BRIEF COMMUNICATION

A recurrent de novo ATP5F1A substitution associated with neonatal complex V deficiency

Matthew A. Lines[1,2], Alexanne Cuillerier[3], Pranesh Chakraborty[4,5,6], Turaya Naas[6], M. Laura Duque Lasio[7], Jean Michaud[8], Chantal Pileggi[3], Mary-Ellen Harper[3], Yan Burelle[3,9], Tomi L. Toler[7], Neal Sondheimer[10,11,12], Heather P. Crawford[13], Francisca Millan[14] and Michael T. Geraghty[4,5]✉

Mitochondrial disorders are a heterogeneous group of rare, degenerative multisystem disorders affecting the cell's core bioenergetic and signalling functions. Spontaneous improvement is rare. We describe a novel neonatal-onset mitochondriopathy in three infants with failure to thrive, hyperlactatemia, hyperammonemia, and apparent clinical resolution before 18 months. Exome sequencing showed all three probands to be identically heterozygous for a recurrent de novo substitution, c.620G>A [p.(Arg207His)] in *ATP5F1A*, encoding the α-subunit of complex V. Patient-derived fibroblasts exhibited multiple deficits in complex V function and expression in vitro. Structural modelling predicts the observed substitution to create an abnormal region of negative charge on ATP5F1A's β-subunit-interacting surface, adjacent to the nearby β subunit's active site. This disorder, which presents with life-threatening neonatal manifestations, appears to follow a remitting course; the long-term prognosis remains unknown.

European Journal of Human Genetics (2021) 29:1719–1724; https://doi.org/10.1038/s41431-021-00956-0

INTRODUCTION

Mitochondrial disorders (MDs) are a group of >200 rare genetic diseases affecting cellular bioenergetics, metabolism and other essential processes [1]. A provisional genetic diagnosis can be made in ~25–50% of cases [2]. Although most MDs are progressive, spontaneous improvement is typical of two disorders, *TRMU* deficiency (OMIM #613070) and *MTTE*-related infantile mitochondrial myopathy (OMIM #500009) [3, 4]. Here, we describe a novel remitting MD in three neonates with failure to thrive, hyperammonemia, lactic acidosis, respiratory defects in fibroblasts, and recurrent *de novo* substitutions of the complex V α subunit gene, *ATP5F1A*.

MATERIALS AND METHODS

Procedures were compliant with the revised Helsinki Declaration of 2000. The study was approved by the Children's Hospital of Eastern Ontario Research Ethics Board (#11/04E). All biochemical and genetic testing was performed routinely as part of usual care. Fibroblast cultures were obtained from all participants via routine skin biopsy. Non-MD control fibroblasts were obtained from a healthy volunteer ('Control 1'), and from individuals with the non-mitochondrial conditions Fryns Syndrome ('Control 2') and 46,XY disorder of sexual development ('Control 3'). Cell lines were assayed for complex V activity as described (Supplementary Material). Genetic variant information is accessible in ClinVar with variation ID 432972/accession VCV000432972.3.

RESULTS

Clinical findings

The patients are three unrelated term neonates presenting between postnatal days nine and eighteen with feeding intolerance, failure to thrive, hyperammonemia and lactic acidemia (Table 1; Supplementary Fig. 1). Blood lactate was variably elevated at presentation (3.3–8.5 mmol/L). Metabolic tests showed hyperalaninemia (1245–1624 µmol/L), hyperprolinemia, hyperglutaminemia (two probands; 1725–1742 µmol/L), depletion of urea cycle intermediates (citrulline, arginine and ornithine) and orotic aciduria. Urine organic acids showed hyperexcretion of lactate with or without pyruvate and Krebs cycle intermediates. In one individual, hyperammonemia was mild (90 µmol/L) and resolved spontaneously; the other two children required protein restriction, intravenous fluids, dextrose, nitrogen-scavenging medications, and supplementary citrulline and/or arginine. Neither received

