ORIGINAL ARTICLE

Mitochondrial *PITRM1* peptidase loss-of-function in childhood cerebellar atrophy

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ABSTRACT

Objective To identify the genetic basis of a childhood-onset syndrome of variable severity characterised by progressive spinocerebellar ataxia, mental retardation, psychotic episodes and cerebellar atrophy.

Methods Identification of the underlying mutations by whole exome and whole genome sequencing. Consequences were examined in patients' cells and in yeast.

Results Two brothers from a consanguineous Palestinian family presented with progressive spinocerebellar ataxia, mental retardation and psychotic episodes. Serial brain imaging showed severe progressive cerebellar atrophy. Whole exome sequencing revealed a novel mutation: pitrilysin metallopeptidase 1 (*PITRM1*) c.2795C>T, p.T931M, homozygous in the affected children and resulting in 95% reduction in *PITRM1* protein. Whole genome sequencing revealed a chromosome X structural rearrangement that also segregated with the disease. Independently, two siblings from a second Palestinian family presented with similar, somewhat milder symptoms and the same *PITRM1* mutation on a shared haplotype. *PITRM1T931M* carrier frequency was 0.027 (3/110) in the village of the first family evaluated, and 0/300 among Palestinians from other locales. *PITRM1* is a mitochondrial matrix enzyme that degrades 10–65 amino acid oligopeptides, including the mitochondrial fraction of amyloid-beta peptide. Analysis of peptide cleavage activity by the *PITRM1T931M* protein revealed a significant decrease in the degradation capacity specifically of peptides ≥40 amino acids.

Conclusion *PITRM1T931M* results in childhood-onset recessive cerebellar pathology. Severity of *PITRM1*-related disease may be affected by the degree of impairment in cleavage of mitochondrial long peptides. Disruption and deletion of X linked regulatory segments may also contribute to severity.

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INTRODUCTION

Autosomal recessive spinocerebellar ataxias (ARCA) are a clinically and genetically heterogeneous group of neurodegenerative disorders associated with cerebellar atrophy or hypoplasia, imbalance and uncoordinated gait.[1] The reported overall prevalence of ARCA is estimated to be 2.2–5.3 per 100 000 births.[2] Common forms of recessive ataxias include ataxia-telangiectasia, Friedreich ataxia and ataxia oculomotor apraxia. Wide phenotypic variability is observed even with mutations in the same ARCA-related gene.[3] Uncommon ARCA forms constitute a heterogeneous group of disorders with early onset ataxia as the predominant feature; intellectual disability is present in >60% of these patients.[1] Mutations in >45 genes are known to cause ARCA, yet the molecular causes of ARCA in many patients remain unknown.[3] Recently, homozygosity for *PITRM1*^{R183Q} was described in a Norwegian brother and sister in their late 60s with recessive spinocerebellar ataxia.[4] Both had mild mental retardation in childhood and gradually developed spinocerebellar ataxia and obsessional behaviour with psychotic episodes.

Pitrilysin metallopeptidase 1 (*PITRM1*), also known as human presequence protease hPreP, is a 117 kDa mitochondrial matrix enzyme widely expressed in multiple human tissues including skeletal muscle, heart, intestine and cerebellum.[5] *PITRM1* functions as a mitochondrial peptidase, degrading peptides of up to 65 amino acids.[6] Substrates include mitochondrial presequences (oligopeptides cleaved from proteins imported into mitochondria), short unstructured peptides and several forms of amyloid-beta peptide (Aβ).[7 8] Mitochondrial dysfunction is increasingly recognised as a hallmark of neurodegeneration and is a prominent feature in primary genetically determined mitochondrial disease as well as in many prevalent neurodegenerative diseases including Parkinson's disease and Alzheimer's disease (AD).[7 9] In AD, the accumulation of mitochondrial Aβ plaques precedes the characteristic extensive extracellular Aβ deposition and is evident before the onset of clinical symptoms both in patients and in Aβ precursor protein transgenic mice.[10 11]

The *PITRM1R183Q* mutation observed in the Norwegian family resulted in a marked reduction in *PITRM1* protein level in patients' fibroblasts and skeletal muscle cells. In vitro, its catalytic activity was comparable to wild-type protein. In a yeast model system, the R183Q substitution resulted in lower mitochondrial oxygen consumption and cytochrome content and reduced capacity to degrade Aβ.[4] A recent study showed that the structural and electrostatic properties of the conserved

strand-loop-strand motif containing *PITRM1* residue R183 are critically important for *PITRM1* function. They showed that the mutational disruption of electrostatic interactions in proximity of *PITRM1* residue R183 contributes to the loss of enzyme activity and may contribute to the loss-of-function phenotype observed in *PITRM1* R183Q-dependent neuropathy.[12]

Here, we report a novel *PITRM1* mutation causing ARCA in four children from two independently ascertained families. Functional studies performed on patients' fibroblasts, with recombinant protein, and in a yeast model demonstrated that this mutation leads to loss of function and strengthens the connection between impairment of mitochondrial peptide degradation and neurodegenerative diseases.

METHODS

Subjects

Families were seen at the medical genetics and paediatric neurology clinics at Shaare Zedek Medical Center and at Al Makassed Hospital, Jerusalem, Israel. Clinical data, blood samples and skin biopsies were obtained after written consent.

