Residual Ataxia Telangiectasia Mutated Protein Function in Cells from Ataxia Telangiectasia Patients, with 5762ins137 and 7271T→G Mutations, Showing a Less Severe Phenotype*

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Grant S. Stewart, James I. K. Last, Tatjara Stankovic, Neva Haites‡, Alexa M. J. Kidd‡, Philip J. Byrd, and A. Malcolm R. Taylor§

From the CRC Institute for Cancer Studies, the University of Birmingham, Vincent Drive, Edgbaston, Birmingham B15 2TT and the ‡Department of Medical Genetics, Grampian University Hospitals, Aberdeen AB25 2ZD, United Kingdom

We have assessed several ataxia Telangiectasia mutated (ATM)-dependent functions in cells derived from ataxia telangiectasia patients, carrying either an ATM 5762ins137 splice site or a 7271T→G missense mutation, with a less severe phenotype compared with the classical disorder. ATM kinase *in vitro*, from 5762ins137 cells, showed the same specific activity as ATM in normal cells, but the protein was present at low levels. In contrast, mutant ATM kinase activity in the 7271T→G cells was only about 6% that of the activity in normal cells, although the level of mutant protein expressed was similar to normal cells. Phosphorylation of the DNA double strand break repair proteins Nbs1 and hMre11, following DNA damage, was observed in normal and 7271T→G cells but was almost absent in both 5762ins137 and classical ataxia telangiectasia cells. The kinetics of p53 response was intermediate between normal and classical ataxia telangiectasia cells in both the 7271T→G and 5762ins137 cells, but interestingly, c-Jun kinase activation following DNA damage was equally deficient in cell lines derived from all the ataxia telangiectasia patients. Our results indicate that levels of ATM kinase activity, but not induction of p53 or c-Jun kinase activity, in these cells correlate with the degree of neurological disorder in the patients.

Ataxia telangiectasia (A-T) is a human autosomal recessive disorder in which affected individuals exhibit a diverse range of clinical symptoms affecting multiple organ systems (1). The principal manifestation of A-T presents as a progressive truncal and limb ataxia as a consequence of degeneration of the cerebellum. This ultimately results in the patients being wheelchair-bound from the early teenage years. Other clinical signs of A-T are the presence of oculocutaneous telangiectasia, selective immunodeficiency, an increased sensitivity to ionizing radiation (3–5).

Across all A-T patients there is a degree of variation between them in both the severity and expression of the clinical features. In particular, the degree of telangiectasia, the extent of immunodeficiency, the susceptibility to recurrent sinopulmonary infections, longevity, the rate of neuronal degeneration, and the development of tumors show the greatest degree of heterogeneity among patients. In some cases this variation presents as a clearly milder form of A-T. These individuals have a combination of the following: a slightly later age of onset of the clinical symptoms, a slower rate of disease progression, an extended life span, when compared with most classical A-T patients, and a reduced or absent hypersensitivity to ionizing radiation (3–5).

Given the predominance of null mutations underlying the classical form of ataxia telangiectasia (6), it is highly likely that milder, “variant” forms arise from less severe mutations that retain some normal protein function. In support of this, we have previously reported two different ATM gene mutations that are specifically associated with a milder clinical syndrome (3, 4). The first mutation, present in the heterozygous state in 15% of A-T families in the United Kingdom, is an intronic missense mutation that activates a cryptic splice donor/acceptor site resulting in the insertion of 137 nucleotides of intronic sequence at position 5762. It appears, however, that this mutation is “leaky” and also allows normal splicing to occur, albeit at a reduced level. It was proposed that the presence of some normal ATM protein was sufficient to reduce the severity of the disease (4). In a similar context, a leaky splice mutation (3576G→A) was identified in four Italian A-T patients with a milder phenotype (5), which again allows residual amounts of normally spliced ATM transcript.

In contrast, the second ATM mutation we reported, associated with a mild variant A-T phenotype (3), is a T to G transition at position 7271, which has so far been found in three A-T families, and homozygous in one, in the UK. In this case the genotype-phenotype relationship is thought to result from some normal activity retained by the mutant ATM protein, which appears to be expressed at levels comparable to that in a
normal individual, at least in affected individuals that are homozygous for this mutation (3).

The mechanism for the milder clinical course in both the 5762ins137 and the 7271T→G patients is currently unknown. Our current study, therefore, was devised to try and identify cellular pathways dependent on ATM that may be important for moderating the symptoms arising from loss of ATM function.