[1]Medical Genetics, Department of Pediatrics, Alberta Children's Hospital, Calgary, AB, Canada. [2]Department of Medical Genetics, Cumming School of Medicine, University of Calgary, Calgary, AB, Canada. [3]Department of Cellular and Molecular Medicine, Faculty of Medicine, University of Ottawa, Ottawa, ON, Canada. [4]Metabolics and Newborn Screening, Children's Hospital of Eastern Ontario, Ottawa, ON, Canada. [5]Department of Pediatrics, University of Ottawa, Ottawa, ON, Canada. [6]Newborn Screening Ontario, Ottawa, ON, Canada. [7]Division of Genetics & Genomic Medicine, Washington University School of Medicine, St. Louis, MO, USA. [8]Department of Pathology and Laboratory Medicine, University of Ottawa, Ottawa, ON, Canada. [9]Interdisciplinary School of Health Sciences, Faculty of Health Sciences, University of Ottawa, Ottawa, ON, Canada. [10]Division of Clinical and Metabolic Genetics, The Hospital for Sick Children, Toronto, ON, Canada. [11]Program in Genetics and Genome Biology Program, Sick Kids Research Institute, Toronto, ON, Canada. [12]Departments of Pediatrics and Molecular Genetics, University of Toronto, Toronto, ON, Canada. [13]Innovative Genetics Care, The Woodlands, TX, USA. [14]GeneDx, Gaithersburg, MD, USA. ✉email: mgeraghty@cheo.on.ca

Received: 9 October 2020 Revised: 12 August 2021 Accepted: 23 August 2021
Published online: 6 September 2021

Table 1. Clinical and laboratory features.

	Individual 1	Individual 2	Individual 3
Clinical presentation			
Sex	M	F	F
Perinatal history	NRFHR, mild temperature instability	normal	NRFHR, C/S
Birth weight (SD)	−2.9	+0.5	−1.0
Age at presentation	18 days	9 days	13 days
Feeding difficulties	+	+	+
Failure to thrive	+	+	+
Acute encephalopathy	−	-	+ (hyperammonemia)
Chronic diarrhoea	+	-	+
Anaemia	+	-	+
Age at most recent assessment	3 years	14 months	18 months
Age at symptom resolution	12 months	10 months	15–18 months
Clinical status	asymptomatic	asymptomatic	asymptomatic
Most recent weight (SD)	−1.0	−0.8	+0.2
Developmental status	normal	normal	normal
Metabolic laboratory testing[a]			
Hyperammonemia	+ (267 μmol/L)	+ (90 μmol/L)	+ (520 μmol/L)
Encephalopathy	-	-	+ (seizures after EBM gavage feeds)
Lactic acidosis (mmol/L)	+ (range: 1.0–11.0; $\overline{x} = 3.6$ mmol/L) ($n = 36$)	+ (range: 1.2–8.5 mmol/L; $\overline{x} =$ 3.5 mmol/L) (n = 17)	+ (range: 3.2–5.8 mmol/L; $\overline{x} =$ 4.3 mmol/L) ($n = 7$)
Plasma amino acids (μmol/L):			
Alanine	1624 (↑↑) [83–447]	1245 (↑↑) [119–439]	1675 (↑↑) [75–500]
Proline	530 (↑) [87–375]	439 (↑) [104–348]	590 (↑) [20–330]
Glycine	574 (↑) [133–409]	229 [103–386]	530 [100–550]
Glutamine	1742 (↑↑) [240–1194]	979 [303–1459]	1725 (↑) [124–1000]
Citrulline	BDL (↓)	BDL (↓)	2 [0–50]
Arginine	19 [6–140]	18 (↓) [30–147]	17 (↓) [50–160]
Ornithine	23 (↓) [29–168]	NR	18 (↓) [25–250]
Urine orotic acid (mmol/molCr)	7.1 (↑) [<2.0]	NR	9.3 (↑) [1.0–3.2]
Urine organic acids	Increased lactic, pyruvic, 2-ethyl-3-hydroxypropionic, ethylmalonic, Krebs cycle intermediates including fumaric	Increased lactic and pyruvic acids	Increased lactic acid
Acylcarnitine profile	Persistent ($n = 4$) minor increases in C3, C3DC, C4, C6, C6OH, and C10	Normal ($n = 3$) apart from trivial increases in C6OH, C3:1, and/or C3	Normal ($n = 1$)
Plasma GDF-15 (pg/mL)	>6000 (↑↑) [<750] (age 3 weeks)	2030 (↑↑) [<750] (7 months)	NR
	3952 (↑↑) (age 2 months)		
	3017 (↑↑) (age 5 months)		
	2731 (↑↑) (age 13 months)		
Residual/subclinical laboratory findings (at most recent assessment)	lactate 2.5 mmol/L; elevated GDF-15 (2731 pg/mL) (last tested age 13 months)	lactate 2.7 mmol/L; hyperalaninemia (685 μmol/L)	lactate 3.7 mmol/L
Muscle biopsy			
Histology and EM	Increased lipid droplets, irregular loss of sarcomeres with replacement by glycogen, large, 'swollen' mitochondria with abnormal figures	NR	NR
Complexes I–IV	normal		
Primary skin fibroblasts			
Complex I	NR	NR	normal
Complexes II–IV	normal	NR	normal
PDH, PC and L/P ratio	normal	NR	NR