Genomic analyses

Genomic DNA was extracted from peripheral blood mononuclear cells. Chromosomal microarray analysis (CMA) was performed for all affected individuals and for both parents of family A, as previously described.[13] Whole exome sequencing was carried out for all affected individuals in families A and B and for both parents of family A, and whole genome sequencing was carried out for all affected individuals, as previously described.[14 15] *PITRM1*^{T931M} was validated with Sanger sequencing. Segregation in families and mutation frequency in ethnically matched controls from the home village of family A were assayed using a *Bsm*AI restriction digest. Haplotypes were identified using PLINK V.1.9.[16]

Chromosome X inactivation and analysis

Skewing of X inactivation in females was performed on genomic DNA extracted from peripheral blood leucocytes and fibroblasts from the proband of family A and from leucocytes of his mother. Details are provided in online supplementary methods.

RNA and protein expression

Total RNA was extracted from peripheral blood leucocytes drawn into Tempus Blood RNA tubes (ABI). cDNA was prepared using ImPromII Reverse Transcriptase (Promega).

qRT-PCR was performed using Power SYBR master mix (Applied Biosystems) and normalised to glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Experiments were performed in triplicate (see online supplementary table 1).

Protein lysates were extracted from primary fibroblast cell lines. Western blot analysis was performed using rabbit polyclonal anti-*PITRM1* (Sigma, R04409) and anti-GAPDH (Abcam, ab8245), along with an antimouse horseradish peroxidase (HRP)-conjugated secondary antibody (Jackson Immunoresearch and Atlas). Protein quantification was performed using ImageJ[17] on three independent experiments. GAPDH was used as a loading control.

Purification of recombinant human *PITRM1*

PITRM1^{T931M} was constructed by site-directed mutagenesis using the QuikChange II kit (Agilent Technologies) and confirmed by sequencing. Production and purification of *PITRM1*^{WT} and *PITRM1*^{T931M} were performed as previously described.[8]

PITRM1 activity

For analysis of Aβ degradation, *PITRM1* samples (*PITRM1*^{WT} or *PITRM1*^{T931M}) were incubated with Aβ residues 1–42, Aβ residues 1–40, pOTC or pF_1b-54 in degradation buffer at 37°C and results visualised using gel electrophoresis as previously described.[4 18] Experiments were performed in duplicate. In the fluorescence-based assay, samples (*PITRM1*^{WT} or *PITRM1*^{T931M}) were mixed with pF_1b residues 43–53, and the increase in fluorescence recorded as previously described.[4 19] Details are described in the online supplementary methods.

Yeast studies

cym1S882T and *cym1S882M* mutant alleles were constructed, cloned and inserted, together with *CYM1* WT allele and the empty plasmid pFL38, in strain W303-1B *cym1*Δ as previously described.[4] Oligonucleotides for plasmid construction are listed in supplementary table 1.

