Genotype, extrapyramidal features and severity of variant Ataxia-Telangiectasia

Authors

Dr Katherine Schon BM BCh, MA, MRCP[2], Dr Nienke van Os MD[3], Dr Nicholas Oscroft MD[1], Dr Helen Baxendale BSc, MBBS, PhD, MRCP, MRCPCH[1], Dr Daniel Scoffings MBBS, MRCP, FRCR[4], Dr Julian Ray MB BS, BSc, FRCP[5], Dr Mohnish Suri MBBS, MD, FRCP[7], Dr William P. Whitehouse BSc, BM BS, DCH, FRCP, FRCPCH [8,9], Dr Puja R. Mehta MB BChir, MA, MRCP[1], Natasha Everett[1], Dr Leonardo Bottolo PhD[10,11,12], Dr Bart P. van de Warrenburg MD, PhD[3], Dr Philip J. Byrd BSc, PhD [13], Dr Corry Weemaes MD [14], Prof Michel A. Willemsen MD PhD[3], Dr Marc Tischkowitz MD, PhD [2,10] , Prof A. Malcolm Taylor PhD[13], Dr Anke E. Hensiek MD PhD[1,6]

Author Affiliations

1. Ataxia Telangiectasia Service, Respiratory Support and Sleep Centre, Papworth Hospital. Papworth Everard, Cambridge, CB23 3RE UK
2. East Anglian Medical Genetics Service, Cambridge University Hospitals NHS Foundation Trust, Hills Road, Cambridge, CB2 0QQ, UK
3. Departments of Neurology & Pediatric Neurology, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Centre, Nijmegen, The Netherlands
4. Department of Radiology, Cambridge University Hospitals NHS Foundation Trust, Hills Road, Cambridge, CB2 0QQ, UK
5. Department of Neurophysiology, Cambridge University Hospitals NHS Foundation Trust, Hills Road, Cambridge, CB2 0QQ, UK
6. Department of Neurology, Cambridge University Hospitals NHS Foundation Trust, Hills Road, Cambridge, CB2 0QQ, UK
7. Nottingham Clinical Genetics Service, National Paediatric Ataxia-Telangiectasia Clinic, Nottingham, UK
8. School of Medicine, University of Nottingham, Queen's Medical Centre, Nottingham, NG7 2UH, UK

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9. Department of Paediatric Neurology, Nottingham Children's Hospital, Nottingham University Hospitals NHS Trust, NG7 2UH, UK
10. Department of Medical Genetics, Cambridge Biomedical Campus, Cambridge CB2 0QQ, UK
11. The Alan Turing Institute, British Library, 96 Euston Road, London NW1 DB, UK
12. MRC Biostatistics Unit, University of Cambridge, Robinson Way, Cambridge Biomedical Campus, Cambridge, CB2 0QQ, UK
13. Institute of Cancer and Genomic Sciences, College of Medical and Dental Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK
14. Department of Pediatrics, Radboudumc Amalia Children's Hospital, Nijmegen, The Netherlands.

Corresponding Authors

Dr Anke Hensiek

Department of Neurology, Cambridge University Hospitals NHS Foundation Trust, Hills Road, Cambridge, CB2 0QQ, UK

Email : ahensiek@nhs.net

Professor Malcolm Taylor

Institute of Cancer and Genomic Sciences, College of Medical and Dental Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK

Email: a.m.r.taylor@bham.ac.uk

Telephone: 0121-414-4488

Fax: 0121-414-4486

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Abstract

Objective

Variant Ataxia-Telangiectasia is caused by mutations that allow some retained ATM kinase activity. Here, we describe the clinical features of the largest established cohort of individuals with variant Ataxia-Telangiectasia and explore genotype-phenotype correlations.

Methods

Cross-sectional data were collected retrospectively. Patients were classified as variant Ataxia-Telangiectasia based on retained ATM kinase activity.

Results

The study includes 57 individuals. Mean age at assessment was 37.5 years. Most had their first symptoms by age ten (81%). There was a diagnostic delay of more than ten years in 68% and more than 20 years in a third of probands.

Disease severity was mild in a third of patients and 43% were still ambulant 20 years after disease onset. Only a third had predominant ataxia and 18% had a pure extrapyramidal presentation. Individuals with extrapyramidal presentations had milder neurological disease severity. There were no significant respiratory or immunological complications, but 25% of individuals had a history of malignancy. Missense mutations were associated with milder neurological disease severity but with a higher risk of malignancy, compared to leaky splice site mutations.

Interpretation

Individuals with variant Ataxia-Telangiectasia require malignancy surveillance and tailored management. However, our data suggest the condition may sometimes be mis- or underdiagnosed due to atypical features, including exclusive extrapyramidal symptoms, normal eye movements and normal AFP levels in some individuals. Missense mutations are associated with milder neurological presentations but a particularly high malignancy risk and it is important for clinicians to be aware of these phenotypes.

Key Words

Ataxia-Telangiectasia, ATM, prognosis, genotype-phenotype correlation

Abbreviations

A-T	Ataxia Telangiectasia
A-T NEST	Ataxia-Telangiectasia Neurological Examination Scale Toolkit
SARA	Scale for Assessment and Rating of Ataxia
AFP	Alpha Fetoprotein

Introduction

Ataxia-Telangiectasia is a rare autosomal recessive disorder caused by mutations in the *ATM* gene on chromosome 11q22.3 (MIM 208900)[1]. The ATM protein is a serine-threonine protein kinase, which phosphorylates more than 700 substrates and is a key player in the cellular response to double stranded DNA damage[2]. The clinical and genetic features of Ataxia-Telangiectasia vary and two forms of the disease have been described.

Classic (or typical) Ataxia-Telangiectasia presents with a severe phenotype and has an estimated incidence of 1 in 300, 000[3]. Individuals with classic Ataxia-Telangiectasia have absent ATM kinase activity[4], either due to two null mutations or mutations which result in protein without ATM kinase activity. It is a multisystem neurodegenerative disease which also causes immunological defects, respiratory problems, oculocutaneous telangiectasia and an increased risk of malignancy[5,6]. Affected children are usually wheelchair bound before teenage and have severe neurological disability including cerebellar ataxia, extrapyramidal features, oculomotor dyspraxia and polyneuropathy[7]. Most individuals with classic Ataxia-Telangiectasia die before the age of 30; malignancy or respiratory failure are the main causes of death[8,9].

In addition to classic Ataxia-Telangiectasia a second form, variant Ataxia-Telangiectasia has been described as a cause of neurological dysfunction[10,11]. A study of 51 patients with A-T (including 9 with retained ATM kinase activity) showed that individuals with retained ATM kinase activity have a milder neurological phenotype[4]. Variant Ataxia-Telangiectasia results either from leaky splice site mutations which allow expression of some normal ATM protein or missense mutations which produce a mutant ATM protein with activity[12]. It has been speculated that the presence of some ATM kinase activity relates to a milder neurological phenotype and a lower risk of systemic complications. Furthermore, a range of atypical neurological presentations have been reported to occur in some individuals with variant A-T.

Previously published reports of variant A-T are limited to small cohorts of less than 15 individuals[7,13–16] or case studies of unusual presentations[17–21].

The true clinical spectrum of variant Ataxia-Telangiectasia is unknown and it is not clear which factors determine the extreme clinical variability that has been reported. It is unclear whether affected individuals require specific surveillance for systemic complications and malignancy, similar to existing management recommendations of classic Ataxia-Telangiectasia[22,23].

Here, we report the clinical features of the largest established cohort of patients with variant Ataxia-Telangiectasia. We provide prognostic information and explore genotype-phenotype correlations to inform management guidelines.

