



LJMU Research Online

Poole, OV, Hardy, SA, Bugiardini, E, Woodward, CE, Hargreaves, IP, Merve, A, Quinlivan, R, Taylor, RW, Hanna, MG and Pitceathly, RDS

Multisystem mitochondrial disease caused by a rare m.10038G>A mitochondrial tRNA Gly (MT-TG) variant

<http://researchonline.ljmu.ac.uk/id/eprint/12600/>

Article

Citation (please note it is advisable to refer to the publisher's version if you intend to cite from this work)

Poole, OV, Hardy, SA, Bugiardini, E, Woodward, CE, Hargreaves, IP, Merve, A, Quinlivan, R, Taylor, RW, Hanna, MG and Pitceathly, RDS (2020) Multisystem mitochondrial disease caused by a rare m.10038G>A mitochondrial tRNA Gly (MT-TG) variant. *Neurology Genetics*. 6 (2). ISSN

LJMU has developed [LJMU Research Online](http://researchonline.ljmu.ac.uk/) for users to access the research output of the University more effectively. Copyright © and Moral Rights for the papers on this site are retained by the individual authors and/or other copyright owners. Users may download and/or print one copy of any article(s) in LJMU Research Online to facilitate their private study or for non-commercial research. You may not engage in further distribution of the material or use it for any profit-making activities or any commercial gain.

The version presented here may differ from the published version or from the version of the record. Please see the repository URL above for details on accessing the published version and note that access may require a subscription.

For more information please contact researchonline@ljmu.ac.uk

<http://researchonline.ljmu.ac.uk/>

Multisystem mitochondrial disease caused by a rare m.10038G>A mitochondrial tRNA^{Gly} (*MT-TG*) variant

Olivia V. Poole, MD,* Alejandro Horga, MD, PhD,* Steven A. Hardy, PhD, Enrico Bugiardini, MD, Cathy E. Woodward, BSc, Iain P. Hargreaves, PhD, FRCPATH, Ashirwad Merve, PhD, FRCPATH, Rosaline Quinlivan, FRCP, Robert W. Taylor, PhD, FRCPATH, Michael G. Hanna, FRCP, and Robert D.S. Pitceathly, PhD, MRCP

Correspondence

Dr. Pitceathly
r.pitceathly@ucl.ac.uk

Neurol Genet 2020;6:e413. doi:10.1212/NXG.0000000000000413

Most pathogenic mitochondrial DNA (mtDNA) variants occur in the 22 mtDNA-encoded tRNA (mt-tRNA) genes. However, despite more than 270 reported mt-tRNA gene mutations, only 5 reside within mt-tRNA^{Gly} (*MT-TG*).¹ We report a rare *MT-TG* variant and evaluate this, in addition to all previously reported *MT-TG* variants, against the published criteria used to help determine the pathogenicity of the mt-tRNA variants.²

Case report

A 39 year old woman, born to nonconsanguineous parents, was reviewed in a specialist mitochondrial disorders clinic. She presented with hearing loss in her late teens followed by visual impairment, with bilateral cataracts, retinal dystrophy, and subsequent bilateral retinal detachments in her twenties; hypothyroidism in her thirties; and secondary amenorrhea. Clinical examination was otherwise normal, apart from short stature. There was no family history of neuromuscular or neurologic disease. Blood tests, including creatine kinase, plasma amino acids, acylcarnitine profile, very long chain fatty acids, and white cell enzymes, were normal. Plasma lactate was elevated (3.70 mmol/L, reference range 0.5–2.2 mmol/L). Nerve conduction studies and EMG showed no evidence of generalized myopathy or large fiber neuropathy. Histochemical analyses of muscle tissue revealed ragged-red and cytochrome *c* oxidase (COX) deficient fibers (figure A). Spectrophotometric determination of mitochondrial respiratory chain enzyme activities as a ratio to citrate synthase activity³ confirmed decreased activities of complexes I (0.076, reference range 0.104–0.268) and IV (0.006, reference range 0.014–0.034). Analysis of the next generation sequencing data (Illumina MiSeq) of the entire mitochondrial genome extracted from the muscle³ revealed a rare m.10038G>A variant (GenBank reference accession number: NC_012920.1) in *MT-TG* (figure B) that was present at variable heteroplasmy levels across tissue types: 15% blood, 40% urinary epithelial cells, and 92% skeletal muscle. Maternal transmission was confirmed: 3% mutant load was present in the mother's urinary epithelial cells (methodology detects heteroplasmy levels $\geq 1\%$). Heteroplasmy levels within individual laser-captured COX-positive and COX-deficient muscle fibers were quantified by pyrosequencing.⁴ Single fiber segregation studies confirmed a higher mutation load in COX-deficient (mean $95.30\% \pm 0.50\%$, SD, $n = 27$) compared with COX-positive fibers (mean $78.92\% \pm 4.43\%$, SD, $n = 26$; $p = 0.0005$, figure C).