RESULTS

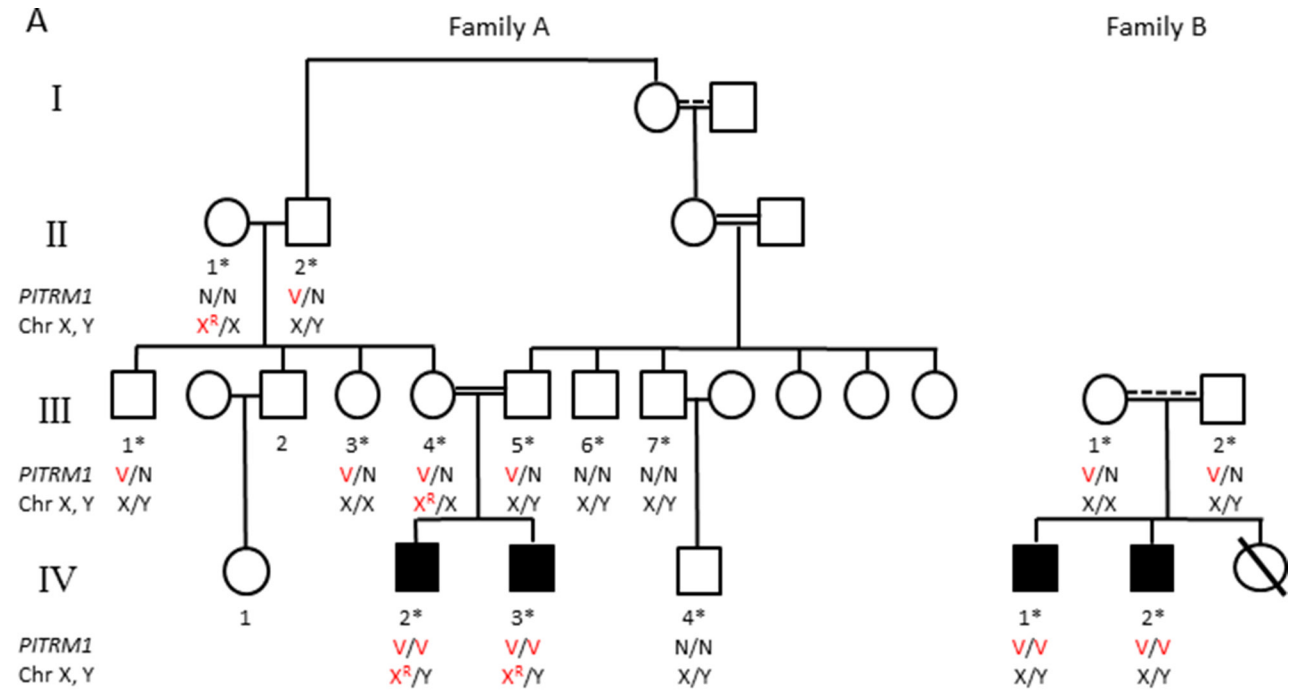
Clinical presentation

Family A

Patient A.IV.3 is the son of consanguineous Palestinian Arab parents. He was born at full term in a vaginal breech delivery after an uneventful pregnancy (figure 1A). Birth weight was 2.7 kg. His development was severely delayed with no regression in early childhood. He presented at the age of 5 months with mild tremor, titubation and brisk deep tendon reflexes. Independent but non-functional walking was gained by the age of 11 years. At age 15 years, he had severe intellectual disability, total expressive aphasia (pronouncing only a few dysarthric words), severe ataxia and dysmetria. He had several psychotic episodes of unexplained anger attacks, each lasting several weeks. There is no history of seizures. Muscle tone and strength were normal. Brain CT at age 6 months was normal. Brain MRI at age 9 years revealed severe cerebellar atrophy (figure 1B); magnetic resonance spectroscopy was normal. Blood tests showed constantly elevated muscle enzyme levels: creatine phosphokinase (CPK) 1400–2900 (normal values 5–130 U/L),[20] lactate dehydrogenase (LDH) 890 (normal values 150–500 U/L) and elevated lactate and pyruvate: pyruvate 0.149 (normal values 0.076±0.026 mmol/L), lactate 1.72 and 3.03 (normal values for age 0.8–1.5 mmol/L). Blood pH, carnitine, acylcarnitine and free fatty acids were within normal limits. Muscle biopsy and mitochondrial oxidative enzyme levels were normal (see online supplementary figure 1). Electromyography (EMG) and nerve conduction velocity tests at the age of 19 years demonstrated only mild symmetric amplitude reduction in sensory responses over the peroneal and sural nerves. Motor conduction velocities (peroneal and tibial nerves) and F-wave responses were normal. The EMG was normal.

The proband's older brother, A.IV.2, had similar but somewhat milder clinical presentation. Pregnancy and delivery were uneventful. He began walking at age 5 years. At age 18 years, he had moderate-to-severe intellectual disability, severe ataxia and psychotic obsession episodes, with no seizures. Brain CT scan at age 22 months showed mild cerebral atrophy, brain MRI at age 18 years demonstrated severe cerebellar atrophy (figure 1B). Routine lab and muscle enzyme levels were within normal limits. Repeated EEG tests of both brothers were normal.

Chromosomal microarray analysis and fragile-X testing were normal for both children. Family history was pertinent for the maternal grandmother reported to have Parkinson's disease. X-inactivation was tested in the mother, revealing 95:5 skewing



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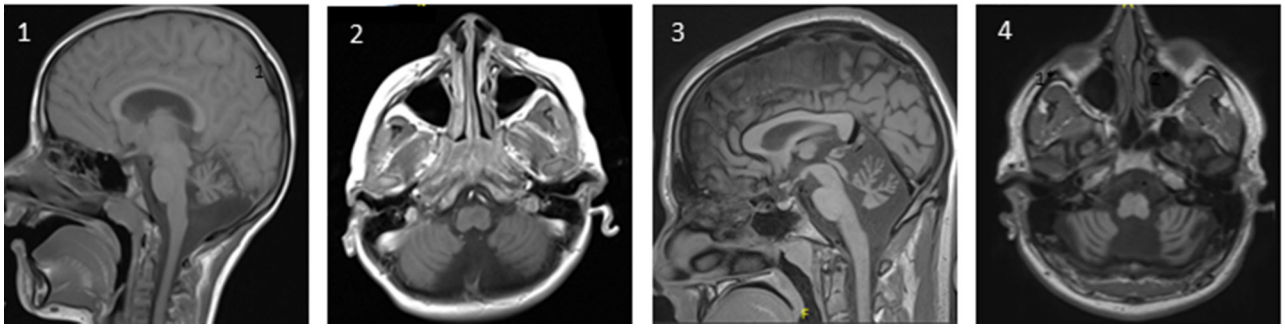

Figure 1 *PITRM1*^{T931M} in two families with progressive ataxia and cerebellar atrophy. (A) *PITRM1*^{T931M} cosegregates with the disease in families A and B under a model of autosomal recessive inheritance. In family A, a rearrangement on chromosome Xp22 also segregates with the disease. Both families are consanguineous; parents in family B are double third cousins. Their daughter is deceased with no DNA available. Letters under the symbols indicate genotypes. For *PITRM1*, V indicates *PITRM1*^{T931M} and N indicates *PITRM1*^{WT}. For Chr X, Y, X^R indicates the rearranged X-chromosome. ■ affected individuals, * sampled individuals. (B) Brain MRI, T1 phase from affected children from family A: IV-2 at age 15 years (1, 2) and IV-3 at age 18 years (3, 4). Both sagittal and axial views demonstrate severe cerebellar atrophy.

in peripheral blood leucocytes and fibroblasts (A.III.4, figure 1A). Both brothers were treated with antipsychotic medications.

