and could be called 'mild classic' (figure 5).

Patients with the *ATM* c.3576G>A mutation are less likely to develop a malignancy, but when they do it is at the similarly young age as in patients with classic A-T.

Most patients with the *ATM* c.3576G>A mutation were of Mediterranean origin, and a founder effect has been confirmed by haplotype analyses in Turkish and Italian patients.[16 25 36 37]

The reason for the milder phenotype in these *ATM* c.3576G>A patients is unclear. If the c.3576G>A allele itself was indeed important, then perhaps patients homozygous for the *ATM* c.3576G>A mutation would be milder compared with compound heterozygous patients. However, this seems not true since some of the longest survivors (24.It6a-b, and 24.It7 and 24.It12) were compound heterozygous and not homozygous for the *ATM* c.3576G>A mutation and have a truncating second mutation. Indeed almost all compound heterozygous patients with the *ATM* c.3576G>A mutation had a second null mutation (ie, frameshift and nonsense). Therefore, it is likely that other factors, such as modifying genes or environmental factors, play a role in the course of the disease in these patients.

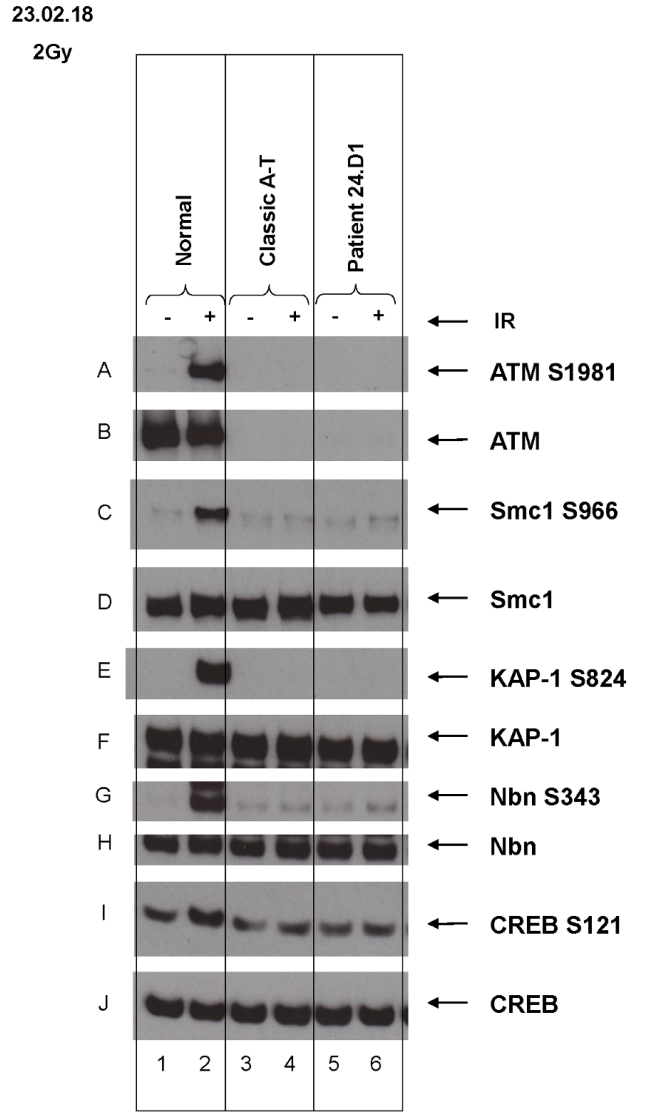

Figure 4 Absence of ATM activity/signalling in cells from patient 24.D1 who is homozygous for the *ATM* c.3576G>A splice site mutation. *ATM*, ataxia-telangiectasia mutated.

ATM protein levels could be detected by western blotting in all but six patients. Three were compound heterozygous and three were homozygous for the *ATM* c.3576G>A mutation. However, two other compound heterozygous patients with a second truncating mutation did have expression of ATM protein, but the expression level was very low. In the compound heterozygous patient who had a higher ATM expression level,[29] the second mutation was a missense mutation that would account for this increase. ATM protein expression was absent in the patient that has survived the longest so far (24.It12). Altogether no clear correlation was observed between the ATM protein expression level and the clinical phenotype in patients with the *ATM* c.3576G>A mutation.

Figure 5 *ATM* c.3576G>A and c.8147T>C mutations in the spectrum of A-T. A-T, ataxia telangiectasia.

In the paper by Demuth *et al*, three homozygous patients with the *ATM* c.3576G>A mutation were suggested to have very low ATM kinase activity, since their cell lines responded to irradiation and to treatment with bleomycin by increased phosphorylation of p53 serine 15, accompanied by p53 stabilisation in western blot anayses.[27] Possibly, some leakiness of the mutation caused measurable expression of ATM kinase activity in the cells from these homozygous patients. In contrast, in six other homozygous patients with presence of ATM protein who were tested, no ATM kinase activity could be detected. When we retrospectively looked at the blots from the homozygous Dutch patients, some variation in signal was seen, but this was not consistent enough to be sure that there was any authentic residual ATM kinase activity. A more sensitive ATM kinase/signalling assay is needed to investigate this further as we could be at the very limit of detection of activity with the current assays. Alternatively, final assessment of this mutation may require analysis in cells different from lymphoblastoid cell lines, as splicing is tissue specific. The three patients with detectable residual ATM kinase activity did not clearly show a milder clinical phenotype compared with other homozygous *ATM* c.3576G>A patients without detectable residual ATM kinase activity.