Methods

Probands

Clinical data were retrospectively collected from case notes for all individuals with variant Ataxia-Telangiectasia who have attended the National Adult Ataxia-Telangiectasia service (Papworth Hospital, UK), the Ataxia-Telangiectasia Specialist Centre (Nottingham City Hospital, UK) and the Excellence Centre of Movement Disorders (Radboud UMC, Nijmegen, Netherlands). Analysis includes single cross-sectional data using the most recent recorded clinical assessment.

Molecular genetics studies

Patients were classified as having variant Ataxia-Telangiectasia based on retained ATM kinase activity. Patients with a mutation in the initiator methionine codon were included because of some uncertainty of ATM activity associated with these mutations. *ATM* mutations were identified by Sanger sequencing of PCR amplified ATM exon sequences. A lymphoblastoid cell line was derived from each patient's blood and immunoblotting for ATM expression and ATM activity assays were performed using methods as previously described[24]. Chromosomal radiosensitivity was measured following exposure to 1Gy gamma rays at cell cycle phase G2.

Neurological Assessment

Clinical neurological assessment was performed by a neurologist with a special interest in Ataxia-Telangiectasia. The Scale for Assessment and Rating of Ataxia (SARA)[25] and Ataxia-Telangiectasia Neurological Examination Scale Toolkit (A-T

NEST) scores[11] were recorded. As part of the neurological examination, a subjective assessment of eye movement abnormalities was recorded by the examining neurologist as normal eye movements, mildly abnormal eye movements (including nystagmus, slowing of saccades and mild oculomotor dyspraxia) or severely abnormal eye movements (including marked oculomotor apraxia).

Neurological Phenotype

Individuals were categorized into three neurological phenotypic groups to reflect those neurological symptoms, which have been reported to most commonly occur in variant Ataxia Telangiectasia:

Group A: Cerebellar ataxia and/or peripheral neuropathy with minimal or no extrapyramidal involvement

Group B: Cerebellar ataxia and/or neuropathy plus additional extrapyramidal features

Group C: Extrapyramidal signs without significant ataxia and/or peripheral neuropathy

Cross-sectional Clinical Neurological Disease Severity

An overall assessment of current severity was made using level of mobility and self-care.

Mild: Individuals still ambulant with/without a walking aid and could use the upper limbs for most activities without help. A-T NEST scores within this group were >55, SARA scores < 22.

Moderate: Individuals used a wheelchair frequently but were able to transfer, walk a few steps with help, and use the upper limbs for most activities, such as feeding themselves. A-T NEST scores within the moderate group were 42-75, SARA scores 15-33.

Severe: Individuals were permanently wheelchair bound, were unable to transfer unaided and had significant limitation of upper limb function, requiring assistance with most activities of daily living. A-T NEST scores within this group were <46, SARA scores > 30.

Neurological investigations

Results of nerve conduction studies and EMG were reviewed. Neuropathy was graded depending on the severity of the nerve conduction abnormality and of any accompanying EMG impairment. The neuropathy grade was determined by amplitude measures and how widespread the abnormalities were. For example, in the case of the sensory changes:

- impairment of sural amplitude only – mild
- absent sural responses – mild-moderate
- impaired upper limb digital potential and absent sural responses – moderate
- impaired/absent upper limb potential and impaired radial amplitude – moderate-severe
- absent sensory responses in upper limb and lower limb – severe.

A similar approach was adopted for motor potentials. If nerve conduction studies were relatively preserved, whilst EMG abnormality was detected, then the sub-categorization of a suspected motor neuronopathy was documented.

MRI brain scans were conducted to rate atrophy of the cerebellar vermis and hemispheres on sagittal 3D T1W sequence. Cerebellar atrophy was assessed separately for the vermis and jointly for both hemispheres and rated as absent, mild (mild widening of sulci and fissures, normal size of folia), moderate (moderate widening of sulci and fissures associated with volume loss of folia) or severe (marked widening of sulci and fissures with marked volume loss of folia). White matter hyperintensities on FLAIR sequence were classified as punctate, beginning confluent or confluent[26]. Cerebral microbleeds were assessed on SWI or T2*W-gradient echo sequence[27].

Respiratory assessment

Patients attending Papworth were assessed by a consultant respiratory physician. They underwent arterial blood gas measurement, pulmonary function testing, overnight oximetry and low dose CT thorax. Five of the Dutch patients had spirometry.

Immunological assessment

Immunological investigations included immunoglobulin levels (IgG, IgA, IgM, IgG2, IgE), serum electrophoresis if indicated, lymphocyte subset analysis and assessment of serum antibody to pneumococcus, tetanus and haemophilus vaccine. History of immunisations, infections and treatment was recorded.

General assessment

Past medical history including diabetes and malignancy was documented in all patients. Level of mobility and age at first wheelchair use was recorded. Examination for conjunctival telangiectasia, measurement of weight, height and BMI, blood tests for alpha-fetoprotein and LFTs and liver ultrasound scan were carried out in the majority of patients but this was not standardized between centres.

Ethical Considerations

The study was approved by the Health Research Authority.

Statistical Analysis

In order to test the effect of the genetic covariates (presence of a missense mutation with retained ATM kinase activity, number of mild mutations, ATM protein levels and chromosomal radio-sensitivity) on neurological and other clinical features, we corrected the regression models for fixed (age, gender and age of onset) and random-effects (hospitals and families). We tested the effect of each genetic covariate on quantile normalised SARA score, A-T NEST score, age at first wheelchair use and alpha-fetoprotein levels using a linear mixed-effects model (lmer function, R project *lme4* package[28]). For peripheral neuropathy, conjunctival telangiectasia and malignancy we used a generalised linear mixed-effects model (glmer function, R project *lme4* package[28]) treating the response as a dichotomous variable. Associations between the neurological group and each genetic covariate were tested using a multinomial logistic regression (multinom function, R project *nnet* package[29]) with hospitals and families treated as fixed effects. For overall severity and eye movements responses we used a mixed regression model for ordinal data, the ordered regression model implemented in the cmml function, R project *ordinal* package[30].

Results

A. Demographic features and employment

The study included 57 individuals (23 males, 34 females) from 50 families (Table 1). Mean age was 37.5 years (SD 12.3 years, range 11-58 years) with four patients aged under 18 years. Six women had offspring (five from the UK cohort, one from the Dutch cohort). None of the men had offspring. Three individuals are deceased. Eleven individuals were in employment and two were students. Seven had university degrees. Thirty-nine patients were recruited from Papworth, six from Nottingham and 12 from the Dutch cohort.

B. Neurological features

1) Onset, duration, mobility and severity

Age of onset is shown in Figure 1a. Most individuals (46/57) had their first symptoms by age ten years. Individuals with disease onset before ten years were considered to have early onset disease. Six had onset between 11 and 16 years. Five had adult onset disease.

Disease duration at last assessment ranged from 10-54 years. Disease duration was 10-20 years in 16 individuals, 20-30 years in 13 and >30 years in 28.

Age at diagnosis ranged from two to 47 years (Figure 1b). The diagnosis was made within one year of symptom onset in seven individuals and in one to nine years in 17. There was a diagnostic delay of 10-20 years in 12 and >20 years in 17 (not documented in four).

42 individuals used a wheelchair and 15 were still ambulant. Median age at first getting a wheelchair was 20 years (range eight to 51 years) (Figure 1c). 11 individuals got their first wheelchair within ten years of symptom onset, 17 in 10-20 years and 14 after more than 20 years. Seven individuals were currently ambulant after a disease duration of 10-20 years, and eight after >20 years.