Family B

Patient B.IV.1, the oldest child in his family, was born after an uneventful pregnancy at full term by caesarean section due to failure in delivery progression (Figure 1A). Birth weight was 4.4 kg. His parents are Palestinian Arabs, double third cousins, from a different locale than family A. Starting at 2 years of age, he was noted to have mild motor and cognitive impairment, speech delay, unsteady gait and dysmetria. Brain MRI at age 12 years showed normal anatomy. His younger brother, patient B.IV.2, now aged 4 years, has mild motor and speech delay with normal cerebellar function. Brain MRI at age 4 years was normal. Neither brother had seizures. A sister, with normal mental and motor development, died at the age of 5 years during a first episode of prolonged seizures without fever or meningeal signs. No further information or DNA samples were available from this child. Comprehensive metabolic work-up of both brothers, including LDH, CPK, lactate, pyruvate, pH, ammonia, amino acids and acylcarnitine in blood and urine organic acids, was normal. The older brother was 46;XY, with normal CMA and fragile-X testing. No other neurodegenerative diseases were reported in the family.

Gene discovery

Family A

Variants from whole exome sequences of the affected children and their parents were selected for autosomal or X linked

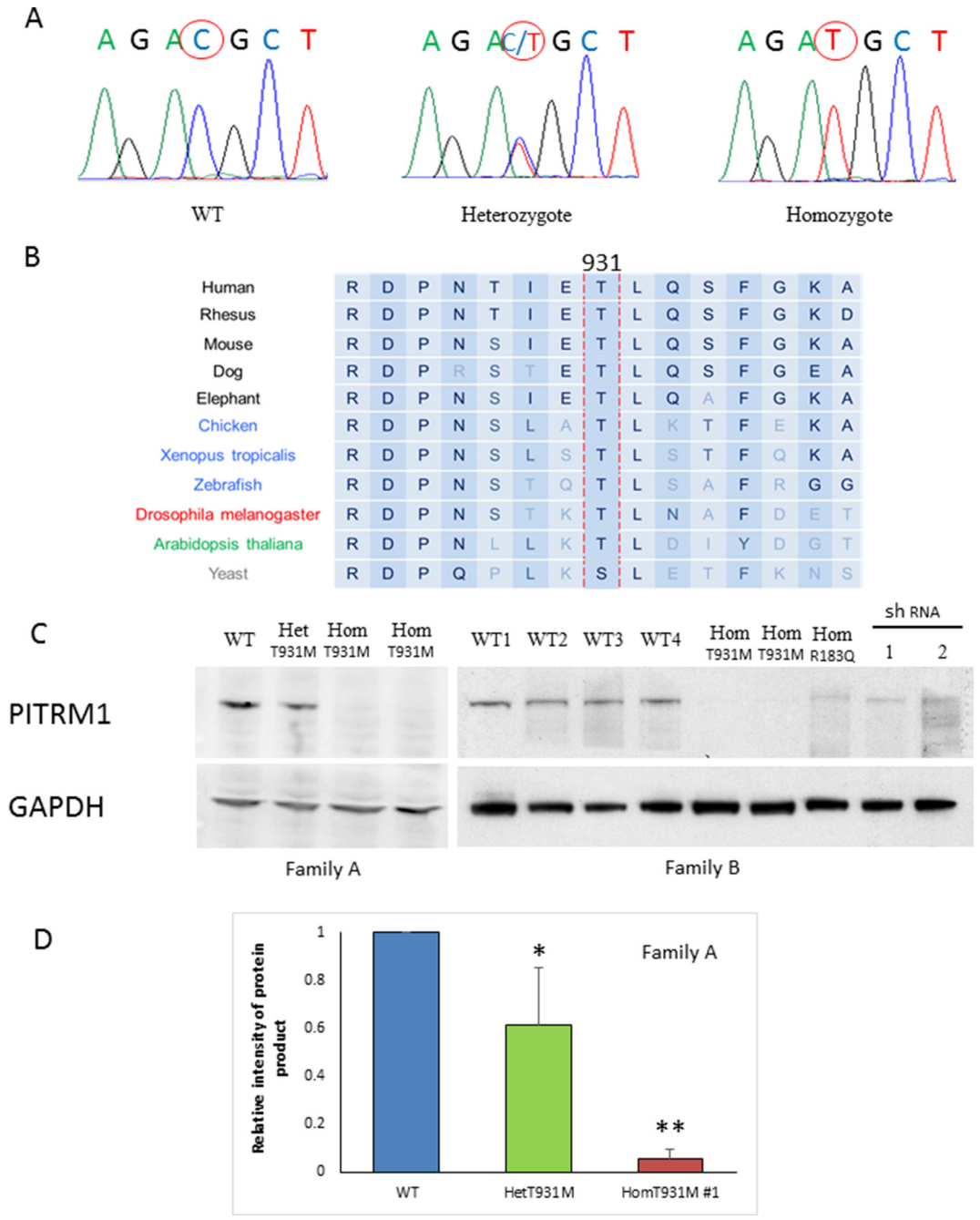

Figure 2 *PITRM1*^{T931M} evolutionary conservation and protein levels. (A) All affected children were homozygous for *PITRM1*^{T931M}. (B) Alignment of human *PITRM1* with proteins from mammals (black), non-mammals vertebrates (blue), invertebrates (red), protozoa (orange), plants (green) and fungi (grey). Threonine or serine are present in the position corresponding to T931. (C) The effect of the mutation on *PITRM1* protein levels was evaluated by western blot analysis of total lysate from fibroblasts from homozygotes (Hom) and heterozygotes (Het) and relatives with wild-type sequence (WT) from family A and from relatives with WT sequence from family B (WT1, WT2, WT3). The WT4 lane carries WT2 cells treated with shRNA and transduced with the empty vector plKO.1. The next three lanes carry fibroblasts from two *PITRM1*^{T931M} homozygotes and a *PITRM1*^{R183Q} homozygote. The last two lanes (shRNA1, 2) represent WT2 and WT3 cells transduced with *PITRM1* shRNAs. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as a loading control in all experiments. Results shown are representative of three experiments. (D) Quantification of *PITRM1* western blot product. Relative intensity of protein product are compared with WT. *PITRM1* levels were reduced by 39% in a *PITRM1*^{T931M} heterozygote (*p<0.05), and by 95% in *PITRM1*^{T931M} homozygotes (**p<0.001). The *PITRM1*^{R183Q} and shRNA lanes were loaded as controls and were not quantified in this experiment.

recessive inheritance, frequency <1% on a public database (ExAC[21]), evolutionary conservation and potential damaging effect on gene function (see online supplementary table 2). *PITRM1* c.2795C>T, p.T931M at chr10:3,181,221 was the strongest candidate variant (figure 2A). This variant appeared particularly promising given the report of a different mutation in *PITRM1* in a Norwegian family with a similar phenotype.[4] *PITRM1*^{T931M} segregated with the disease in family A under a recessive model (figure 1A). Furthermore, threonine at this site is highly conserved in evolution, where only threonine or serine, which share a hydroxyl group in the side chain, are present in the corresponding position (figure 2B). Substitution

of threonine, a polar uncharged side chained amino acid, by methionine, a hydrophobic side chained amino acid, is non-conservative. This variant does not appear in any public database.[21–23]

Family B

Whole exome sequencing was performed on genomic DNA from the two affected children (figure 1A). Variants were filtered as above, yielding two candidate variants (see online supplementary table 2), including *PITRM1* c.2795C>T, p.T931M. Both brothers were homozygous for *PITRM1*^{T931M}, which segregated with disease under a recessive model. Finding the same mutation in two independent families from neighbouring geographic areas suggested the possibility of a shared founder. Analysis of exome data indicated that *PITRM1*^{T931M} occurred on the same haplotype in both families, with a shared homozygous segment of 3.6 MB at chromosome 10 at 1 714 886–5 324 817 bp (hg19).

Among healthy individuals from the same village as family A, the frequency of heterozygous carriers of *PITRM1*^{T931M} was 0.027 (3/110). There were no homozygotes among controls.