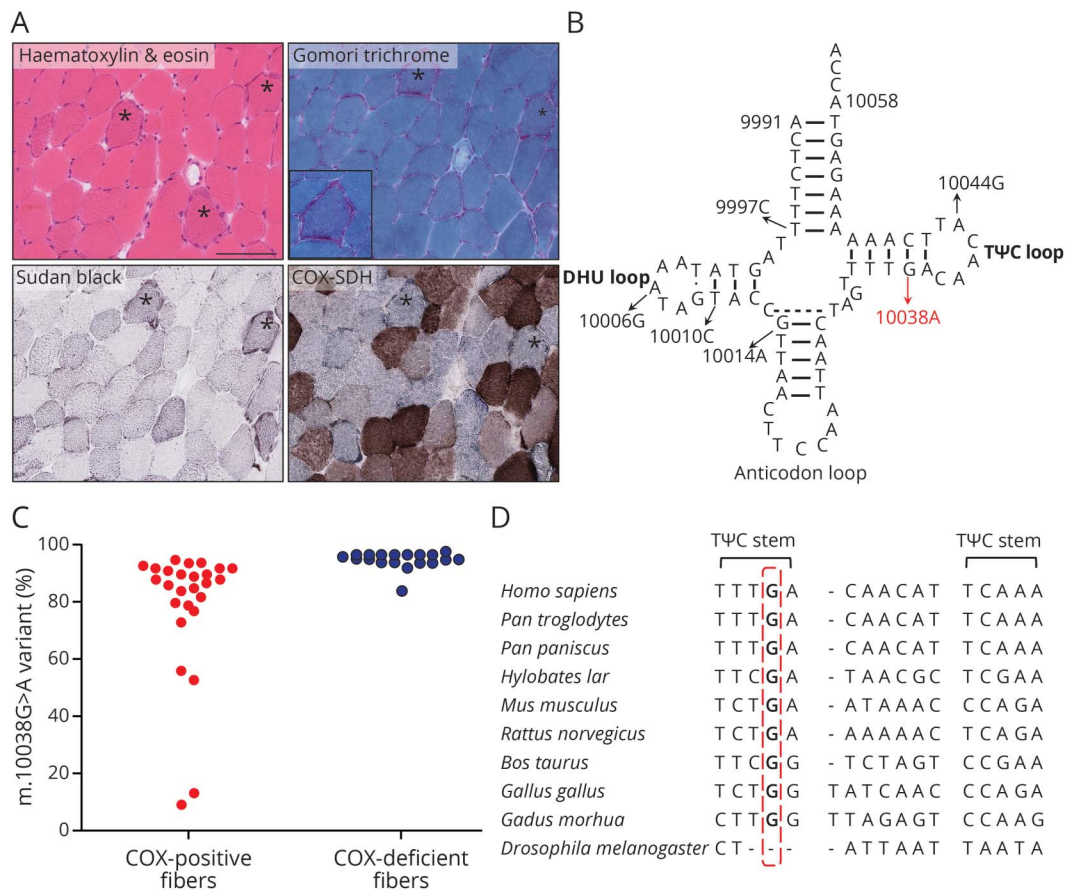
*These authors contributed equally to the manuscript.