17 individuals had mild disease, 26 had moderate disease and ten had severe disease. Classification of disease severity was not available for four individuals. There was one outlier in the severe group, a paediatric patient who used a wheelchair since age 8, and had an A-T NEST score of 72.

2) Neurological phenotype

Three distinct patterns of neurological deficits were observed. The neurological features and treatments in each group are summarised in Table 2.

Group A (predominant cerebellar ataxia and/or peripheral neuropathy with minimal or no extrapyramidal involvement)

Nineteen individuals (33%) were in Group A. Severe oculomotor dyspraxia was common (11/19, 58%) and 6 patients had mild eye movement abnormalities (32%). A 15-year old with mild disease had normal eye movements. The other patient with normal eye movements presented initially with a severe reaction to radiotherapy for breast cancer and had a mild phenotype consisting of peripheral neuropathy with only minimal limb ataxia[31]. Disease severity was moderate or severe, except in the individual described above and two paediatric patients aged 11 and 15 years. Mean disease duration was 32 years.

Group B (disability determined by a mixture of ataxia and/or peripheral neuropathy plus additional extrapyramidal features)

Twenty-eight individuals (49%) were in Group B. Their extrapyramidal features included dystonic posturing/neck dystonia (12 patients), dystonic tremor (11 individuals), choreiform movements (9 patients) and orofacial dystonia (5 patients). Two patients had a resting tremor. In this group, 12/28 (43%) exhibited prominent oculomotor dyspraxia, 43% had mildly abnormal eye movements and four patients had normal eye movements. Disease severity was widely distributed. Six individuals had mild disease, 15 moderate disease and four individuals were severely affected (not documented in three). Mean disease duration was 30 years.

Group C (predominantly extrapyramidal signs)

Ten individuals (18%) were in Group C. One individual exhibited marked truncal dystonic spasms which limited his ability to walk[32]. Another patient had a clinical phenotype typical of myoclonic dystonia, which improved markedly following deep brain stimulation[33]. One individual had marked orofacial dystonia but otherwise an essentially normal neurological examination with no limb ataxia or dystonia[17]. One exhibited neck and limb dystonia, which was treated with botulinum toxin injections and another had hemichorea. Three siblings presented with severe resting tremor (onset between 16 and 34 years) and anterior horn neuronopathy[18]. One individual

presented with resting tremor at age 12 years and then developed chorea-athetosis and dystonia. Another presented initially with chorea-athetosis.

Two patients (20%) had marked oculomotor dyspraxia, two had mildly abnormal eye movements and 6/10 of individuals had normal eye movements. Disease severity was classified as mild in 8/10 and six individuals in this group were in employment at the time of assessment. Mean disease duration was 28 years.

3) Neurological investigations

Neurophysiology

Neurophysiology studies were performed in 34 individuals. Eighteen had an axonal sensorimotor polyneuropathy of varying severity. Three showed predominant involvement of the motor neurons. Nine had an axonal sensorimotor polyneuropathy with likely spinal muscular atrophy component. Four had normal/ nearly normal neurophysiology studies.

Detailed neurophysiology results for 21 patients assessed through Papworth are shown in Supplementary Table 1.

Radiology

Brain MRI scans were available in a total of 35 individuals (including 23 performed through the Papworth service, ten Dutch individuals and two patients through the Nottingham clinic). Cerebellar atrophy was found in 29 individuals. Six scans were reported as showing a normal cerebellum (age 19 to 46 years), of which one also showed atrophy of the left corpus nucleus caudatus. Seven individuals had white matter changes (single lesion in two, a few punctate lesions in five). Cerebral microbleeds were noted in two individuals. One had multiple, mainly in the occipital subcortical white matter (age 53). The other had a single microbleed in the right external capsule (age 55).

Brain MRI scans performed through Papworth were reviewed by a consultant radiologist. Cerebellar atrophy was observed in 23/23 scans. Atrophy of the cerebellar vermis was classified as mild in 8, moderate in 9 and severe in 6. Atrophy of the cerebellar hemispheres was classified as mild in 11, moderate in 7 and severe in 5 (Figure 2a, Figure 2b, Figure 2c).

C. Other Clinical Features

1.Endocrine function

Mean height percentile was 48.1 (SD 31.1, 53 patients evaluated). Mean body mass index was 23.3 (SD 4.8, 49 patients evaluated). Six individuals were underweight (BMI<18.5) and four had gastrostomy feeding due to dysphagia and/or poor appetite. Two patients had type II diabetes. None had type I diabetes.

2. Conjunctival telangiectasia

Conjunctival telangiectasia were observed in 35/56 individuals with documented assessment (63%).

3. Respiratory Function

No patient had severe lung disease. CT thorax was performed in 39 patients. Thirty showed normal results, six showed mild bronchiectasis, one showed mild emphysema (ex-smoker), one showed patchy air trapping and one showed two left upper lobe nodules (likely intrapulmonary lymph nodes).

Arterial blood gases showed mean PaO_2 12.4kPa (SD 1.34kPa) and $PaCO_2$ 5.17kPa (SD 0.52kPa). Of 39 results, only one was abnormal with mild hypoxaemia. Overnight oximetry in 37 subjects did not show hypoventilation with mean oxygen saturations of 96.4% (SD 1.35%) and mean 4% desaturation index of 3.8 (SD 3.26).

Pulmonary function data from 42 individuals showed mean FEV_1 80.2% (SD 18.7%), FVC 79.7% (SD 21.6%) and KCO 95.5% (SD 19.3%). Twenty spirometrys were restrictive however patients struggled with spirometric technique.

4. Immunological Function

Infection history was assessed in all patients and showed episodes of severe chickenpox (2), sinusitis (1), recurrent boils (1), frequent urinary tract infections (1) and sore throats 12 times per year (1). Two individuals were taking azithromycin prophylaxis for lower respiratory tract infections. No individuals were receiving immunoglobulin therapy.

Subtle signs of immune dysregulation and possible immune deficiency were seen in a

number of patients. Immunoglobulin results were available in 52 patients. Polyclonal increases were observed in IgG (1), IgA (7) and IgM (12). One further patient had increased IgG and IgA. Four patients had a monoclonal gammopathy of unknown significance. One individual had mild panhypogammaglobulinaemia. A slight reduction in total IgG was observed in one and IgA in five individuals. One had IgA deficiency (<0.07mcg/ml). IgE was below level of detection in 8 patients.

Lymphocyte subpopulation results were available in 44 patients. Slight reductions in CD4 and CD8 counts were found in 2 and 12 patients respectively and 16 patients had CD19 counts just below the normal range.

5. Alpha-Fetoprotein Levels

Mean serum alpha-fetoprotein level was 176 microgram/L (range 2 to 600, SD 146) in 45 individuals with a result available. Three (6.6%) had levels in the normal range

6. Liver Ultrasound, Liver Function Tests

Liver ultrasound was performed in 36 patients. Seven showed fatty liver and three detected liver lesions (haemangioma, neuro-endocrine tumour, metastatic prostate cancer). 15/44 patients with LFT results available had mildly abnormal results.

D. Malignancy

Fourteen patients have been diagnosed with a malignancy. Solid tumours included five female unilateral oestrogen-receptor positive breast cancers (age 28-44 years), one dermatofibrosarcoma protuberans of the breast (age 29), one neuroendocrine tumour (age 48), one pancreatic cancer (died age 48), one pelvic mass (possible germ cell tumour at age 11) and one prostate cancer (age 52). Lymphoid malignancies included T-cell non-Hodgkin's lymphoma (age 2), Acute Lymphoblastic Leukaemia (age 9), Chronic Myeloid Leukaemia (age 39) and Chronic Lymphoblastic Leukaemia (patient also had breast cancer and died aged 47 years). One patient died of Acute Lymphoblastic Leukaemia aged 51.