While evidence suggests that the *ATM* c.3576G>A mutation is associated with a milder clinical phenotype, it is not clear how. It may be that the mutation is leaky, and detection of ATM activity is inefficient or that these patients also carry other modifying genes contributing to the milder phenotype.

C.8147T>C; p.(Val2716Ala) missense mutation

The *ATM* c.8147T>C mutation is a pathogenic missense mutation that so far has been described in a compound heterozygous A-T state only. The Val2716Ala mutation is located in the N-terminal part of the PI-3 kinase domain adjacent to Lys2717 which, together with Asp2720, His2872, Asp2870, Asn2875 and Asp2889, is predicted to bind ATP or the essential Mg+ion. A mutation in any of these will probably result in loss of ATM activity.[38 39] Occasional mutant amino acids (such as Phe2827Cys and Arg2832Cys) in the ATM PI-3 kinase-like domain, however, as well as Leu3035Phe in the FATC domain cause reduction in the level of ATM activity/signalling but not its abolition.[7] The Val2716Ala mutation also results in a reduction of ATM activity and is being a conservative change may mean that its effect on adjacent Lys2717 ATP binding is not sufficient to result in abolition of all activity.

This mutation is suggested to be a Dutch founder mutation.[5] Homozygosity for this mutation has not been described yet, suggesting that homozygosity for this mutation may be minimally disease causing.

Interestingly, respiratory failure was not a cause of death in either patient with the *ATM* c.8147T>C mutation, in contrast to 44.4% and 55.6% of known deaths due to lung disease in the groups with patients with classic A-T and patients with the *ATM* c.3576G>A mutation, respectively. One patient died at the age of 7 years from a malignancy of unspecified origin[27] and seems to be an exceptional case within this group. She had no immunodeficiency, no recurrent infections and only a mild neurological phenotype with ataxia disappearing at the age of 6 years. Ataxia disappeared at 6 years of age in two other patients described by Demuth *et al*.[27] We could not find other descriptions of this phenomenon in A-T. Three French patients with the *ATM*

c.8147T>C mutation had an immunodeficiency,[34] but only one of them had recurrent infections.

The prevalence of lymphoid malignancies in patients with the *ATM* c.8147T>C mutation was low in contrast to patients with classic A-T,[40] consistent with absence of immunodeficiency in them. Five of six patients with a malignancy developed breast cancer, which is a prevalent type of cancer in A-T patients with residual ATM kinase activity.[40]

The present study includes one German patient who was still alive at the age of 78 years, an exceptionally advanced age, even for variant A-T, and the oldest patient reported to date. This patient had a combination of *ATM* c.8147T>C with a frameshift *ATM* mutation in the last exon where there is a possibility of expression of a slightly truncated protein; whether or not this contributed to her milder phenotype is not known (online supplementary figure S1). Since the clinical phenotype in compound heterozygous patients with the *ATM* c.8147T>C mutation is relatively mild, the phenotype of any patient with A-T homozygous for this mutation might be even milder. Identification of such patients may possibly broaden the clinical spectrum of A-T to hitherto unrecognised phenotypes, such as pure and simple neurological disorders, for example, 'torticollis only' or 'peripheral neuropathy only', or a cancer predisposition syndrome with intolerance to radiation therapy.

The fact that serum AFP levels were elevated in almost all patients with the *ATM* c.3576G>A and c.8147T>C mutations confirms that serum AFP assessment is a reliable first screening tool for patients in whom A-T is suspected. AFP levels were lower in patients with both mutations compared with classic A-T, even with the relatively large number of young patients with classic A-T in this cohort, while it is well known that AFP levels increase with age in A-T.[41] In separate analyses for patients <20 years and ≥20 years of age, the statistically significantly differences in serum AFP levels between classic A-T and patients with both *ATM* mutations did not change.

Strengths and limitations

For the comparisons between A-T patients with the *ATM* c.8147T>C mutation and patients with classic A-T, we did not include patients with the *ATM* c.3576G>A mutation in the classic A-T group. This may suggest that the patients with classic A-T are a selected group. Therefore, we conducted separate analyses where we added the patients with the *ATM* c.3576G>A mutation to the classic A-T group. This did not lead to substantial differences in the results, except for the HR of malignancy (HR 0.48, 95% CI 0.20 to 1.18) and for the difference in median AFP level (p=0.662, additional data available on request).

This study is the first to provide an overview of all published and well-documented patients with the *ATM* c.3576G>A and c.8147T>C mutations, and it shows that these mutations are relatively common in A-T. Furthermore, this study shows that international collaborations are necessary in order to gain sufficient evidence for what is expected based on clinical observations in small numbers of cases. Besides genetic databases such as the LOVD database, an international clinical database for rare disorders such as A-T would simplify this kind of study. Increasing insights in genotype–phenotype correlations will hopefully lead to better understanding of the pathophysiological mechanisms that underlie A-T. In addition, ascertainment bias could be prevented in this manner, since unpublished cases would not be missed.

CONCLUSION

This study shows that classic A-T patients with the *ATM* c.3576G>A mutation had a milder clinical phenotype in terms of prolonged survival and lower susceptibility to the development of malignancies, respiratory disease and immunodeficiency. The underlying reason for this remains unsolved; possibly very low levels of ATM activity or the effect of unidentified modifying genes may be important. They contrast with variant A-T patients carrying the c.8147T>C missense mutation who additionally showed a later onset and slower progression of neurological symptoms. This phenotype is associated with a clear cellular retention of ATM kinase activity. In the era of next-generation sequencing, one may expect that very early (or even preclinical) diagnosis of A-T will occur in the near future. Therefore, reliable phenotype prediction may become increasingly important, particularly when it deviates positively from the classic A-T phenotype .

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