Reference intervals are denoted in square brackets.
BDL below detectable limit, *C/S* Caesarean section, *EBM* expressed breast milk, *F* female, *GDF-15* growth and differentiation factor 15, *L/P* lactate/pyruvate, *M* male, *NR* not reported, *NRFHR* non-reassuring foetal heart rate, *PC* pyruvate carboxylase, *PDH* pyruvate dehydrogenase, *SD* standard deviations.
[a]See Supplementary Fig. 1 for additional details.

haemodialysis, although both required hypercaloric, protein-restricted feeds and nitrogen-scavenging agents to maintain control of hyperammonemia. One individual was tested genetically for suspected ornithine transcarbamylase (OTC) deficiency, with normal results.

The patients experienced failure to thrive which proved refractory to anti-reflux medications, hypercaloric hypoallergenic feeds, gavage feeding, and (in one case) parenteral nutrition. Two individuals developed chronic non-bloody diarrhoea of indeterminate aetiology. Two of three probands had an unexplained anaemia. Pearson syndrome was considered in one case and duly excluded by mtDNA studies in muscle (see below).

Each proband was investigated for suspected mitochondriopathy. Plasma growth and differentiation factor 15 (GDF-15) was markedly increased in two individuals. Muscle biopsy in one case showed several histologic and ultrastructural abnormalities (Supplementary Fig. 2). Complex I–IV activities in muscle and lactate:pyruvate ratio in fibroblasts were normal. Mitochondrial DNA sequencing in muscle was negative for pathogenic point variants and rearrangements.

The major clinical symptoms appeared to remit by late infancy in all cases. Weight re-entered the normal range between ten and eighteen months; diarrhoea remitted before one year, and lactate stabilised near the upper normal limit after several months. One proband experienced recurrent hyperammonemia at age fifteen months, after single missed doses of her glycerol phenylbutyrate, arginine, and citrulline. As of their most recent assessment (age range: fourteen months to three years), the probands' growth and developmental parameters are normal. They continue to exhibit subtle laboratory abnormalities including mild intermittent hyperlactatemia (three individuals), hyperalaninemia (one individual), and elevated GDF-15 (one individual). One proband (individual 3; age 18 months) remains on medical hyperammonemia prophylaxis with arginine, citrulline, and glycerol phenylbutyrate.