E. Genetics

1. Family History

Nineteen individuals had a family history of Ataxia-Telangiectasia in a sibling and four had affected siblings who died from malignancies (primary hepatoma, ectopic pituitary tumour, and two with lymphomas). There was parental consanguinity in two families. Twenty out of 114 parents had been diagnosed with a cancer (18%). Mothers of ten patients developed breast cancer (aged 39-64 years).

2. Cytogenetic Features

Chromosomal radiosensitivity was normal in 15/48 individuals tested (31%) and raised in 33/48 (69%). Two had a high level, the same as expected in classic Ataxia-Telangiectasia.

3. Mutation Analysis

Mutation analysis (Table 1) revealed mutations in 111/114 alleles. Patients were classified based on the mutation present causing the retained kinase activity.

Individuals in Genetic Group 1 all have a leaky splice site mutation, which allows some normal ATM protein to be expressed with kinase activity. Fourteen patients in this group have the c.5763-1050A>G mutation.

Individuals in Genetic Group 2 all have a missense mutation, which results in the presence of a mutant protein with some ATM kinase activity.

Individuals in Genetic Group 3 have mutations affecting the initiator methionine codon. These mutations allow expression of a very low level of truncated protein[31]. Patients with these mutations have been noted to have a milder clinical course[24] and there is some uncertainty of ATM activity associated with these mutations.

Individuals in Genetic Group 4 have one confirmed mutation and one mutation which has not been identified or not been completely characterised. Patient 50 has a missense mutation Val2716Ala which is associated with retained ATM activity. The other six patients showed a low level of ATM protein and low level of ATM kinase activity. cDNA was examined in these cases. Two have alterations found at the cDNA level, but no potentially causative mutations found in genomic DNA. The mutations giving protein with activity are likely to be intronic splice site mutations.

Twelve patients (1, 2, 18a,b, 26, 31, 33, 35 and 37a-c, 41) had two 'milder 'mutations, which are both known to be associated with retained ATM kinase activity.

4. Genotype-Phenotype Correlation

Cell lines from 51/51 patients tested showed retained expression of some ATM protein. This ranged from just detectable (~5%) to near normal.

ATM kinase activity was tested in cell lines from 45 patients. Cells derived from patients 34 and 42 showed possibly a low level of ATM kinase activity. All of the other cell lines showed clear retained kinase activity.

The four patients (34, 42, 43, 44) with absent or possibly just detectable ATM kinase activity had more severe disease. Onset of symptoms was between 1 and 2.5 years, with first wheelchair use at eight years in two patients and 11 and 13 years in the other two. Two have PEG feeding tubes.

There are two major subgroups of variant patients: those with a missense mutation producing some ATM protein with retained activity (Group 2, and 1 in Group 4) and those with a leaky splice site allowing expression of a low level of normal ATM with some activity (Group 1, and most likely six in Group 4).

Individuals with at least one missense mutation which produces a mutant protein with residual kinase activity had milder neurological disease compared to the rest of the cohort who have leaky splice site mutations or mutations in the initiator methionine codon. There were statistically significant differences in overall severity ($p = 0.0256$, OR CI = (0.0265,0.7901)) and age at first wheelchair use ($p = 0.0012$, CI = (0.2694, 1.0926), and they were more likely to have normal eye movements ($p = 0.0011$, OR CI = (0.0333,0.4315)). These individuals were also more likely to have a malignancy ($p = 0.0389$), with an odds ratio of 4.94 (OR CI = (1.0849,22.5292)) compared with the rest of the cohort (see, Figure 3, Table 3 and Supplementary Table 2).

Although there were no statistically significant differences in neurological group, it is striking that predominant extrapyramidal signs were only seen in patients with missense mutations (Group 2 and patient 50).

Individuals with a high ATM protein level had lower alpha-fetoprotein levels (p = 0.0235, CI = (-1.7628,-0.1270)), and slightly higher A-T NEST scores (p-value = 0.0319, CI = (0.0713,1.5825)).

There were no statistically significant associations between chromosomal radiosensitivity or number of mild mutations with neurological or other clinical features.

Discussion

This study describes the largest published cohort of individuals with variant Ataxia-Telangiectasia who have undergone detailed genetic and clinical evaluation. We show that all individuals included in this survey have neurological involvement, whilst systemic features are mild or absent, apart from an increased risk of malignancy.

Our study confirms that disease severity in variant Ataxia-Telangiectasia is milder compared to the classic form. Our cohort includes 31 patients aged >= 40 years, 26% were still ambulant, six had children and 11 were in paid or voluntary employment. Consistent with other reports[13], the majority of individuals included in this study had their symptom onset in early childhood . However, disease progression is then much slower than in the classic form.

We formally graded disease severity according to a combination of neurological rating scales (A-T NEST, SARA) and assessment of mobility and self-care. According to this grading, 32% of patients had mild disease and only 19% severe disease. Similar to Multiple sclerosis, another multisystem neurological disease, we have chosen to grade disease severity mainly according to functional impairment and mobility[34]. Further studies will be required to validate our severity rating.

All individuals in our cohort showed neurological abnormalities on clinical examination. A range of neurological systems were affected (cerebellum, peripheral nerves, eye movements, extrapyramidal system), but none universally. For example, ten individuals had no significant cerebellar features, eleven had normal eye movements and eight had no evidence of peripheral nerve involvement. A large proportion had extrapyramidal symptoms and in some, extrapyramidal presentation was the predominant or only clinical feature. The most common extrapyramidal

symptoms were dystonia and dystonic tremor. Chorea and parkinsonism were rare. The presence of exclusive extrapyramidal symptoms has not been reported in classic A-T. This suggests possible differences in neuronal vulnerability and pathways between the classic and variant form of the disease and could be further explored with imaging studies.

With the limitation of small sample size, our study suggests that disease severity is milder in individuals with a purely extrapyramidal presentation compared to those who have ataxia. This finding could partly reflect effective treatments (botulinum toxin, medication and deep brain stimulation) for extrapyramidal symptoms. Longitudinal studies will be important in order to evaluate whether individuals with exclusive extrapyramidal or cerebellar symptoms eventually progress to develop a more mixed presentation.

Our study is the first to report on neurological investigations in a large number of patients with variant Ataxia-Telangiectasia. We found that cerebellar and vermian atrophy was the most common imaging abnormality, although 5 individuals had a normal cerebellar appearance. Cerebral microhaemorrhages were shown in 2/19 patients whose scan included GRE and SWI and white matter abnormalities occurred in 7/23 patients. Reported radiological findings in classic Ataxia-Telangiectasia include cerebellar atrophy and multiple punctate haemosiderin deposits, but in some individuals also very extensive white matter abnormalities[35] which were speculated to represent 'oedema from vascular leakage'[36]. It is possible that that the extracerebellar abnormalities we observed could represent an earlier stage of a process that progresses more floridly in some individuals with classic Ataxia-Telangiectasia.

Neurophysiology studies showed that most patients tested had an axonal sensorimotor polyneuropathy (18/34), three had predominant anterior horn involvement and nine had a combination of both. Interestingly, neurophysiology was normal in four included individuals, all of whom had mild disease progression but otherwise a mixture of associated neurological symptoms.

Our study confirms that the risk of malignancy is significantly increased in variant Ataxia-Telangiectasia. The cancers in our cohort were predominantly female pre-menopausal breast cancer and haematological malignancies. There were only two

childhood tumours which is consistent with Reiman's study[24] which showed a protective effect of retained ATM kinase activity against childhood tumours.