From the Department of Neuromuscular Diseases (O.V.P., A.H., E.B., R.Q., M.G.H., R.D.S.P.), UCL Queen Square Institute of Neurology and The National Hospital for Neurology and Neurosurgery, London, United Kingdom; Wellcome Centre for Mitochondrial Research (S.A.H., R.W.T.), Translational and Clinical Research Institute, Newcastle University, Newcastle Upon Tyne; Neurogenetics Unit (C.E.W.), and Neurometabolic Unit (I.P.H.), The National Hospital for Neurology and Neurosurgery; Division of Neuropathology (A.M.), UCL Queen Square Institute of Neurology; Department of Histopathology (A.M.), Camelia Botnar Laboratory, Great Ormond Street Hospital; and Dubowitz Neuromuscular Centre (R.Q.), Great Ormond Street Hospital, London, United Kingdom.

Go to Neurology.org/NG for full disclosures. Funding information is provided at the end of the article.

The Article Processing Charge was funded by the authors.

This is an open access article distributed under the terms of the Creative Commons Attribution License 4.0 (CC BY), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.



(A) Staining of the muscle sections with haematoxylin and eosin demonstrated ragged-red fibers (*) which were confirmed in the Gomori trichrome preparation (*) with the inset showing high magnification of left top fiber. A number of fibers, often with ragged-red morphology, showed a mild increase in the number of lipid droplets with Sudan black (*). Sequential COX and SDH histochemistry demonstrated frequent COX-deficient fibers, some of which had a ragged red-like appearance (*). The bar represents 100 μm for all stains with the inset 50 μm. (B) Two-dimensional cloverleaf structure of mitochondrial DNA-encoded tRNA^{Gly}. Black arrows indicate previously reported pathogenic variants. Red arrow indicates the rare variant identified in our patient. (C) Single muscle fiber segregation studies. COX-deficient fibers, (blue circles) harbor a higher mutational load compared with COX-positive fibers (red circles). (D) Evolutionary conservation of the m.10038G residue across species. COX = cytochrome c oxidase; SDH = succinate dehydrogenase.

Discussion

The m.10038G nucleotide is highly conserved across species (figure D, figure e-1, links.lww.com/NXG/A248) and the G>A transition at this location disrupts a C-G Watson-Crick base pair in the TΨC stem of the tRNA molecule. The m.10038G>A variant is rare (absent from 3,450 in-house and 48,882 GenBank¹ sequences) and detectable at variable heteroplasmy levels across different tissues, with the highest levels in the postmitotic muscle. Histochemical and biochemical evidence of reduced complex I and IV activities in the muscle is supportive of impaired mitochondrial protein synthesis, whereas single fiber studies confirmed segregation of the COX defect with higher mutant levels. Consequently, the mutation would be considered “definitely pathogenic” based on the accepted criteria for assigning pathogenicity to tRNA mutations (table e-1, links.lww.com/NXG/A249).²

Review of the 5 previously reported *MT-TG* variants according to these criteria (table e-1, links.lww.com/NXG/A249) reveals

that only 2 variants; m.9997T>C and m.10010T>C, should be considered “definitely pathogenic.” Although the m.9997T>C variant has only been reported in one family, *trans*-mitochondrial cybrid studies support its pathogenic effects. The m.10010T>C variant has been reported a number of times, and its pathogenicity has been confirmed using single fiber segregation studies. The m.10006A>G variant has only been reported in individuals harboring additional mtDNA variants, and thus may represent a benign polymorphism or be insufficient to cause disease in isolation. Current evidence suggests that the m.10014A>G variant is a benign polymorphism. Finally, although the scoring system indicates that the m.10044A>G variant is “possibly pathogenic,” it has been detected in healthy controls, and thus may represent a haplogroup specific polymorphism.⁵

It remains unclear why pathogenic variants occur more frequently in specific mt-tRNA genes. Possible explanations include intensive investigation of mt-tRNAs in which pathogenic variants have previously been identified, and survivor bias and

the absence of maternal transmission of mutations in mt-tRNA genes linked with severe biochemical deficiencies, with isolated cases dissuading clinicians from further investigation of an underlying mitochondrial disorder.⁶ Variants in mt-tRNA^{Gly} affecting its canonical or noncanonical functions may also potentially be better tolerated than other mt-tRNAs or, conversely, be severely deleterious and embryonic lethal.