Exome sequencing

Clinical exome trio analysis (proband and parents) was performed in each case. All probands were identically heterozygous for the same *de novo* substitution, *ATP5F1A*(NM_001001937.1):c.620G>A, p.(Arg207His). This variant, which is absent from gnomAD [5], intersects all RefSeq transcripts of *ATP5F1A*, and carries pathogenic predictions (PHRED-scaled CADD 32; SIFT 0; PolyPhen-2 1.000).

Bioenergetic studies in patient fibroblasts

ATP5F1A encodes subunit α of mitochondrial ATP synthase (complex V), the rotary ATPase responsible for >90% of cellular ATP synthesis. To assess the functional consequences of the observed p.Arg207His substitution, we studied complex V activity in patient-derived fibroblasts using multiple methods (Fig. 1). Patient cells exhibited defects in mitochondrial oxygen consumption, ATPase activity, and ATP5F1A expression. Nondenaturing PAGE (Fig. 1E) further showed a smaller proportion of monomeric complex V in patient cells, with an increased proportion participating in higher-order complexes. The significance of the latter finding is unclear.

Structural modelling

We modelled the potential structural consequences of the recurrent p.Arg207His substitution based on a previously-solved structure of ATP5F1A (Fig. 2) [6]. In the model, the sidechain of residue 207 resides at the α-β subunit interface, opposite the nearby β subunit's active site. At matrix pH (~7.8), replacement of Arg207 (pKa 12.5) with a histidine (pKa 6.0) is a nonconservative substitution predicted to create a negatively-charged patch at the α–β interaction surface.

DISCUSSION

Complex V is uniquely crucial to cellular energy metabolism, and complex V disorders are very rare. This report of a comparatively mild condition extends the clinical spectrum of *ATP5F1A* as defined by two previous reports of lethal multisystem MD in children with biallelic disease [2, 7]. The other described genes for isolated complex V deficiency are *ATP5F1D* and *ATP5F1E* (encoding subunits δ and ε), *TMEM70* and *ATPAF2* (encoding assembly factors), and *MT-ATP6* and *MT-ATP8* (mitochondrial genes for subunits a and A6L of F_O) [8–12]. Of the five nuclear complex V disorders, all except *TMEM70* deficiency (~50 individuals) are rare congenital disorders comprising growth restriction, encephalopathy, cardiomyopathy, pulmonary hypertension, and/or congenital malformations. mtDNA-based forms of complex V deficiency are broadly variable by degree of heteroplasmy; nevertheless, these too are lifelong conditions with a significant associated burden of illness [12, 13].

The long-term prognosis of heterozygous *ATP5F1A* deficiency is unknown. All patients in this study were young, and subclinical biochemical abnormalities persisted in each case despite clinical resolution. Untreated, the condition described here is likely to be highly deleterious, considering: (i) its uniform presentation with life-threatening neonatal symptoms, (ii) absence of the causal variant in general-population databases, and (iii) de novo occurrence in each case. Given the nonspecific clinical presentation, exome or panel testing is anticipated to be the means of diagnosis in most cases. Clinical pitfalls relating to this disorder could include: (i) early assignment of an overly poor prognosis, (ii) overestimation of recurrence risks, (iii) misdiagnosis as 'mutation-negative' *OTC* deficiency, and (iv) false-negative respiratory chain testing where complex V is not specifically assessed.

Heterozygous *ATP5F1A*-related MD joins two other reversible neonatal MDs associated with the genes *TRMU* (OMIM #613070), and *MTTE* (OMIM #500009) [3, 4]. In *MTTE*-related mitochondriopathy, spontaneous recovery is proposed to entail activation of the integrated stress response via mTOR, an adaptive process [14]. In this study we did not observe the specific amino acid deficiencies (of cysteine, glutamine, and glutamate) described in *MTTE* deficiency [14].