MATERIALS AND METHODS

Cell Lines—Lymphoblastoid cell lines (LCLs) and skin fibroblast strains were derived in our laboratory from normal individuals and patients with ataxia telangiectasia. LCLs were routinely maintained in RPMI medium supplemented with 10% fetal calf serum, glutamine, penicillin, and streptomycin.

Immunoblot Analysis—Whole cell extracts (from ~4 × 10⁶ cells) were prepared as described (3). Briefly, cells were sonicated in UTB buffer (9 mM urea, 150 mM β-mercaptoethanol, 50 mM Tris/HCl (pH 7.5)), and cellular debris was removed by centrifugation. Proteins were fractionated in 6% SDS-polyacrylamide gels. Proteins were transferred to nitrocellulose, and immunoblots were performed with p53 (donated by Dr. D. Lane), p-Chk2, Nbs1, c-Abl, and RPA (9). In an attempt to under- stand the underlying reason for the milder clinical phenotypes exhibited by patients with the 5762ins137 and 7271T→G mutations (4), the second mutation in patient 62-4 (G mutation) was expressed at levels comparable to normal in cells from patients 109II-2, 109II-5, and 109II-6, homozygous for the mutation and approximately half that of normal in patient 46II-2 who was heterozygous for the 7271 mutation and had a second null mutation, 3910del7 (3). In an attempt to understand the underlying reason for the milder clinical phenotypes exhibited by patients with the 5762ins137 and 7271T→G mutations (4), the second mutation in patient 62-4 (G mutation) was expressed at levels comparable to normal in cells from patients 109II-2, 109II-5, and 109II-6, homozygous for the mutation and approximately half that of normal in patient 46II-2 who was heterozygous for the 7271 mutation and had a second null mutation, 3910del7 (3). The normal level of ATM protein in the 7271 heterozygous patient 136II-1 suggested that the second unknown mutation was also an expressed missense point mutation that did not affect the stability of the protein (Fig. 1C).

To evaluate the kinase activity of the residual ATM protein...
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A similar assay was carried out using cells from patients with the 7271T→G mutation, except that similar amounts of protein were used in each immunoprecipitate given that the level of mutant protein in these patients was comparable to that found in normal cells (3). The mutant protein expressed in cells from the homozygous 7271 patients 109 II-1, 109 II-5, and 109 II-6 showed only ~4–6% of normal ATM kinase activity when using PHAS-I as a substrate (Fig. 2B). In addition, this mutant ATM protein was also able to undergo weak autophosphorylation (data not shown). The kinase activity of the mutant ATM protein in the 7271T→G heterozygous patient 46 II-2 was too low to detect over and above nonspecific background phosphorylation of PHAS-I observed in the negative control (data not shown). In contrast, the kinase activity in the heterozygous patient 136 II-1 was ~10–15% of normal ATM activity suggesting that the second expressed mutant allele, in addition to the 7271T→G mutation, significantly contributed to the residual ATM function in these cells (data not shown). Therefore, the residual ATM protein expressed in cells derived from both the 5762ins137 and the 7271T→G patients retained detectable in vitro protein kinase activity. In addition, the second ATM mutation present in the cells may also affect the level of remaining activity.

The in Vivo Kinase Activity of the Residual ATM Protein in Cells Derived from Variant A-T Patients—To assess the activity of the residual ATM protein in these variant A-T patients in vivo, we examined the ATM-dependent phosphorylation of the double strand break repair proteins, Nbs1 and hMre11, after DNA damage. The phosphorylation of Nbs1 (17, 20–22) and hMre11 (23) can be detected by a change in protein mobility on an SDS-PAGE gel. The bandshift of Nbs1 and hMre11 proteins is clearly evident in normal cells but not in cells from a classical A-T after exposure to 50 Gy of γ-radiation (Fig. 3, A and B). To confirm that the mobility shift of both hMre11 and Nbs1 was specifically due to phosphorylation, immuno precipitates of hMre11 and Nbs1 following exposure of normal and classical A-T cells to ionizing radiation were treated with A protein phosphatase. Phosphatase treatment resulted in normal mobility of hMre11 and Nbs1 (Fig. 3C), therefore confirming that retardation of the hMre11 and Nbs1 protein after DNA damage was specifically due to phosphorylation. The three 5762ins137 cell lines analyzed showed a small detectable level of Nbs1 but not hMre11 phosphorylation following DNA damage (Fig. 3A). Immunoprecipitation and phosphatase treatment of Nbs1 in one of the 5762ins137 cell lines (40-4) following DNA damage demonstrated a small amount of damage-induced Nbs1 phosphorylation (Fig. 3C). However, immunoprecipitation of hMre11 in this cell line following exposure to ionizing radiation failed to show any damage-induced mobility shift (Fig. 3C). In contrast, phosphorylation of Nbs1 and hMre11 was observed at a higher level in irradiated cells derived from patients that were either homozygous or heterozygous for the 7271T→G mutation, albeit at reduced levels when compared with normal (Fig. 3B). Thus in both the 5762ins137 and the 7271 cells, the ATM protein did retain some normal activity in vivo as well as in vitro, although it was more evident in patients with the 7271T→G mutant protein.