The detection of the rare mt-tRNA gene variants in suspected mitochondrial disease has become more commonplace, given the widespread availability of whole mtDNA high throughput sequencing. Furthermore, whole genome sequencing, which includes capture and deep sequencing of the mitochondrial genome, is identifying mtDNA variants in those in whom mitochondrial disease may not previously have been considered. However, despite advances in the DNA sequencing technology, the challenge of assigning pathogenicity to the rare mtDNA variants remains. It is therefore crucial that pathogenic variants are reported after confirmation using “gold-standard” techniques, for example, single fiber or *transmitochondrial* cybrid studies, given the implications to genetic counseling and the available reproductive options for mtDNA mutations, including mitochondrial donation. Moreover, generating a comprehensive data set for “definitely pathogenic” mt-tRNA variants would potentially advance the understanding of the molecular mechanisms underpinning the susceptibility of individual genes to deleterious variants and facilitate the development of targeted therapies to treat this group of disorders.

Acknowledgment

The authors would like to sincerely thank the family that participated in the study.

Study funding

Part of this work was undertaken in the University College London Hospitals/University College London Queen Square Institute of Neurology sequencing facility, which received a proportion of funding from the Department of Health’s National Institute for Health Research Biomedical Research Centres funding scheme. This research was supported by the National Institute for Health Research University College London Hospitals Biomedical Research Centre. O.V. Poole has received funding from the Lily Foundation. R.W. Taylor is supported by the Wellcome Centre for Mitochondrial Research (203105/Z/16/Z), the Medical Research Council (MRC) International Centre for Genomic Medicine in Neuromuscular Disease, Mitochondrial Disease Patient Cohort (UK) (G0800674), the UK NIHR Biomedical Research Centre for Ageing and Age-related disease award to the Newcastle upon Tyne Foundation Hospitals NHS Trust, the MRC/EPSC Molecular Pathology Node and The Lily Foundation. R.D.S. Pitceathly is supported by a Medical Research Council Clinician Scientist Fellowship (MR/S002065/1). The clinical and diagnostic mitochondrial services in London and Newcastle upon Tyne are funded by the UK NHS Highly Specialised Commissioners to provide the “Rare Mitochondrial Disorders” Service.

Disclosure

The present study is not industry sponsored. O.V. Poole, A. Horga, S.A. Hardy, E. Bugiardini, C. Woodward, I.P. Hargreaves, A. Merve, R. Quinlivan, R.W. Taylor, M.G. Hanna, and R.D.S. Pitceathly report no disclosures. Go to Neurology.org/NG for full disclosures.

Publication history

Received by *Neurology: Genetics* July 7, 2019. Accepted in final form January 23, 2020.

Appendix Authors

Name	Location	Contribution
Olivia V. Poole, MD	UCL Queen Square Institute of Neurology and The National Hospital for Neurology and Neurosurgery, London, United Kingdom	Study concept and design, Major role in acquisition of data, Analysis/interpretation of data, Revising the manuscript for intellectual content
Alejandro Horga, MD	UCL Queen Square Institute of Neurology and The National Hospital for Neurology and Neurosurgery, London, United Kingdom	Study concept and design, Major role in acquisition of data, Analysis/interpretation of data, Revising the manuscript for intellectual content
Steven A. Hardy, PhD	Wellcome Centre for Mitochondrial Research, Translational and Clinical Research Institute, Newcastle University, Newcastle Upon Tyne, United Kingdom	Major role in acquisition of data, Analysis/interpretation of data, Revising the manuscript for intellectual content
Enrico Bugiardini, MD	UCL Queen Square Institute of Neurology and The National Hospital for Neurology and Neurosurgery, London, United Kingdom	Major role in acquisition of data, Analysis/interpretation of data, Drafting/revising the manuscript for intellectual content
Cathy E. Woodward, BSc	The National Hospital for Neurology and Neurosurgery, London, United Kingdom	Major role in acquisition of data, Analysis/interpretation of data, Revising the manuscript for intellectual content
Iain P Hargreaves, PhD, FRCPath	The National Hospital for Neurology and Neurosurgery, London, United Kingdom	Major role in acquisition of data, Analysis/interpretation of data, Drafting/revising the manuscript for intellectual content
Ashirwad Merve, PhD, FRCPath	UCL Queen Square Institute of Neurology, London, United Kingdom	Major role in acquisition of data, Analysis/interpretation of data, Drafting/revising the manuscript for intellectual content
Rosaline Quinlivan, FRCP	UCL Queen Square Institute of Neurology and The National Hospital for Neurology and Neurosurgery, London, United Kingdom	Analysis/interpretation of data, Drafting/revising the manuscript for intellectual content
Robert W. Taylor, PhD, FRCPath	Wellcome Centre for Mitochondrial Research, Translational and Clinical Research Institute, Newcastle University, Newcastle Upon Tyne, United Kingdom	Major role in acquisition of data, Analysis/interpretation of data, Drafting/revising the manuscript for intellectual content