The p.(Arg207His) substitution in our probands occurred de novo in each case. Possible explanations could include: (i) coincidence, (ii) selection bias towards similar cases, (iii) hypermutability of the specific genomic position (i.e., deamination of a CpG dinucleotide), or (iv) unique functional characteristics specific to the variant itself. At present, there is insufficient information to conclude whether the p.(Arg207His) substitution is hypomorphic versus dominant-negative. Although *ATP5F1A* is statistically depleted of truncating variants (gnomAD pLI: 1.00 and 0.98, respectively), multiple (>10) truncating variants are nevertheless found at low frequency among healthy adult controls [5]. In mice, homozygous *Atp5f1a* deficiency is embryonic-lethal; heterozygotes, although viable, are smaller versus controls [15, 16]. The possibility that $ATP5F1A_{p.(Arg207His)}$ is dominant-negative with respect to complex V assembly, stability, or function remains to be tested in a suitable animal model.

Another MD gene with distinct recessive and dominant phenotypes is *SLC25A4*, encoding the ATP/ADP translocase (ANT1). In a manner similar to *ATP5F1A*, recurrent de novo substitutions of *SLC25A4* have been shown to cause a congenital mitochondriopathy distinct from the adult-onset (monoallelic) and childhood-onset (biallelic) forms of ANT1 deficiency [17]. Because many mitochondrial inner membrane proteins function in macromolecular complexes, it stands to reason that structurally dominant-negative alleles may also occur in other, unrecognised, forms of MD.

