Supplementary methods

Abnormal movements polygraphic recording

Abnormal movement recording was performed in 13 patients, in the same neurophysiology department (EA, YAB, Saint-Antoine Hospital) with a Neuropack (Nihon Kohden, Japan) device.

Polymyography - Upper limbs movement was recorded with a unidirectional linear piezo-resistive accelerometer (Acc) (PCB Piezotronics, USA), attached to the proximal phalanx of the index. Acc signal was band-pass filtered at 0.5–100 Hz. Electromyographic (EMG) signals were obtained from pairs of 9-mm diameter silver/silver chloride electrodes (Medtronic, Minneapolis, MN, USA) placed 2 cm apart over muscle bellies. The selection of recorded muscles was guided by the clinical location of movement disorders, (1) infraspinatus, biceps brachii, triceps brachii, extensor carpi radialis (ECR), flexor carpi radialis (FCR) and first dorsal interosseous (FDI) muscles for UL study, (2) sternocleidomastoid (SCM) and splenius muscles, bilaterally, for cervical study, (3) vastus lateralis, tibialis anterior and gastrocnemius for LL study. EMG signals were band pass filtered at 20-500 Hz. Patients were recorded during rest, posture and action/intention. The effect of mental calculation or verbal enumeration on tremor was tested at rest. Stimulus-sensitive myoclonus was systematically searched for with a slight distal touch, a pinprick and passive mobilization of the wrist. Postural and action/intention tremor/myoclonus were analyzed during tonic contraction, slow elementary non goal-directed movements, goal-directed movements and spiral drawing. The frequency of tremor/myoclonus and the burst duration were measured by manual analysis of EMG and Acc epochs. Temporo-spatial organization of myoclonus was also studied. Co-contractions between agonist and antagonist muscles, spasms and tonic activity were systematically looked for as a marker of dystonia, whereas the overflow evaluation was based on clinical observation.
Cortical tests – Jerk-Locked Back-Averaging (JLBA) and C-reflex studies were conducted as previously described. JLBA was obtained on 4 out of 11 recorded patients with myoclonus (see table 2); it was systematically performed when the upper limit duration of the EMG bursts was below 100 ms (patients 7, 9 and 10). C-reflex study was performed in 10 out of 11 recorded patients with myoclonus.

Oculographic recording

Oculographic recording was performed in 13 patients. The subjects were seated in complete darkness facing a screen located 60 cm in front of them, with a head and a chinrest. Eye movements were recorded with a video-based monocular eye tracker (500Hz, SMI, Germany). The protocol consisted of a visually guided saccade task (VGST), an antisaccade task (AST) and a smooth pursuit task (SPT). In the VGST, subjects were asked to follow as fast and as accurately as possible a visual target stepping from a central to a 13° lateral right or left position A 200 ms blank interval (gap) occurred between central fixation offset and lateral target onset. In the AST, the same design was used except that subjects were instructed not to follow the lateral target but instead to look as fast as possible towards the opposite direction. A minimum of 18 trials in each direction was recorded in each subject and in each task. In the SPT, subjects were asked to follow as accurately as possible a target that moved horizontally with a slow sinusoidal velocity (peak velocity: 15 and 28°/s). In the VGST, saccade latency and gain (ratio of initial saccade amplitude on target step) were automatically determined (Eyebrain software). Saccades with latencies below 90 ms or perturbed by blinks were discarded. In the AST, correct antisaccade latency and antisaccade error rate (i.e. the percentage of misdirected saccades) were similarly analysed.

Nerve conduction study

Motor and sensory nerve conduction studies (NCS) were performed in 12 patients. NCS were realized with a Medtronic KEYPOINT device (Alpine Biomed, USA). Skin impedance was
maintained below 5KΩ and limb temperature at 32°C. Motor nerves examination included at least bilateral peroneal and right posterior tibial nerves at the lower limbs (LL), and right median and ulnar nerves at the upper limbs (UL), using supra-maximal stimulation. Additionally, other nerves were sometimes studied. For each motor nerve, distal latency (DL), peak to baseline amplitude of the compound muscle action potentials (CMAPs), motor nerve conduction velocity (MNCV) and F-wave latency (F-WL) were recorded with classical procedure.

Sensory nerves study included sural nerves at the LL bilaterally as well as right median and ulnar nerves at the UL. For each sensory nerve, distal latency (DL), peak to peak amplitude of the sensory nerve action potential (SNAP) and sensory nerve conduction velocity (SNCV) were measured. Sensory nerve action potentials (SNAPs) were recorded with an orthodromic procedure for median and ulnar nerves and antidromically for sural nerves.