Phenotypic Characterization of Cells from Variant A-T Patients with the 5762ins137 or 7271 T→G ATM Mutations; Induction of p53 after Exposure to Ionizing Radiation—ATM activation results in the induction of p53 and certain downstream responsive genes. A-T cells exhibit a defective induction of p53 and GADD45 after exposure to ionizing radiation (24). However, in keeping with the heterogeneity that is characteristic of

![Figure 1](http://www.jbc.org/)
A-T, the kinetics of the damage-induced p53 accumulation in these cells does show some variability. It would appear that the defect in cells from classical A-T patients is either a delay in the accumulation of stable p53 or in both the timing of peak p53 accumulation and also a reduction in the maximal level of detectable p53 protein (24–27).

Table I

| Patient | Mutation 1 | Mutation 2 |
|---------|------------|------------|
| Cell lines with 5762ins137 mutations |
| 1–3 | 5762ins137 | Stop1930 | 3801delG | Stop1268 |
| 14-4 | 5762ins137 | Stop1930 | ND | ND |
| 38-3 | 5762ins137 | Stop1930 | ND | ND |
| 40-4 | 5762ins137 | Stop1930 | 8787ins14 | Stop2933 |
| 44-4 | 5762ins137 | Stop1930 | ND | ND |
| 45-3 | 5762ins137 | Stop1930 | ND | ND |
| 52-4 | 5762ins137 | Stop1930 | 9139–T | Stop3047 |
| 59-4 | 5762ins137 | Stop1930 | 6412delAG | Stop2144 |
| 62-4 | 5762ins137 | Stop1930 | 7636del9 | delISR |
| 79-3 | 5762ins137 | Stop1930 | 2282delCT | Stop763 |
| 153-3 | 5762ins137 | Stop1930 | ND | ND |

| Cell lines with 7271T→G mutation |
| 109 II-1 | 7271T→G | V2424G | 7271T→G | V2424G |
| 109 II-5 | 7271T→G | V2424G | 7271T→G | V2424G |
| 109 II-6 | 7271T→G | V2424G | 7271T→G | V2424G |
| 46, II-1 | 7271T→G | V2424G | 3910del7 | Stop1304 |
| 46, II-2 | 7271T→G | V2424G | 3910del7 | Stop1304 |
| 136, II-1 | 7271T→G | V2424G | ND | ND |

| Cell lines with 7636del9 mutations |
| 26-4 | 7636del9 | DelISR | ND | ND |
| 39-4 | 7636del9 | DelISR | 136del4 | 136del4 |

| Cell lines from classical A-T with two truncating ATM mutations |
| 92-3 | 2249ins9 | ter752 | 2249ins9 | ter752 |
| 113-3 | 2639del1200 | ter880 | 2639del1200 | ter880 |
| 118-3 | 794ins4 | Stop266 | 2839del83 | Stop947 |

Similarly, cells from all six 7271T→G patients exhibited an intermediate p53 response following exposure to ionizing radiation, again showing a rapid increase in p53 protein during the first 2 h post-irradiation. p21 and MDM2 were also up-regulated normally in all 7271T→G cell lines, peaking at 2–4 h after γ-radiation treatment (Fig. 4B).

The intermediate p53 responses following radiation exposure observed in cells derived from both the 5762ins137 and the 7271T→G patients suggested that the residual low level of normal ATM protein or the mutant 7271 ATM protein expressed in these patients, respectively, was capable of moderating the severity of the A-T cellular phenotype.

p53 Serine 15 Phosphorylation in Cells Harboring the 5762ins137 or 7271T→G ATM Mutation—Following DNA damage, p53 is phosphorylated on multiple sites, which allows the p53 protein to stabilize and also become transcriptionally active (reviewed in Ref. 28). The modification of serine 15 after ionizing radiation exposure is primarily mediated by ATM and its related kinase, ATR (10, 11, 29). It has been proposed that the initial phosphorylation of p53 is a result of the rapid activation of ATM following DNA damage, and this phosphorylation is then maintained at later times post-irradiation by ATR (29). This is supported by the fact that serine 15 phosphorylation in ATM-deficient cells after exposure to ionizing radiation is delayed but not abrogated (30).