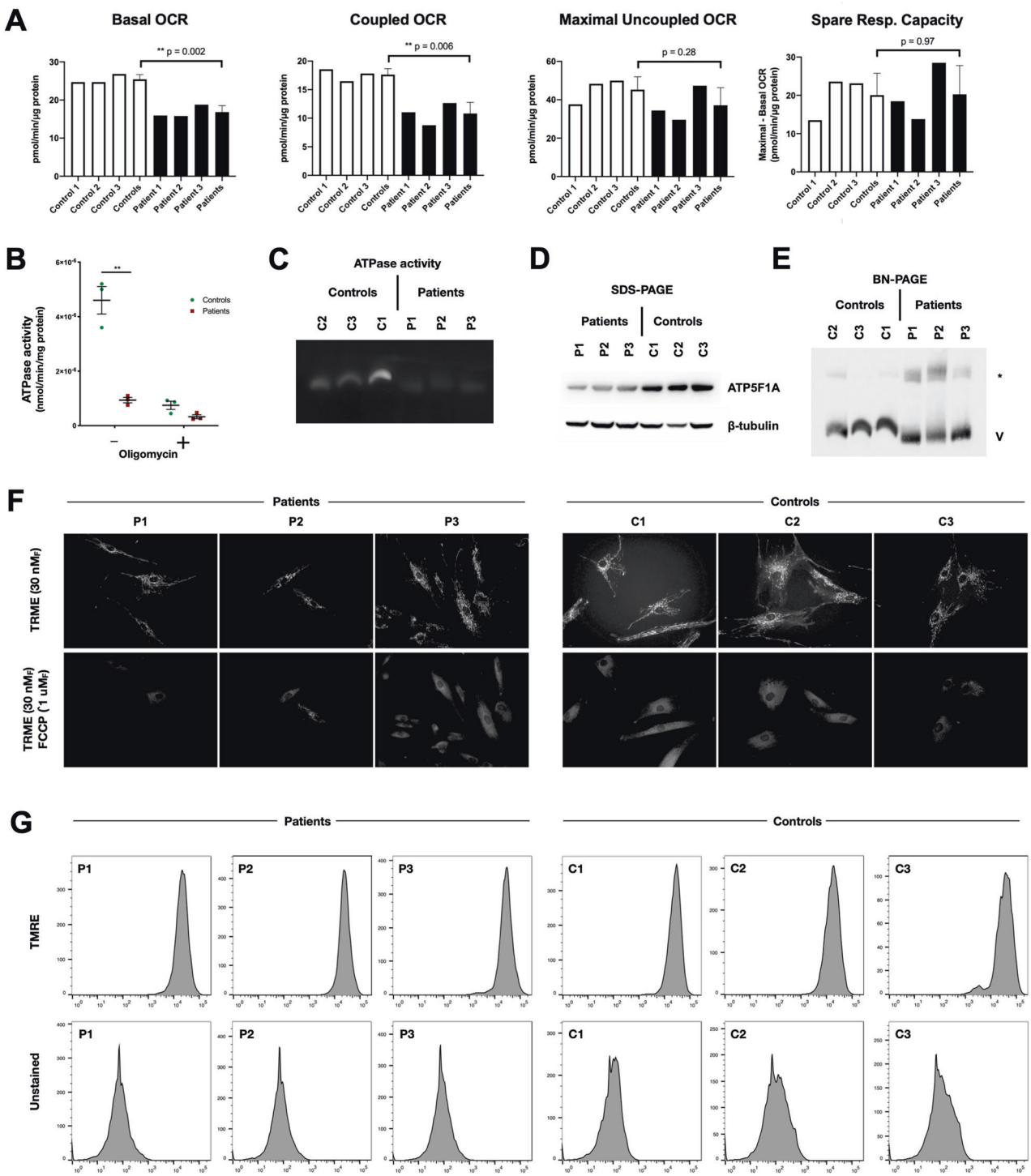

Fig. 1 Complex V defect in patient-derived fibroblasts. a Extracellular flux analysis showed deficits in basal and coupled (complex V-mediated) oxygen consumption rate (OCR), but no difference in maximal uncoupled OCR or spare respiratory capacity. **b** Patient-derived fibroblasts exhibited reduced complex V activity measured as oligomycin-sensitive ATP synthase. **c** In-gel ATPase activity following nondenaturing electrophoresis was lower in patient-derived cells. **d** ATP5F1A expression was lower as assessed by immunoblot analysis. **e** Blue native PAGE showed a diminished proportion of monomeric complex V, and an increased abundance of a high-molecular-weight complex (*) of unknown composition. **f** Fluorescence microscopy of mitochondria stained with a voltage-sensitive cationic lipophilic dye (TMRE) with and without a pharmacological uncoupler (FCCP) demonstrated no difference in resting mitochondrial inner membrane potential in patient cells versus controls. Similarly, patient and control cells exhibited no significant difference in TMRE fluorescence intensity as assessed by flow cytometry (**g**).