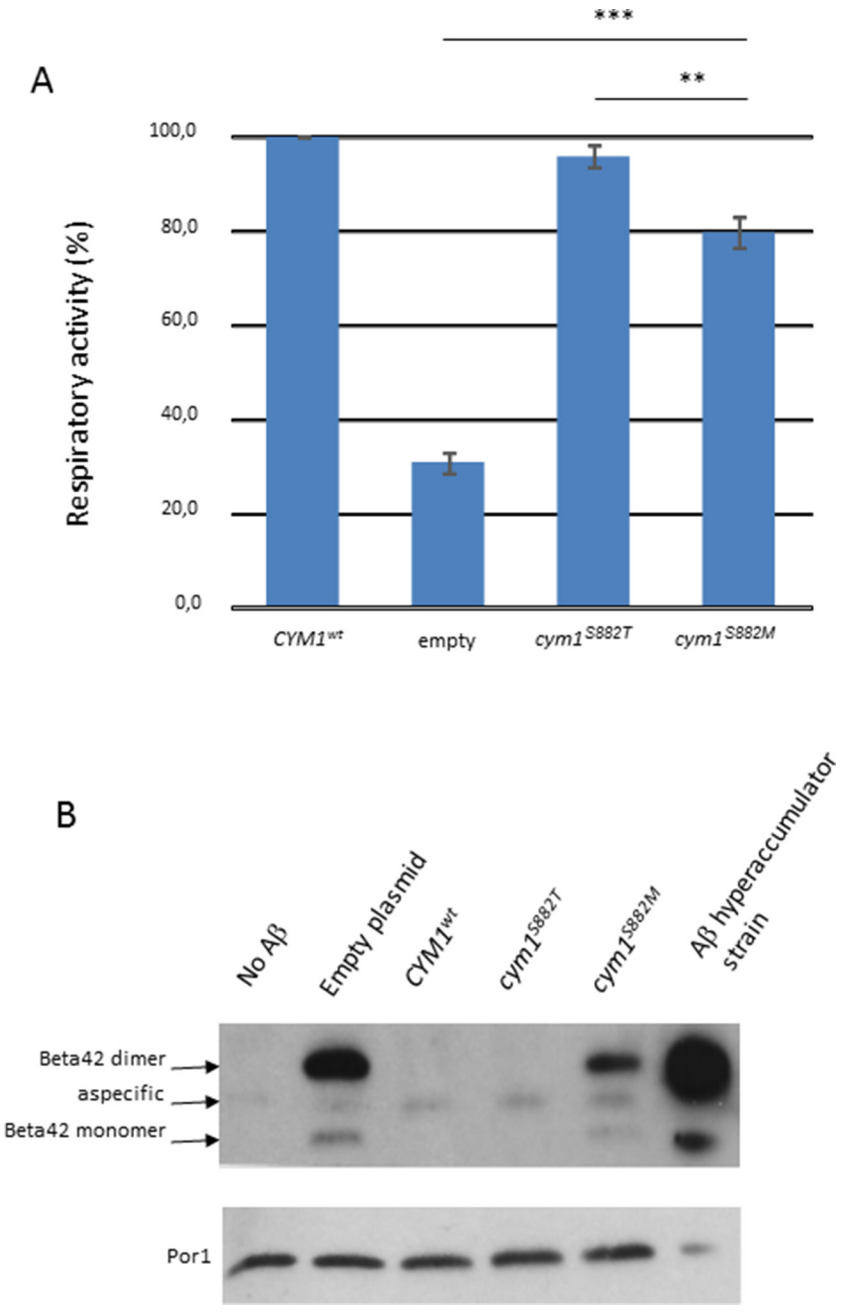

Figure 3 *PITRM1*^{T931M} respiratory capacity and proteolytic activity in a yeast model. *CYM1* is the yeast orthologue of *PITRM1*, and *PITRM1*^{T931} corresponds to *CYM1*^{S882}. Yeast strains examined were: *cym1*^{WT}, *cym1*^{S882T} with serine 882 substituted with threonine, as found in humans, and *cym1*^{S882M} corresponding to the T931M mutation. (A) Mitochondrial respiratory activity of W303-1B *cym1Δ* strains transformed with *CYM1*^{WT} and mutant alleles. Results are means of five independent experiments±SD normalised to the strain transformed with *CYM1*^{WT} allele **p<0.01; ***p<0.001 in a one-way ANOVA with Bonferroni test. (B) A representative western blot analysis of the previous strains transformed with a plasmid-borne gene encoding the Aβ42 peptide localising in mitochondria. Aβ42 accumulates in mitochondria transfected with *cym1*^{S882M}, but not with *CYM1*^{WT} plasmids, indicating that *cym1*^{S882M} has reduced peptide-degrading capacity. No Aβ: *CYM1*^{WT} strain transformed with a plasmid devoid of the Aβ42 gene. Aβ42 hyperaccumulator strain: strain mutated in mitochondrial proteases, which hyperaccumulates the Aβ42 peptide.