In light of the intermediate p53 response observed in cells from both the 5762ins137 and the 7271T→G patients following DNA damage, we determined the extent of serine 15 phosphorylation in these cells following genotoxic stress. p53 was rapidly phosphorylated on serine 15 within 1 h post-irradiation in normal cells and was maintained at a high level even until 8 h post-irradiation (Fig. 5). In contrast, both the classical A-T cell line and 7636del9 cell line showed a significantly reduced serine 15 phosphorylation response. The 5762ins137 patient (40-4) elicited normal serine 15 phosphorylation kinetics but with the overall level of phosphorylation being reduced. Interestingly, cells from the three 7271T→G patients tested exhibited different phosphorylation responses. Cells from the 7271T→G homozygous patient (109 II-1) showed a rather gradual, although significant, phosphorylation of serine 15 during the first 2 h post-irradiation that could be detected easily (Fig. 5), and cells from the 7271T→G heterozygous patient 46II-2 also showed some delay in the phosphorylation response. Cells from the 7271T→G heterozygous patient, 136 II-1, however, demonstrated a more rapid induction of serine 15 phosphoryl-
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A. ATM kinase activity in vitro. A, normal in vitro specific kinase activity of the residual ATM protein expressed in variant A-T patients with the 5762ins137 ATM gene mutation. Immunoprecipitates were carried from whole cell lysate using the Ab-2 anti-ATM monoclonal antibody (Oncogene Science). The kinase assay was carried out using 1 μg of whole cell lysate from patients 135-3, 40-4, and 39-4 was used per immunoprecipitate to compensate for the low levels of ATM expression, and 3 μg of whole cell lysate from patient 45-3 due to higher level of residual ATM expression. 1 μg of whole cell lysate from a normal LCL was used as a standard. The kinase assay was carried out using 1 μg of PHAS-I (Stratagene) substrate. The level of phosphorylated PHAS-I was measured by scanning densitometry. The background PHAS-I phosphorylation obtained from an immunokinase assay using the same amount of whole cell lysate from a classical A-T (118-3) as compared with the test sample was subtracted from the PHAS-I phosphorylation obtained from the test patients. The level of immunoprecipitated ATM was also quantified, and a ratio of ATM level to PHAS-I phosphorylation was calculated and expressed as a percentage of normal ATM kinase activity. B, residual in vitro kinase activity of the 7271T→G mutant ATM protein. Immunoprecipitates were carried from 1 μg of whole cell lysate using the Ab-2 anti-ATM monoclonal antibody (Oncogene Science). The kinase assay was carried out using 1 μg of PHAS-I substrate. The activity of the 7271T→G mutant ATM protein from patients 109II-1, 109II-5, and 109II-6 was calculated by densitometric scanning of the phosphorylated PHAS-I and compared with normal ATM kinase activity and the background kinase activity found in a classical A-T (118-3).

B. Number of downstream targets including JNK and the AP-1 transcription factor (9, 31–33).

To characterize further the cellular defects in the variant A-T patients harboring the 5762ins137 and the 7271T→G ATM mutations, the ability of cells from these patients to activate JNK after γ-radiation exposure was examined. The activity of JNK was stimulated ~7-fold by ionizing radiation in normal cells, whereas neither cells from the 5762ins137 nor the 7271T→G patients showed any significant activation of JNK following DNA damage (Fig. 6, A and B). Furthermore, the kinetics of JNK activation over a 4-h period in cells from these patients was not significantly different from those observed for a classical A-T patient (data not shown). Also, the basal level of JNK activity was elevated in cells derived from the majority of
5762ins137 and 7271T→G patients tested comparable to those observed in classical A-T cells (Fig. 6, A and B). It was inferred that a deficiency in the damage-induced c-Abl/JNK signaling pathway did not overtly affect the severity of the A-T clinical phenotype.