Individuals with a missense mutation which causes production of a mutant ATM protein with retained kinase activity were about five times more likely to have a malignancy than individuals in the other genetic groups. It has been previously reported that a specific missense mutation c. 7271T>G, p.(Val2424Gly) causes a high risk of breast cancer[16]. Canadian Mennonites who were homozygous for the c.6200 C>A (p. Ala2067Asp) missense mutation also showed a high risk of malignancy[14]. This is consistent with the idea that specific missense mutations cause a high risk of malignancy, which suggests a possible gain of function mechanism.

We found that individuals with a missense mutation have milder neurological features, as measured by overall severity and A-T NEST score. These patients are more likely to remain ambulant and more likely to have normal eye movements.

The four individuals (33, 40, 41 and 42) who have borderline low/absent ATM kinase activity had more severe neurological features and two required gastrostomy feeding. Patients with a mutation in the initiator methionine were included because of the evidence of longevity in some of these patients and that their cells express a low level of N-terminally truncated ATM protein, although, as it turns out, without measurable activity using the assay described. A more sensitive assay would be helpful in detecting differential low levels of ATM activity/signalling associated with different ATM proteins. This may also be true of some ATM missense mutations (e.g. c.5228C>T; p.(Thr1743Ile) where current assays do not detect any activity associated with the expressed protein. It is possible that there may be sufficient activity retained to have some positive clinical effect. Individuals (3, 4, 5, 16, 20, 22, 23a, 23b, 27, 31, 32) with a high ATM level (around 50% or more) showed higher A-T NEST scores (less severe disease) and lower alpha-fetoprotein levels. Taken together, these results indicate that the reduced severity of the neurological features may reflect the level of ATM kinase activity, which is consistent with the fact that classic Ataxia-Telangiectasia (where ATM kinase is absent) generally presents with a more severe phenotype than variant Ataxia-Telangiectasia.

Each of the five sets of siblings included here showed similar neurological features. For example, three affected sisters all have ataxia together with peripheral neuropathy, dystonic tremor and essentially normal eye movements. Three Dutch siblings all presented with resting tremor and distal muscle weakness due to anterior horn cell involvement[18]. This similarity between siblings is consistent with the fact that the neurological phenotype is partly determined by shared 'genetic background'. However, we did not find significant genotype-phenotype association with regards to neurological group in the cohort overall, indicating an additional influence of shared environmental factors or disease modifying genes.

Systemic complications were mild or absent, apart from the increased risk of malignancy. This is consistent with previous reports [4,13,14] and has significant implications on surveillance and management of these patients. None of the individuals in this study had significant respiratory disease. Functional testing with arterial blood gases and overnight oximetry was essentially normal. Even though 42 out of 53 tested individuals had a detectable abnormality on immunological testing, in general this was subtle and of doubtful clinical significance. No individual required treatment with immunoglobulin replacement and only two used prophylactic antibiotics for recurrent pulmonary infections. Our cohort includes only two patients with diabetes mellitus, which suggests a similar prevalence to in the general population. However, more than a quarter of included individuals had abnormalities in their liver function tests, the relevance of which needs to be further investigated in future studies.

Our results suggest that variant Ataxia-Telangiectasia is likely to be under or misdiagnosed. The time from first symptoms to diagnosis was over ten years in more than half of our cohort and we included individuals with only very mild neurological symptoms. Clinicians may not be familiar with the wide range of clinical presentations of variant Ataxia-Telangiectasia, where eye movements can be normal, conjunctival telangiectasia absent, neurological presentation mainly extrapyramidal and alpha-fetoprotein levels within normal range.

Individuals with variant Ataxia-Telangiectasia require surveillance for malignancy and management guidance – including breast screening in women, minimising exposure to ionising radiation and facilitating specialist assessments. Missense

mutations are associated with milder neurological presentations but have a particularly high malignancy risk and it is important for clinicians to be aware of these phenotypes.

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Author Contributions

KS, NO, HB, MW, MT, AMRT and AH contributed to the conception and design of the study.
All authors contributed to the acquisition and analysis of data.
KS, NvO, NO, HB, DS, JR, LB, MT, AMRT and AH contributed to drafting the text and preparing the figures.

Potential Conflicts of Interest

Nothing to report.

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Legends

Table 1 – Patient demographics, *ATM* Mutations, and Protein Changes in 57 Variant Ataxia-Telangiectasia Patients

Table 2 – Neurological Features

Table 3 – Comparison of clinical features in individuals either with one or more missense mutations which produce mutant protein with residual kinase activity or with a leaky splice site mutation also expressing ATM protein with activity

Figure 1 – Age at (A) symptom onset, (B) diagnosis with Ataxia-Telangiectasia and (C) first using a wheelchair were obtained from each individual's clinical notes

Figure 2

A– MRI scans showing examples of mild, moderate and severe cerebellar atrophy. The top row shows midline images to assess vermian atrophy and the bottom row shows parasagittal images for assessing hemispheric atrophy.