**Magnetic resonance imaging**

All patients underwent brain MRI. All MRIs included T1, T2-weighted and FLAIR images. Axial, sagittal and coronal images were performed.

**123I-ioflupane SPECT**

Dopamine transporter SPECT imaging was performed in 5 patients in a single Nuclear Medicine department. All subjects received potassium perchlorate to block thyroid uptake prior to injection. Images were acquired 3 - 4 h after intravenous injection of 150–185 MBq of 123I-FP-CIT (DaTSCAN; GE Healthcare) on a head camera. Transverse slices with clear visualization of the striatum were provided. A visual assessment of images was performed according to previously proposed criteria² as following: i. normal: tracer uptake bilaterally in putamen and caudate nuclei and largely symmetric; ii. abnormal image grade 1: asymmetric uptake with normal or almost normal putamen activity in one hemisphere and with a more marked reduction in the contralateral putamen ; abnormal image grade 2: Significant bilateral
reduction in putamen uptake with activity confined to the caudate nuclei; abnormal image
grade 3: Virtually absent uptake bilaterally affecting both putamen and caudate nuclei.

**Biological testing**

Blood samples were collected for all the patients. The following tests were performed: (i) class A, M and G immunoglobulins plasma levels, (ii) alpha-foetoprotein plasma level and (iii) high resolution karyotype.

**Western blotting**

Cells were collected by centrifugation at 1500 rpm for 10 minutes at 4°C and washed in cold phosphate buffered saline (PBS). Cell pellets were lysed in radioimmunoprecipitation assay buffer (RIPA) [100mM Tris-HCl/pH7.5, 0.1M NaCl, 1mM EDTA, 1% Triton, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS)], supplemented with NaF, Na$_3$VO$_4$, phenylmethanesulfonylfluoride (PMSF) and a cocktail of protease inhibitors (cOmplete®, Roche). 50µg of protein extracts were resolved by 4-15% SDS-PAGE (SDS-PAGE Mini-
PROTEAN® TGX™ #456-1086 Biorad) and transferred to 0.2 µm nitrocellulose membranes (Biorad). Membranes were blocked in Odyssey blocking buffer (LI-COR #927-40000) for 1h at RT and incubated overnight at 4°C with the primary antibodies at the following concentrations: $ATM$ (NB110-55475, 1/1000, Novus Biologicals), Phospho-$KAPI$ Ser824 (A300-767A, 1/1000, Bethyl laboratories), $KAPI$ (A300-275A,1/1000, Bethyl laboratories), and β-actin (A-5316, 1/10000, Sigma) used as a control for protein loading. The secondary antibody coupled to IRDye 800CW (anti-mouse : 926-32212, LI-COR; and anti-rabbit : 926-32213, LI-COR) was revealed using the Odyssey Quantitative Fluorescence Imaging System and band intensities were quantified using Odyssey software.

**Genetic analyses**

The 62 coding exons of $ATM$ and an average of 30 nucleotides spanning each exon/intron junction were analyzed by direct sequencing of genomic DNA (RefSeq U82828.1). Primer
sequences and PCR amplification conditions are available upon request. The search for large gene rearrangements was performed by semiquantitative PCR, using the Multiplex Ligation-dependent Probe Amplification (MLPA) kit (SALSA MLPA KIT P041/P042 ATM, MRC-Holland, Amsterdam, The Netherlands). In addition, ATM missense mutations were indirectly studied through the response to ionizing radiation (IR) of lymphoblastoid cell lines established from patients carrying the mutation at the homozygote state. For compound heterozygote mutations, the presence of one mutation on each parental allele (in trans) was verified when parental DNA was available. In addition, genetic samples of 200 healthy European individuals were used as control for ATM sequencing. As nucleotide variations can lead to splicing abnormalities, the genomic sequence environment of each DNA variant was analyzed using Splice Site Prediction by Neural Network (NNSPLICE available at http://www.fruitfly.org/seq_tools/splice.html), MaxEntScan (MES available at http://genes.mit.edu/burgelab/maxent/Xmaxentscan_scoreseq.html). All missense mutations with unknown biological effects were considered to have a likely pathogenic effect when at least 2 of the following criteria were present: carrier frequency of less than 1% in a series of controls, location in a domain of ATM that is required for function (especially the phosphatidylinositol 3-kinases or domains FRAP-ATM-TRRAP), or a high score from the Align-GVGD algorithm, which quantifies amino acid changes and their conservation among species. The IR cellular response was studied through the KAP1 phosphorylation, an ATM target analyzed according to Jacquemin et al. The ATM mutation detection rate in A-T patients has been estimated to be 96% (292 mutations found in a series of 305 alleles tested).

References