**DISCUSSION**

Our aim was to try and relate the less severe clinical and cellular features of some A-T patients to any residual function of expressed ATM protein in cells from these individuals. In the UK, A-T is characterized by the presence of founder mutations, and so several families with the same mutation could be studied. We examined A-T patients with either the 5762ins137 or 7271T→G mutations in which homozygotes for the 7271T→G mutation showed a less severe progression of cerebellar degeneration compared with patients carrying the 5762ins137 mutation. A recent study by Becker-Catania et al. (34) demonstrated the difficulty in correlating genotype and phenotype in a large cohort of 123 A-T patients using Western blotting on whole cell extracts and radiosensitivity as two experimental parameters. Our results, however, suggest that such a correlation can be made provided that the subgroups of A-T patients are tightly defined, that methods of protein detection are refined, and that different, well characterized ATM-dependent cellular responses to DNA damage are analyzed.

We show that the small amount of normal ATM protein expressed in patients with the 5762ins137 mutation can be detected more reliably by immunoprecipitation or by carrying out Western blotting on nuclear extracts. This, in turn, permits greater confidence in the correlation of expression of normal ATM with cellular or clinical features in these individuals. In contrast, the expression of mutant protein in cells from patients with the 7271 mutation can be adequately seen on an ordinary Western blot.

The small amount of expressed normal ATM protein present in cells derived from 5762ins137 patients, nevertheless, has a high specific activity of kinase function, as might be expected. The large amount of mutant ATM from the 7271T→G homozygous and heterozygous variant A-T patients also retains kinase activity *in vitro* but at much lower specific activity. These kinase activities, although qualitatively different, correlate with the milder clinical phenotypes exhibited by the respective groups of patients. In addition, the intermediate p53 response and up-regulation of p21 and MDM2 in both the 5762ins137 and 7271T→G cells, in conjunction with the *in vivo* phosphorylation of serine 15 following exposure to ionizing radiation, also suggest that the residual ATM protein is capable of mediating

![Image of p53 stabilization and transactivation of p21 and MDM2 in irradiated variant A-T cell lines](http://www.jbc.org/)

4, 8, 12, and 24 h post-irradiation. Whole cell lysate was separated on an SDS-PAGE gel, and Western blots were probed for p53, p21, and MDM2. A graphical representation of the p53 induction kinetics obtained from normal and classical A-T cell lines are given for comparison. The Western blot was re-probed for actin to standardize for protein loading. The band density was quantified by densitometry to give data on fold increase in the graph above. A, cells derived from A-T patients with the 5762ins137 ATM gene mutation exhibit intermediate p53 induction kinetics following exposure to ionizing radiation. The p53 induction kinetics determined for cells from 5762ins137 patients (79-3, 135-3, 45-3, 40-4, 59-4, 14-4, and 44-4) are compared with two cell lines from 7636del9 patients (39-4 and 26-4) and two classical A-T patients (92-3 and 113-3). Western blots of representative p53 responses of cells from normal, classical A-T and 5762ins137 A-T patients are also shown. B, cells derived from variant A-T patients with the 7271T→G ATM gene mutation exhibit intermediate p53 induction kinetics following exposure to ionizing radiation. Patients 109II-1, 109II-5, and 109II-6 are homozygous for the 7271T→G ATM mutation, and patients 46II-1, 46II-2, and 136II-1 are compound heterozygous for the 7271T→G mutation. The curves for the normals and classical A-T (92-3) are the same as in A. Western blots of representative p53 responses of cells from 109II-1, 109II-5, and 109II-6 are also shown.
some normal function that can alter the severity of the A-T cellular phenotype.

The principal feature of both the 5762ins137 and 7271T→G patients is a reduction in the severity of the neurological features and a significant slowing of the cerebellar degeneration that gives rise to the ataxia. p53 and various downstream target gene products are reported to be crucial when mediating ionizing radiation-induced cell death in the cerebellum and central nervous system (35, 36). It has been proposed that loss of p53-dependent signaling in ATM-deficient neurons may allow the accumulation of genomic damage that in turn may lead to functional deficits in later life. Thus, in a specific cell populations such as Purkinje and granule cells where the presence of functional ATM is critical, this process could lead to selective neurodegeneration (35). However, abnormal development of the central nervous system that is seen in Atm null mice (37) has not been reported in mouse models for p53 deficiency (38). Furthermore, the presence of a normal p53 response following exposure of cells to ionizing radiation from patients with ataxia telangiectasia-like disorder (39) and an inability to distinguish between the 5762ins137 and 7271T→G homozygote cells in terms of p53 response suggest that the ATM/p53 pathway is not the sole determinant for preventing progressive cerebellar degeneration.