B – MRI scans showing examples of white matter lesions

C – MRI scans showing microbleeds

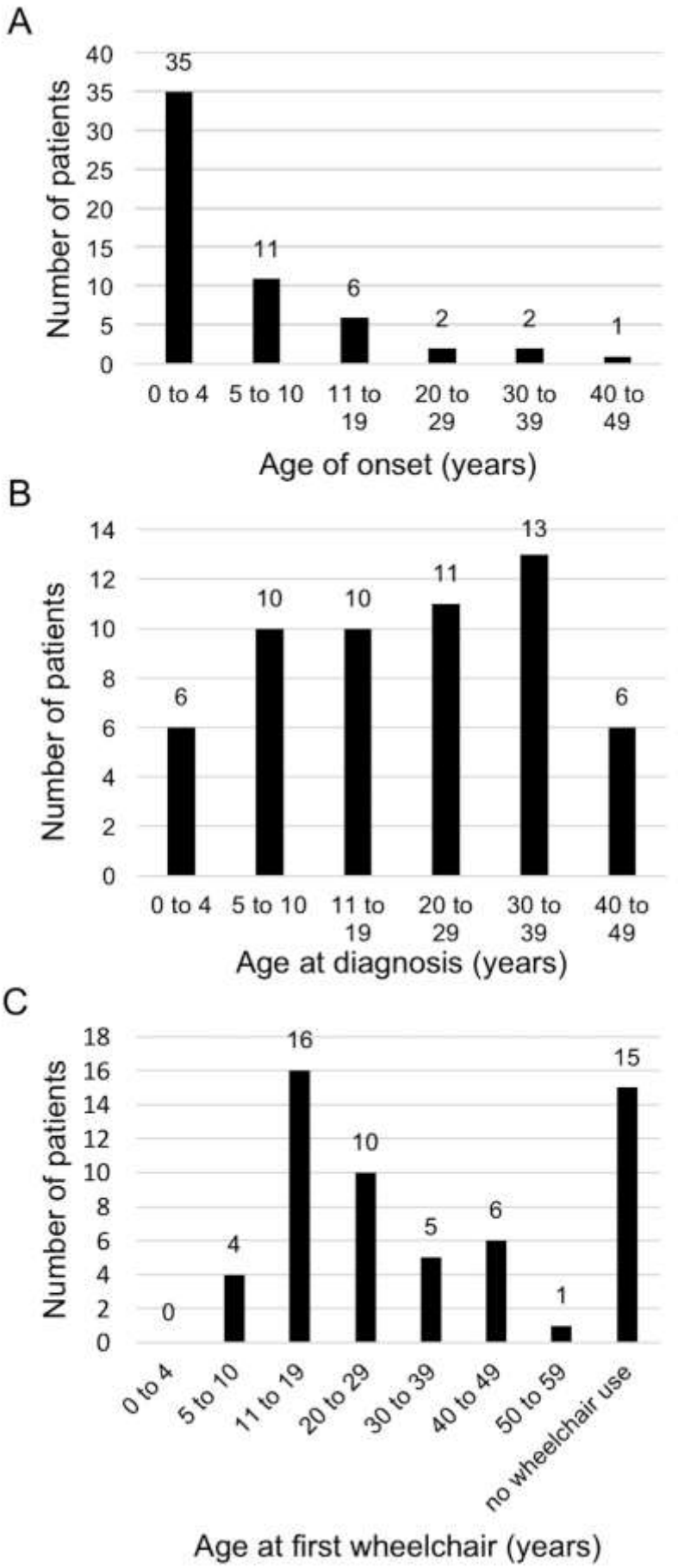
Figure 3

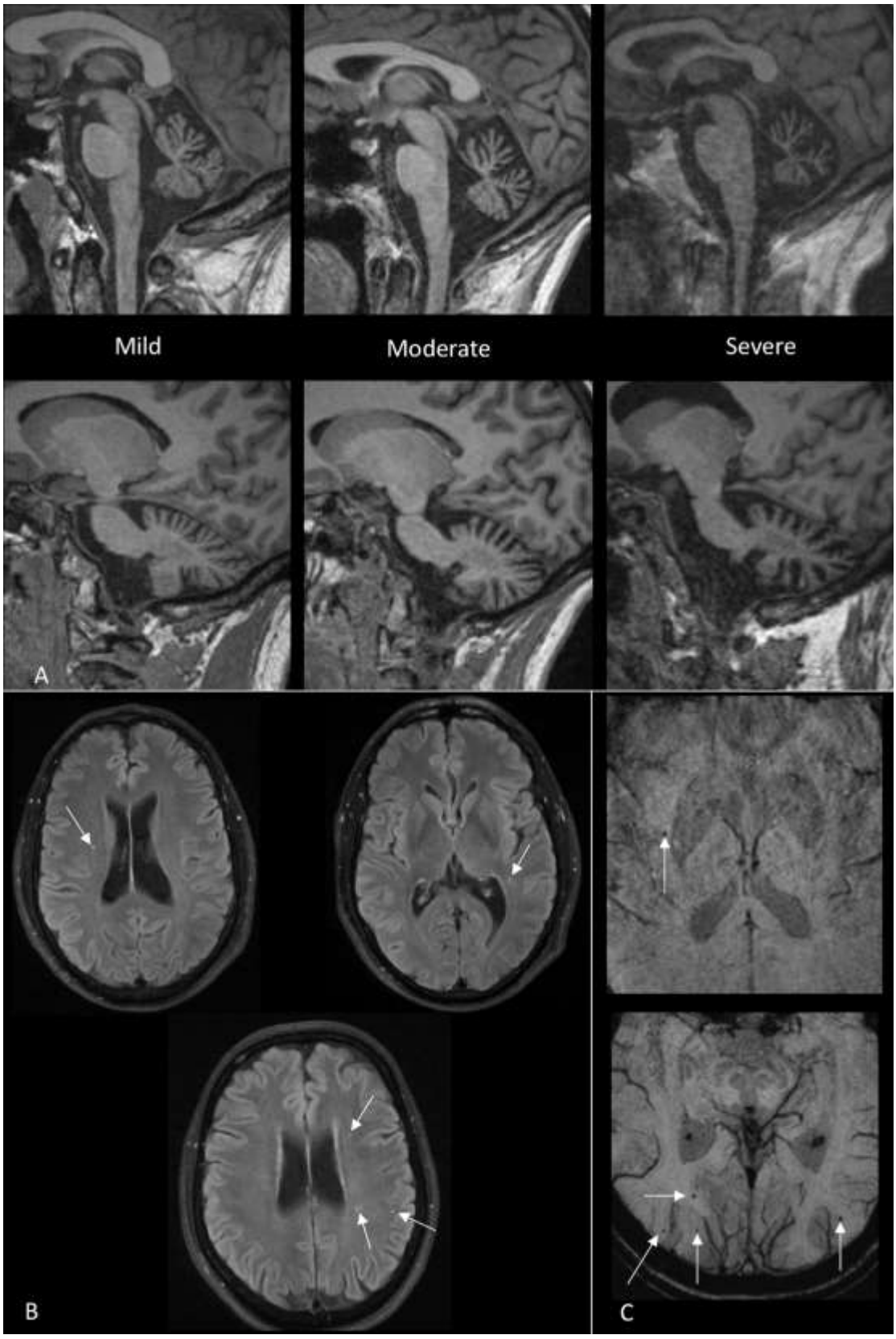
A - Graph shows number of individuals in each neurological phenotypic group in patients with retained ATM kinase activity due to a missense mutation or a leaky splice site mutation.

B - Graph shows numbers of individuals who use a wheelchair or are still ambulant in patients with retained ATM kinase activity due to a missense mutation or a leaky splice site mutation.

C- Graphs shows Cross-Sectional Clinical Neurological Disease Severity in patients with retained ATM kinase activity due to a missense mutation or a leaky splice site mutation.

D - Graph shows number of malignancies diagnosed in patients with retained ATM kinase activity due to a missense mutation or a leaky splice site mutation.