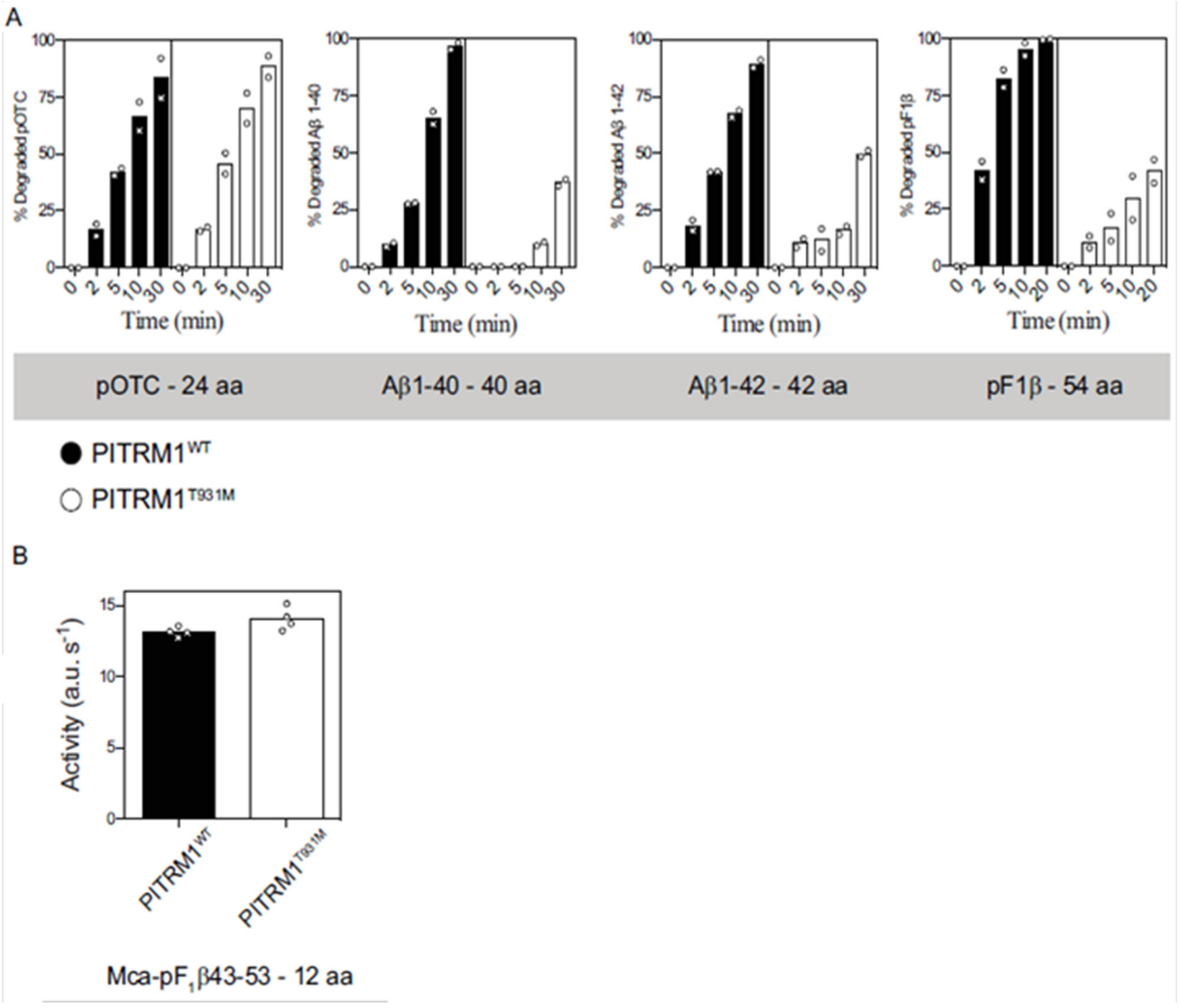

Figure 4 In vitro peptide degradation activity of purified *PITRM1*^{T931M}. (A) Degradation of pOTC, Aβ1–40, Aβ1–42 and $pF_1\beta$ by *PITRM1*^{WT} or *PITRM1*^{T931M} as analysed using a gel-based assay (original data used for quantification shown in online supplementary figure 4). Bars represent the mean of two experiments, each indicated by a circle. (B) Degradation of fluorogenic peptide Mca-pF_1b43-52 by *PITRM1*^{WT} and *PITRM1*^{T931M}. Bars represent the mean of four experiments. Values are expressed as arbitrary units per second (a.u.s^{-1}).

In our experience, in communities with a high level of consanguinity, carrier frequencies for recessive disease alleles in this range are not unusual.[24] No heterozygotes or homozygotes were seen in 300 Palestinian controls from other geographic locales.

The phenotype of family A is more severe than that of family B, despite the affected children in the two families being homozygous for the same mutation. Furthermore, skewed X-inactivation was observed in white blood cells of the mother and maternal grandmother of the affected children of family A. To investigate how skewed X-inactivation might influence disease severity, we carried out whole genome sequencing for the affected brothers of family A and evaluated data for the X-chromosome using CNVnator,[25] Manta-SV[26] and by PCR with diagnostic primers (online supplementary file 1) and Sanger sequencing. Both brothers carry a complex rearrangement on chromosome Xp22.31 characterised by a deletion of 4.75 kb and an adjacent inserted inverted duplication of 3.89 kb (see online supplementary figure 2). This rearranged region does not harbour any protein coding genes, but includes signals of methylation and of nucleosome depletion, characteristic of active regulatory elements. Also, the rearrangement lies in a 30 kb region that is completely intolerant of structural genomic deletions, unlike most of chromosome Xp.[27] The rearrangement was inherited from the children's mother and maternal grandmother (figure 1A). The rearrangement was not present in anyone in family B or in any other Palestinian control, either from the home village of family A or from other locales. The degree of X-chromosome skewing in cells of the mother and grandmother of the affected children of family A was extreme. In only 5% of their white blood cells was the mutant X-chromosome active, suggesting significant selection against cells with the rearrangement.