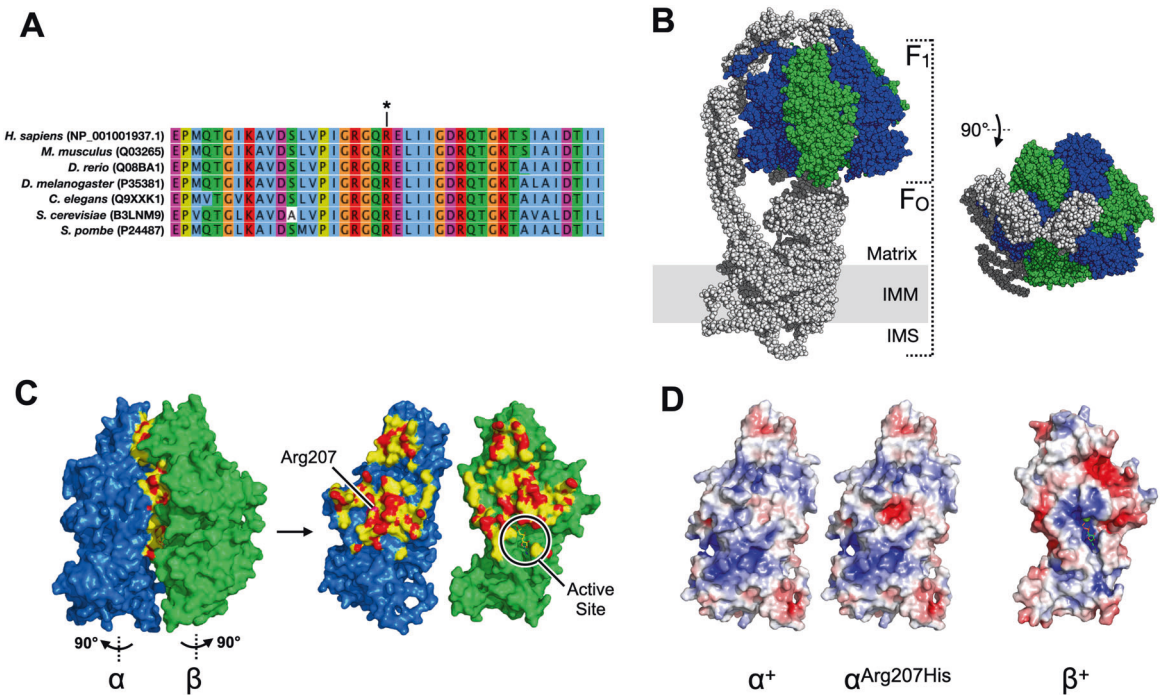

Fig. 2 Predicted functional consequence of p.Arg207His substitution in ATP5F1A. The F_1 catalytic core of complex V is a radial hexamer of alternating α and β subunits ($\alpha_3\beta_3$) surrounding a central rotor (γδε). The three ATP-producing catalytic active sites lie 120° apart on each of the three β subunits, in clefts adjoining the β-α subunit interfaces. **a** Peptide sequences surrounding Arg207 (*) are highly conserved. **b** Cryo-EM structure of complex V (PDB: 6J5J) (ref. [18].). ATP5F1A (α) is shown in blue, and ATP51B (β) in green. **c** Detail of α-β subunit interface, per PDB:1COW (ref. [6].). Residues shown in red and yellow are within 3.6 Å and 5 Å, (respectively) of the opposing subunit. The β-subunit ATPase active site lies indirectly opposite α-Arg207 at the position shown. **d** at mitochondrial matrix pH (7.8), the β-interacting surface of $\alpha^{Arg207His}$ is predicted to display a region of central negative charge (red patch) not present in the unsubstituted protein.

DATA AVAILABILITY STATEMENT

All data generated or analysed during this study are included in this published article and its supplementary information files. Genetic variant information is accessible in ClinVar with variation ID 432972.