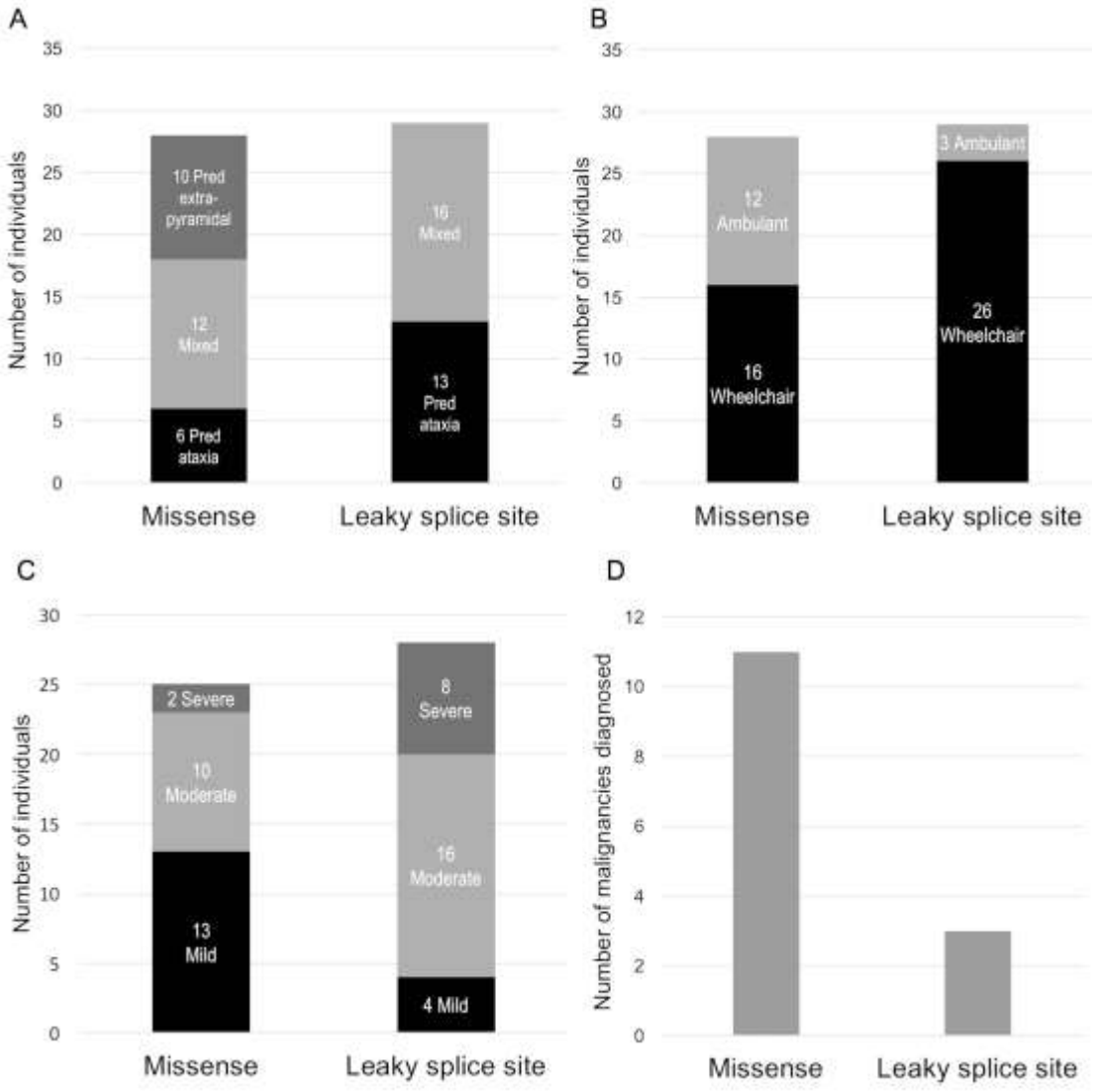


Table 1 - *ATM* Mutations, Protein Changes and ATM kinase activity in 57 Variant Ataxia-Telangiectasia Patients

Patient (gender, age,)	ATM Mutation 1	ATM Mutation 2	Neur
Genetic group 1 - some normal ATM protein, with residual kinase activity present, due to a leaky splice site mutation			
1 (F,38)	c.5763-1050A>G, p.(Pro1922fs)	duplication of ATM exons 53-61	B
2 (M,41) (Ref 21)	c.5763-1050A>G, p.(Pro1922fs)	c.5763-1050A>G, p.(Pro1922fs)	A
3 (F,36)	c.5763-1050A>G, p.(Pro1922fs)	c.9022C>T, p.(Arg3008Cys)	B
4 (M,47)	c.5763-1050A>G, p.(Pro1922fs)	c.9022C>T, p.(Arg3008Cys)	B
5 (F,55)* (Ref 15)	c.5763-1050A>G, p.(Pro1922fs)	c.9139C>T, p.(Arg3047Ter)	B
6 (F,55)	c.5763-1050A>G, p.(Pro1922fs)	c.2T>C, p.(Met1Thr)	B
7 (F,20)	c.5763-1050A>G, p.(Pro1922fs)	c.1563_1564delAG, p.(Glu522IlefsTer21)	B
8 (F,40)*	c.5763-1050A>G, p.(Pro1922fs)	c.6136delC, p.(Leu2046fsTer1)	A
9 (M,38)*	c.5763-1050A>G, p.(Pro1922fs)	c.6199-6_6227del35, p.(Ala2067GlnfsTer10)	B
10 (F,50)	c.5763-1050A>G, p.(Pro1922fs)	c.8418+2_5delTGAG, p.(Val2757_Met2806del)	B
11 (M,49)	c.5763-1050A>G, p.(Pro1922fs)	c.8786+1G>A, p.(Gly2891fs)	A
12 (M,21)	c.5763-1050A>G, p.(Pro1922fs)	c.8491_8497del7, p.(Phe2831ThrfsTer24)	B
13 (M,60)*	c.5763-1050A>G, p.(Pro1922fs)	c.2284-2285delCT, p.(Leu762fs)	A

14 (F,22)*	c.5763-1050A>G, p.(Pro1922fs)	c.6198+1G>A, p.(Leu2033ProfsTer15)	B
15 (M,31)	c.8418+681A>G, p.(Glu2807ValfsTer4)	c.1564_1565delGA, p.(Glu522IlefsTer43)	B
16 (M,45)	c.1066-6T>G, Loss of exon 9	c.9023G>A, p.(Arg3008His)	A
17 (F,31)	c.6807G>A, Loss of exon 46	c.1158delG, p.(Lys387fs)	B
18a (F,43), 18b (M,40)	c.331+5G>A	c.331+5G>A, exon 4 splice donor defect	A, A
19 (F,48)* (Ref 4,7)	c.496+5G>A, p.Arg111_Glu166del55insLys	c.7875_7876delTGinsGC, p.Asp2625_Ala2626delinsGluPro	B
Genetic group 2- mutant ATM protein present with residual kinase activity from a missense mutation			
20 (F,27)	c.7271T>G, p.(Val2424Gly)	c.103C>T, p.(Arg35Ter)	C
21 (F,48)*	c.7271T>G, p.(Val2424Gly)	c.8266A>T, p.(Lys2756Ter)	B
22 (M,26)	c.7271T>G, p.(Val2424Gly)	c.3G>T, p.(Met1Ile)	A
23a (M,47), 23b (M,36)	c.7271T>G, p.(Val2424Gly)	c.8269-loss of exon 57 by MLPA	A, A
24 (M,41) (Ref 33)	c.743G>T, p.(Arg248Leu)	c.8266A>T, p.(Lys2756Ter)	C
25 (M,52) (Ref 32)	c.743G>T, p.(Arg248Leu)	c.5623C>T, p.(Arg1875Ter)	C
26 (F,27) (Ref 17)	c.590G>A, p.(Gly197Glu)	c.590G>A, p.(Gly197Glu)	C
27 (F,56c) (Ref 31)	c.8672G>A, p.(Gly2891Asp)	c.1A>G, p.(Met1Val)	A
28 (F,25)	c.8480T>G, p.(Phe2827Cys)	c.1564_1565delAG, p.(Glu522IlefsTer21)	B
29a (F,50c), 29b (F,47c), 29c (F,41c)	c.7184A>T, p.(Asp2395Val)	c.6490G>T, p.(Glu2164Ter)	BBB
30 (F,32)	c.875C>T, p.(Pro292Leu)	c.5129_5763-1060del9263 p.(Glu1669AspfsTer16) deletion of exons 35-38	A
31 (M,21)	c.875C>T, p.(Pro292Leu)	c.8494C>T, p.(Arg2832Cys)	B
32 (M,32)	c.6115G>A, p.(Glu2039Lys)	c.8609_8610delAT, p.(Asp2870GlufsTer10)	C
33 (F,11)	c.9103C>T, p.(Leu3035Phe)	c.9103C>T, p.(Leu3035Phe)	A
34 (M,13)(PD)	c.7013T>C, p.(Leu2338Pro)	c.6056A>G, p.(Tyr2019Cys)	B
35 (M,15)	c.8494C>T, p.(Arg2832Cys)	c.1844C>T, p.(Leu615Pro)	B
36 (F,40) (Ref 4,7)	c.2909T>G, p.(Leu970Arg)	c.6908dupA, p.(Glu2304fs)	B
37a (M,39), 37b† (M,48), 37c† (M,48)	c.3136C>T, p.(Leu1046Phe) (Ref 4,7,18)	c.7622T>G, p.(Leu2541Arg)	CCC
38 (F,42) (Ref 4,7)	c.8147T>C, p.(Val2716Ala)	c.5932G>T, p.(Glu1978Ter)	C
39† (F,47)* (Ref 4,7)	c.8147T>C, p.(Val2716Ala)	c.1391_1395delTGTGT, p.(Leu464SerfsTer21)	B
40 (F,47)* (Ref 4,7)	c.8147T>C, p.(Val2716Ala)	c.717_720delCCTC, p.(Val240fs)	B
41 (F,58c) (Ref 4,7)	c.8147T>C, p.(Val2716Ala)	c.2922-1G>A, p.(Asn975_Trp1026del)	B
Genetic group 3 - patients with mutations in initiator methionine, with no likely kinase activity			
42 (F,23)(PD)	c.2T>C, p.(Met1Thr)	c.9139 C>T, p.(Arg3047Ter)	B
43 (F,42)*	c.2T>C, p.(Met1Thr)	c.640_640delT, p.(Ser214ProfsTer16)	A
44 (M,22)*	c.2T>C; p.(Met1Thr)	c.7665delinsGTGA, p.His2555GlnfsX2	A
Genetic group 4 - patients with residual ATM kinase activity with one mutation not detected or incompletely resolved			
45 (F,30)	c.5644C>T, p.(Arg1882X)	not detected	B
46a (M,56), 46b (M,54)	c.170G>A, p.(Trp57Ter)	c.3403del174(del exon 24)-incompletely resolved	A, A
47 (F,26)	c.387delA, p.(Asp130fsTer23)	skipping exons 31 and 32 - incompletely resolved	B
48 (F,31c)	c.2466+6T>A complex, unresolved	not detected	A
49 (F,15)	c.2731dupG, p.(Ala911GlyfsTer9)	possible splice mutation involving exons 33 and 34	A
50 (F,45)* (Ref 4,7)	c.8147T>C, p.(Val2716Ala)	not detected	C