Controlling the p53 signaling pathway is only one component of the ATM response to DNA damage. Another important role for ATM is regulating cellular DNA repair processes. The striking developmental defects of the nervous system exhibited by mice with gene ablations for certain components of the V(D)J recombination/DNA repair machinery, for example, DNA ligase IV or XRCC4, have provided new insight into understanding important physiological processes that are required for normal brain and central nervous system development (40–42). This association is further strengthened by the findings in ataxia telangiectasia-like disorder patients where the presence of cerebellar degeneration is associated with mutations in the DNA double strand break repair protein, hMre11 (39). It is, therefore, intriguing to speculate that the less severe neurological symptoms exhibited by the 5762ins137 and 7271T→G variant A-T patients could be, at least in part, accounted for by some normal DNA repair in damaged neural tissue. The residual phosphorylation of the DNA double strand break repair proteins Nbs1 and hMre11, following ionizing radiation expo-
sure, in cells derived from the 7271T–G patients may be consistent with such a process, although this has not been further substantiated experimentally. The apparent lack of significant Nbs1/hMre11 phosphorylation in the 5762ins137 patient-derived cell lines, however, might argue against this, although it is likely that the level of phosphorylation was beyond the level of sensitivity for Western blotting. It is also the case that the 5762ins137 patients are more severely affected than the 7271T–G homozygous mutant patients. The presence of fewer chromosome 7/14 translocations in both the 5762ins137 and 7271T–G patients may be indicative of a moderate DNA repair defect. Finally, further evidence for a less severe DNA repair defect is that one of the 7271 homozygous patients has had a child (3), indicating that meiotic recombination is not defective, and therefore, it is likely that homologous recombination repair processes are functional.

Conversely, it may be that the role of ATM in vesicular trafficking is more important in maintenance of the central nervous system (43). The report of a patient with an autoimmune reaction to neuron-specific vesicle coat protein β-NAP, developing progressive cerebellar degeneration, merely serves to highlight the importance of vesicle-mediated signaling in neural cell survival (44). The derivation of mouse models would be needed to address these specific questions.

Rather than the emphasis of p53 responses being important for the integrity of neuronal processes, the retention of some normal p53-dependent signaling may be more important for neuronal cell survival (44). The derivation of mouse models would serve to highlight the importance of vesicle-mediated signaling in neural cell survival (44). The derivation of mouse models would be needed to address these specific questions.

In contrast to the intermediate p53 response to DNA damage, the activation of the c-Abl/JNK pathway following ionizing radiation exposure is completely defective in cells from both the 5762ins137 and the 7271T–G patients. It could be argued that the mutant 7271 ATM protein is unable to bind c-Abl and activate it and that the residual normal ATM protein expressed in the 5762ins137 patients is expressed at levels too low to be effective in activating this pathway. However, this seems unlikely because, at least in the case of the 5762ins137 patients, the low levels of normal ATM are sufficient to affect other cellular signaling pathways, such as p53-dependent processes. The contribution of a defective c-Abl/JNK pathway to the A-T phenotype is unknown. Members of the JNK family of stress-activated protein kinases have been reported to be involved in implementing apoptosis in certain cells types, namely neuronal and lymphoid cells (50, 51). However, the lack of immunodeficiency and slow progression of cerebellar degeneration in both the 5762ins137 and 7271T–G patients suggests that this defect in the c-Abl/JNK pathway does not significantly affect the severity of the A-T phenotype. Furthermore, cells from A-T patients, which do not show any cerebellar degeneration, also exhibit a comparable defect in the damage-induced activation of c-Abl/JNK, suggesting that this defect also does not affect the type of nervous system abnormality (39).

In conclusion, these data would suggest that the ATM protein expressed in cells derived from both the 5762ins137 and the 7271T–G variant A-T patients does retain sufficient activity to moderate the severity of the A-T cellular phenotype. This includes both kinase activity and responses resulting in cell cycle arrest. When the patients are ordered in decreasing clinical severity, with classical A-T patients being the most severely affected and homozygous 7271T–G patients being the least severely affected, the level of residual ATM function is greatest in those least severely affected (Fig. 7). The overall clinical and cellular features of A-T patients carrying either the 5762ins137 or 7271T–G mutation are likely to result from a combination of the cellular level of functional ATM protein (including the type and level of ATM expression from the second mutant allele), the age of the patient (the degree of cerebellar degeneration is age-related in childhood), as well as a number of possible modifying factors. Additional biochemical characterization of these cells is needed to identify, precisely, the ATM-dependent pathways that are crucial in modulating the severity of the clinical symptoms. Furthermore, identification of these pathways will provide invaluable information for targeting certain cellular processes when designing potential therapeutic protocols.

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