Protein stability, mitochondrial respiratory activity and peptidolytic activity of the *PITRM1*^{T931M} mutant

PITRM1^{T931M} did not alter transcript expression, based on RT-PCR analysis, but did lead to marked reduction of *PITRM1* protein, based on western blot analysis (figure 2C,D). Compared with

controls, *PITRM1* levels were reduced by 95% in *PITRM1*^{T931M} homozygotes ($p<0.001$) and by 39% in a *PITRM1*^{T931M} heterozygote ($p<0.05$), suggesting instability of the mutant protein. In fibroblasts from two controls stably expressing a *PITRM1*-specific shRNA, *PITRM1* protein levels were reduced by 60%, similar to the level of *PITRM1* protein reduction in fibroblasts from *PITRM1*^{R183Q} homozygous subjects.[4]

Given that *PITRM1* is a mitochondrial protein, we evaluated the effect of the mutation on respiratory oxidative activity in *Saccharomyces cerevisiae*, a common model system for evaluating mitochondrial function. *PITRM1*^{T931} corresponds to serine at residue 882 in the orthologous yeast gene *cym1*. Substitution of this serine with threonine, as found in humans, has no effect on mitochondrial phenotypes. However, substitution of serine with methionine, as found in patients, significantly decreased both the respiratory activity and the activities of the respiratory complexes (figure 3A and online supplementary figure 3).

We analysed the peptidolytic activity of *PITRM1*^{T931M} in comparison to wild-type *PITRM1* using purified recombinant proteins. As shown in figure 4A, *PITRM1*^{T931M} was able to cleave short peptides, such as the 24 amino acid OTC peptide, but compared with *PITRM1*^{WT}, had highly decreased capacity to degrade longer peptides, such as two Aβ species of 40 and 42 amino acids and the 54 amino acid presequence of $pF_1\beta$ (figure 4). The observed difference is likely to be due to the length of the peptide, rather than its sequence, since *PITRM1*^{T931M} was able to degrade an 11-residue peptide derived from $pF_1\beta$ as efficiently as was *PITRM1*^{WT} (figure 4B). In addition, heterologous expression of human Aβ in mitochondria of *cym*^{S882M} yeast yielded higher levels of longer Aβ peptide compared with results in either wild-type or humanised *cym1*^{S882T} yeast (figure 3B). This observation indicates that the threonine to methionine substitution reduces the capacity of *PITRM1* to degrade mitochondrial Aβ peptide in vivo.