Ref= reference to previous publication, †= individual deceased, c= individual has offspring *=kinase activity not tested, Neur =neurology group PD=possibly just detectable kinase activity

Table 2 – Neurological Features and malignancies in each neurological phenotypic group

	A: Cerebellar ataxia and/or peripheral	**B: Cerebellar ataxia and/or peripheral**	**C: Extrapyramidal**

	neuropathy with minimal or no extrapyramidal involvement	**neuropathy plus additional extrapyramidal features**	**signs without significant ataxia and/or peripheral neuropathy**
Number of individuals	19	28	10
Mean age at assessment	38.8 years (range 11 to 56)	35.7 years (range 13 to 56)	39.8 years (range 27 to 52)
Mean disease duration (years)	32	30	28
Onset	Early 16: Late 3	Early 25: Late 3	Early 5: Late 5
Progression	Slow: 5 Slow/Mod: 4 Moderate: 7 Rapid: 3	Slow: 9 Slow/Mod: 2 Moderate: 9 Rapid: 8	Slow: 7 Slow/Mod: 1 Moderate: 1 Rapid: 0
Current severity	Mild: 3 Moderate: 10 Severe: 6	Mild: 6 Moderate: 15 Severe: 4 Not documented: 3	Mild: 8 Moderate:1 Severe: 0 Not documented:1
Mean A-T NEST	61.0 (range 36 to 95)	58.2 (range 34 to 87)	64.3 (range 57 to 72)
Mean SARA	26.0 (range 4 to 38)	22.1 (range 6 to 34)	11.6 (range 6 to 21)
Eye movements	Normal: 1 Mildly abnormal: 6 Moderate/severe oculomotor dyspraxia: 11	Normal: 4 Mildly abnormal: 12 Moderate/severe oculomotor dyspraxia: 12	Normal: 6 Mildly abnormal: 2 Moderate/severe oculomotor dyspraxia: 2
Neurophysiology	N=12 Normal neurophysiology studies: 1 Axonal sensorimotor neuropathy: 8 Axonal sensorimotor neuropathy and likely spinal muscular atrophy: 3	N=14 Axonal sensorimotor neuropathy: 10 Axonal sensorimotor neuropathy and likely/possible spinal muscular atrophy: 4	N=8 Normal neurophysiology studies:1 Axonal sensorimotor neuropathy: 3 Axonal peripheral neuropathy and affected motor neurons: 3 Affected motor neurones only:1
Neurological Treatment	No specific neurological treatment relating to A-T: 14 Amitriptyline for painful neuropathy: 1 Propranolol and primidone for tremors: 1 Clonazepam and baclofen: 1 Amitriptyline and oxybutynin:1	No specific neurological treatment relating to A-T: 16 Single agents: Madopar:1 Trihexiphenidyl:2 Sinemet plus:2 Baclofen:1 Oxybutynin (for drooling):1 Leviteracitam 1 Two agents: Propranolol and primidone:1 Pregabalin and amantadine:1 Amifampridine and trihexiphenidyl: 1 Four agents: Clonazepam, amantadine, pregabalin, trihexyphenidyl	No specific treatment relating to A-T: 6 Deep brain stimulation: 1 Botulinum toxin injections: 1 Botulinum toxin injections and clonazepam:1 Baclofen and benzhexol: 1
Malignancy	Breast cancer 43y Dermatofibroma 29y	Breast cancers 29y, 43y Neuroendocrine tumour	Breast cancers 28y, 33y

	Germ cell tumour 11y ALL 9y	48y T cell non-Hodgkin lymphoma 2y CLL died 47 years CML 39y	Prostate cancer 52y Pancreatic cancer died 48y ALL died 51 years

Table 3 – Comparison of clinical features in individuals either with one or more missense mutations which produce mutant protein with residual kinase activity or with a leaky splice site mutation also expressing ATM protein with activity

	>=1 missense mutation producing mutant protein with retained ATM kinase activity	**Leaky splice site mutation producing protein with retained ATM kinase activity (1)**
	28 individuals	29 individuals
Demographic features		
Male/Female	11/17	12/17
Mean age (range)	38 (13-58)	37 (15-56)
Median age at onset (range)	3 (0.5 to 44)	3 (1 to 22)
Median age at diagnosis (range)	28 (2 to 47)	16 (3.5 to 44)
Neurological features		
Neurology group A/B/C	6/12/10	13/16/0
Number with wheelchair/ still ambulant	16/12	26/3
Median age at first wheelchair among users(range)	30.5 years (8 to 51)	16 years (8 to 40)
Cross-sectional Clinical Neurological Disease Severity (mild/ moderate/ severe)	13/10/2 (not recorded in 3)	4/16 /8 (not recorded in 1)
Median SARA	17	24.5
Median AT-nest	61.5	56
Peripheral neuropathy (present/ absent)	21/5 (not recorded in 2)	25/3 (not recorded in 1)
Eye movements (moderate to severe ocululotor dyspraxia/ mild abnormalities/ normal)	4/13/11	21/7/1
Non-neurological Features		
Conjunctival telangiectasia (present/ absent)	14/12 (1 not documented)	21/8
CT chest	Bronchiectasis 3 Normal findings 9 intrapulmonary nodules 1 Not done 12	Bronchiectasis 3 Normal findings 21 Emphysema 1 Patchy air trapping 1 Not done 3
Personal history of malignancy	Breast cancer (4 patients) CML CLL ALL Non Hodgkins Lymphoma Pancreatic cancer Germ cell tumour Prostate cancer	Breast cancer T cell ALL Dermatofibrosarcoma protuberans
Laboratory features		
Mean alpha-fetoprotein microgram/L (range)	203 (10-600)	118 (2 to 332)
Chromosomal radio-sensitivity (raised/ normal)	16/6 (6 not done)	17/9 (3 not done)

(1) These also include the three group 3 patients