REFERENCES

1. Vafai SB, Mootha VK. Mitochondrial disorders as windows into an ancient organelle. Nature. 2012;491:374–83.
2. Lieber DS, Calvo SE, Shanahan K, Slate NG, Liu S, Hershman SG, et al. Targeted exome sequencing of suspected mitochondrial disorders. Neurology. 2013;80:1762–70.
3. Zeharia A, Shaag A, Pappo O, Mager-Heckel AM, Saada A, Beinat M, et al. Acute infantile liver failure due to mutations in the TRMU gene. Am J Hum Genet. 2009;85:401–7.
4. Horvath R, Kemp JP, Tuppen HA, Hudson G, Oldfors A, Marie SK, et al. Molecular basis of infantile reversible cytochrome c oxidase deficiency myopathy. Brain. 2009;132:3165–74.
5. Lek M, Karczewski KJ, Minikel EV, Samocha KE, Banks E, Fennell T, et al. Analysis of protein-coding genetic variation in 60,706 humans. Nature. 2016;536:285–91.
6. van Raaij MJ, Abrahams JP, Leslie AG, Walker JE. The structure of bovine F1-ATPase complexed with the antibiotic inhibitor aurovertin B. Proc Natl Acad Sci USA. 1996;93:6913–7.
7. Jonckheere AI, Renkema GH, Bras M, van den Heuvel LP, Hoischen A, Gilissen C, et al. A complex V ATP5A1 defect causes fatal neonatal mitochondrial encephalopathy. Brain. 2013;136:1544–54.
8. Oláhová M, Yoon WH, Thompson K, Jangam S, Fernandez L, Davidson JM, et al. Biallelic mutations in ATP5F1D, which encodes a subunit of ATP synthase, cause a metabolic disorder. Am J Hum Genet. 2018;102:494–504.
9. Mayr JA, Havlícková V, Zimmermann F, Magler I, Kaplanová V, Jesina P, et al. Mitochondrial ATP synthase deficiency due to a mutation in the ATP5E gene for the F1 epsilon subunit. Hum Mol Genet. 2010;19:3430–9.
10. Cízková A, Stránecký V, Mayr JA, Tesarová M, Havlícková V, Paul J, et al. TMEM70 mutations cause isolated ATP synthase deficiency and neonatal mitochondrial encephalocardiomyopathy. Nat Genet. 2008;40:1288–90.
11. De Meirleir L, Seneca S, Lissens W, De Clercq I, Eyskens F, Gerlo E, et al. Respiratory chain complex V deficiency due to a mutation in the assembly gene ATP12. J Med Genet. 2004;41:120–4.
12. Holt IJ, Harding AE, Petty RK, Morgan-Hughes JA. A new mitochondrial disease associated with mitochondrial DNA heteroplasmy. Am J Hum Genet. 1990;46:428–333.
13. Jonckheere AI, Hogeveen M, Nijtmans LG, van den Brand MA, Janssen AJ, Diepstra JH, et al. A novel mitochondrial ATP8 gene mutation in a patient with apical hypertrophic cardiomyopathy and neuropathy. J Med Genet. 2008;45:129–33.
14. Hathazi D, Griffin H, Jennings MJ, Giunta M, Powell C, Pearce SF, et al. Metabolic shift underlies recovery in reversible infantile respiratory chain deficiency. EMBO J. 2020;39:e105364.
15. Baran AA, Silverman KA, Zeskand J, Koratkar R, Palmer A, McCullen K, et al. The modifier of Min 2 (Mom2) locus: embryonic lethality of a mutation in the Atp5a1 gene suggests a novel mechanism of polyp suppression. Genome Res. 2007;17:566–76.
16. Bult CJ, Blake JA, Smith CL, Kadin JA, Richardson JE, the Mouse Genome Database Group. Mouse genome database (MGD) 2019. Nucleic Acids Res. 2019;47:D801–D806. 2019
17. Thompson K, Majd H, Dallabona C, Reinson K, King MS, Alston CL, et al. Recurrent de novo dominant mutations in SLC25A4 cause severe early-onset mitochondrial disease and loss of mitochondrial DNA copy number. Am J Hum Genet. 2016;99:860–76.
18. Gu J, Zhang L, Zong S, Guo R, Liu T, Yi J, et al. Cryo-EM structure of the mammalian ATP synthase tetramer bound with inhibitory protein IF1. Science. 2019;364:1068–75.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the participation of the patients and their families. Partial funding was received from Newborn Screening Ontario.

AUTHOR CONTRIBUTIONS

All authors contributed to the study conception and design. Data collection and analysis were performed by M. L., A. C., T. N., M. L. D. L., J. M., C. P., T. T., N. S., H. C., F. M., and M. G. The first draft of the manuscript was written by M. L. and A. C., and all authors

commented on previous versions of the manuscript. All authors read and approved the final manuscript.

ETHICAL APPROVAL

Informed consent was obtained from all subjects, and study procedures were compliant with the revised Helsinki Declaration of 2000. The study was approved by the Children's Hospital of Eastern Ontario Research Ethics Board (#11/04E).

COMPETING INTERESTS

Francisca Millan is employed by GeneDx (MD, USA); the remaining authors declare no conflicts.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41431-021-00956-0.

Correspondence and requests for materials should be addressed to MichaelT. Geraghty

Reprints and permission information is available at http://www.nature.com/reprints

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.