Continued

Appendix *(continued)*

Name	Location	Contribution
Michael G. Hanna, FRCP	UCL Queen Square Institute of Neurology and The National Hospital for Neurology and Neurosurgery, London, United Kingdom	Study concept and design, Analysis/interpretation of data, Revising the manuscript for intellectual content
Robert D.S. Pitceathly, PhD	UCL Queen Square Institute of Neurology and The National Hospital for Neurology and Neurosurgery, London, United Kingdom	Study concept and design, Analysis/interpretation of data, Revising the manuscript for intellectual content

References

1. Lott MT, Leipzig JN, Derbeneva O, et al. mtDNA variation and analysis using mitomap and mitomaster. *Curr Protoc Bioinformatics* 2013;44:1–23.
2. Yarham JW, Al-Dosary M, Blakely EL, et al. A comparative analysis approach to determining the pathogenicity of mitochondrial tRNA mutations. *Hum Mutat* 2011;32:1319–1325.
3. Poole OV, Everett CM, Gandhi S, et al. Adult-onset Leigh syndrome linked to the novel stop codon mutation m. 6579G> A in MT-CO1. *Mitochondrion* 2019;47:294–297.
4. Blakely EL, Yarham JW, Alston CL, et al. Pathogenic mitochondrial tRNA point mutations: nine novel mutations affirm their importance as a cause of mitochondrial disease. *Hum Mutat* 2013;34:1260–1268.
5. Lehtonen MS, Meiniälä M, Hassinen IE, Majamaa KJ. Haplotype-matched controls as a tool to discriminate polymorphisms from pathogenic mutations in mtDNA. *Hum Genet* 1999;105:513–514.
6. McFarland R, Elson JL, Taylor RW, Howell N, Turnbull DM. Assigning pathogenicity to mitochondrial tRNA mutations: when “definitely maybe” is not good enough. *Trends Genet* 2004;20:591–596.

Neurology[®] Genetics

**Multisystem mitochondrial disease caused by a rare m.10038G>A mitochondrial tRNA
Gly (MT-TG) variant**

Olivia V. Poole, Alejandro Horga, Steven A. Hardy, et al.

Neurol Genet 2020;6;

DOI 10.1212/NXG.0000000000000413

This information is current as of March 18, 2020

Neurol Genet is an official journal of the American Academy of Neurology. Published since April 2015, it is an open-access, online-only, continuous publication journal. Copyright © 2020 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology. All rights reserved. Online ISSN: 2376-7839.



Updated Information & Services	including high resolution figures, can be found at: http://ng.neurology.org/content/6/2/e413.full.html
References	This article cites 6 articles, 0 of which you can access for free at: http://ng.neurology.org/content/6/2/e413.full.html##ref-list-1
Subspecialty Collections	This article, along with others on similar topics, appears in the following collection(s): All Genetics http://ng.neurology.org/cgi/collection/all_genetics Endocrine http://ng.neurology.org/cgi/collection/endocrine Metabolic disease (inherited) http://ng.neurology.org/cgi/collection/metabolic_disease_inherited Mitochondrial disorders http://ng.neurology.org/cgi/collection/mitochondrial_disorders Visual loss http://ng.neurology.org/cgi/collection/visual_loss
Permissions & Licensing	Information about reproducing this article in parts (figures, tables) or in its entirety can be found online at: http://ng.neurology.org/misc/about.xhtml#permissions
Reprints	Information about ordering reprints can be found online: http://ng.neurology.org/misc/addir.xhtml#reprintsus

Neurol Genet is an official journal of the American Academy of Neurology. Published since April 2015, it is an open-access, online-only, continuous publication journal. Copyright © 2020 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology. All rights reserved. Online ISSN: 2376-7839.