DISCUSSION

Autosomal recessive spinocerebellar ataxias are a group of neurodegenerative diseases with wide phenotypic and genotypic heterogeneity. Mutations in the same gene may lead to several distinct phenotypes (eg, *FXN*, *POLG* or *ATM*)[1]; conversely, the same phenotype may be caused by mutations in different genes (eg, Friedreich ataxia or ataxia with vitamin E deficiency).[2] The spectrum of the phenotype is also variable with respect to age of onset, disease severity, rate of disease progression and associated extracerebellar and systemic signs.[1] The molecular diagnosis of childhood onset ataxias, cerebellar atrophy and intellectual disability is therefore challenging.

Here, we report a novel mutation in *PITRM1* causing ARCA in four children from two families. All four affected children share features of cerebellar dysfunction. In family A, intellectual disability is moderate, and cerebellar dysfunction is much more severe, with significant ataxia from early childhood, and severe cerebellar atrophy on brain imaging at age 9 years. This clinical presentation seems comparable to that observed in two adult siblings from a previously reported Norwegian family, much older than the patients described in this study.[4] In family B, the children (aged 13 and 4 years) presented with mild intellectual disability. The older child also had tremor and mild ataxia from age 2 years, with normal brain imaging at age 12 years. This may be compatible with an age-dependent phenotype similar to the previously reported Norwegian family.[4] Families A and B share the same mutation, and the clinical variability may be explained by the X-chromosome rearrangement, found only in family A and leading to significant X-chromosome skewing in the carrier females. In only 5% of their white blood cells was the mutant X-chromosome active, suggesting selection against cells with the rearrangement. We speculate that in the affected children, all of whose cells carry only the rearranged X-chromosome, the effect of the *PITRM1* mutation may have been exacerbated either through effects of the deletion on X-chromosome, affecting regulatory regions or, perhaps more likely, through aberrations of cell division or survival.

Combining the two families reported here with the Norwegian cases, *PITRM1* deficiency points to an age-dependent condition that includes intellectual disability, progressive cerebellar dysfunction ultimately leading to cerebellar atrophy and psychiatric manifestations and including obsessive behaviour, anger attacks and psychosis. Although *PITRM1*^{T931M} is a missense mutation, it leads to nearly complete loss of function at the protein level, including almost complete absence of the *PITRM1* protein (figure 2C,D). Furthermore, in both human cells and in yeast, *PITRM1*^{T931M} protein has impaired peptide degradation activity with secondary effects, although slight, on mitochondrial oxidative phosphorylation. Interestingly, the catalytic defect was found only on long peptide substrates (≥40 amino acids), particularly affecting Aβ metabolism. However, oxidative respiratory function in muscle samples of the patients from families A and B was normal, as were their muscle biopsies, suggesting that the mitochondrial effects of this mutation are mild. Its progressive nature may lead to muscle pathology similar to that demonstrated in the previously reported patients at much older ages.[4] This is also suggested by elevated muscle enzymes in the most severely affected patient we describe. Future studies may determine if the neuropathology associated with *PITRM1* deficiency is explained by impaired processing only of Aβ or whether other proteins are involved.

CONCLUSION

We describe a novel *PITRM1* mutation leading to *PITRM1* deficiency as the basis of ARCA in early childhood. This mutation impairs degradation of longer peptides in the mitochondria, strengthening the association (see online supplementary file 8) between mitochondrial dysfunction, particularly altered mitochondrial proteostasis, and paediatric neurodegenerative diseases.

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analysis, wrote the first draft and approved final manuscript version. AA participated in conceptualising the study, identified and recruited patients and family members examined the participants, collected DNA samples and clinical data; wrote the first draft with YL and approved final manuscript version. SG performed genetic analysis and interpretations and wrote sections of the manuscript. BAL identified and recruited patients and family members examined the participants, collected DNA samples and clinical data and wrote sections of the manuscript. PR participated in conceptualising the study, provided insights about the hypothesis and directed genetic testing and analysis. DB performed genetic analysis and interpretations and wrote sections of the manuscript. P-FT performed peptide assays and wrote sections of the manuscript. TW performed genetic analysis and interpretations. SZ performed genetic analysis and interpretations. RR designed and constructed the plasmid bearing the mitochomdrial-targeted A42 peptide coding sequence. RB performed genetic analysis and interpretations and wrote sections of the manuscript. ID recruited patients and family members, examined the participants, collected DNA samples and clinical data. MS recruited patients and family members examined the participants, collected DNA samples and clinical data. AW-S performed genetic analysis and interpretations. FZ performed genetic analysis and interpretations. EB designed and performed the experiments in yeast and wrote sections of the manuscript. EG directed peptide assays and wrote sections of the manuscript. M-CK participated in conceptualising the study; directed genomic analysis, interpreted results, obtained funding and critically revised the manuscript. EL-L participated in conceptualising the study; directed genetic testing, analysis and interpretation of the results; obtained funding and critically revised the manuscript. ZM directed genetic analysis and interpretations and wrote sections of the manuscript. RS participated in conceptualising the study, identified and recruited patients and family members, examined the participants, collected DNA samples and clinical data, directed genetic testing, analysis and interpretation of the results, wrote the first draft and approved final manuscript version.

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Competing interests None declared.

Patient consent Not required.

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