Investigation of the correlation between mildly deleterious mtDNA variations and the clinical progression of multiple sclerosis

Article (Accepted Version)

Submitted for editorial review to *Multiple Sclerosis and Related Disorders*

Investigation of the Correlation Between Mildly Deleterious mtDNA Variations and the Clinical Progression of Multiple Sclerosis

Ilse S. Pienaar[1], Rean Mohammed[3], Rebecca Courtley[2], Michael R. Gledson[4], Richard Reynolds[4,5], Richard Nicholas[4], Joanna L. Elson[3,6]

Running head: mtDNA variation in Multiple Sclerosis progression

Key words: mitochondrial DNA (mtDNA), Variant load model (VLM), Haplogroup, Multiple Sclerosis, Disease progression

[1] School of Life Sciences, University of Sussex, Falmer, United Kingdom
[2] Northern Genetics Service, Newcastle Hospitals NHS Foundation Trust Newcastle upon Tyne, United Kingdom
[3] Department of Brain Sciences, Faculty of Medicine, Imperial College London, London, United Kingdom
[4] Biosciences Institute, Newcastle University, Newcastle upon Tyne, United Kingdom
[5]Centre for Molecular Neuropathology, Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore
[6] Centre for Human Metabolomics, North-West University, Potchefstroom, South Africa

Corresponding author:

Dr. Joanna L. Elson
Lecturer in Mitochondrial Genetics
Biosciences Institute, Newcastle University, Newcastle upon Tyne, United Kingdom
Tel: 0044 191 241 8630; E-mail: Joanna.elson@newcastle.ac.uk
Author orchid: 0000-0002-3551-5624

.

Abstract

Background: Evidence suggests that mitochondrial DNA (mtDNA) variation at a population level may influence susceptibility to, or the clinical progression of Multiple Sclerosis (MS).

Objective: To determine if mtDNA population variation is linked to the clinical progress of MS.

Methods: Using the complete mtDNA sequences of 217 MS patients, we applied the new 'variant load' model, designed as a framework by which to examine the role of mtDNA variation in the context of complex clinical disease.

Results: No significant association was detected between mtDNA 'variant load' and the clinical measures of progression.

Conclusion: Our results show that mtDNA population variation does not play a substantial role in the clinical progression of MS; however, modest effects and/or effects in a subgroup of patients cannot be entirely excluded as a possibility. The results further illustrate the method's applicability to other disease phenotypes, to use in conjunction with quantitative patient measures, to test for a statistical relationship between mildly deleterious mtDNA variation and disease progression.

Introduction

Mitochondrial DNA (mtDNA) is present as 100-1000's copies per cell. Rare inherited mtDNA mutations that cause classic mitochondrial disease are present as a mixture of mutated and wild-type mtDNA molecules within cells, a phenomenon termed heteroplasmy (1). mtDNA has been implicated in MS susceptibility in some case-control studies using the haplotype association model, but not in others (2, 3).

However mtDNA also shows high levels of population variation termed a homoplasmic inheritance pattern. Here variants are present on all mtDNA copies within cells, with some of these changes predicted to be mildly deleterious and potentially have a role in the progression of multifactorial disease (4). Building on this a further approach a 'variant load model' (VLM) has been developed. This suggests that mtDNA variants that are less common at the population level may be regarded as either a factor that increases disease susceptibility, or one that may modulate the course of complex disease. This is considered biologically plausible, as rarer variants are more likely to be mildly deleterious (4, 5). The VLM does not attempt to link a single mildly deleterious variant with disease, but rather suggests that the cumulative effect of such variants might be more common in patients with certain diseases, or a more rapid course of disease.

In this report we consider whether MS disease characteristics are affected by the level of mildly deleterious inherited mtDNA population variants harbored by patients from the UK MS Society Tissue Bank (UKMSTB).

Methods

Patient cohort and brain tissue collection and mtDNA sequencing

MS post-mortem brain tissue samples were provided by the UKMSTB (https://www.imperial.ac.uk/medicine/multiple-sclerosis-and-parkinsons-tissue-bank). The study was approved by the national NRES committee (08/MREC09/31+5). DNA was extracted from snap frozen tissue blocks from the cerebellum (n = 217) at Source Bioscience (https://www.sourcebioscience.com). Next Generation sequencing using Fluidigm technology (Illumina) was carried out at a mean depth of 5,000x per site (5).

Data analyses

Complete mitochondrial genomes were analysed using mtDNA-Server (http://haplogrep.uibk.ac.at) (6). Pathogenicity status was assessed using MitoMap (https://www.mitomap.org/MITOMAP) (7) and accepted

scoring criteria (8). The MutPred software (http://mutpred.mutdb.org), assigned a 'pathogenicity' score between 0-1 (an amino acid change). A score of zero predicts the variant to be benign, a score >0.5 is classified as an "actionable hypothesis", predicting an effect on protein function. The scores >0.5 and >0.7 were then summed to produce the variant load. Low scoring variants were excluded, in accordance to previously published guidelines (5).

Statistical analyses

Correlation analysis was performed using either a Pearson's Correlation Coefficient or Spearman's Rho. Fishers *t*-test and contingency analysis were used as indicated in the text. Analyses were performed using SPSS Statistics software (v25; IBM corporation, Chicago, USA). The threshold for significance was set at 0.05 with appropriate correction for multiple testing being employed where a significant result was found.

Results

Heteroplasmy analysis

The sequence data was examined for variants previously linked to inherited mitochondrial disease (1, 7, 8). A single variant tRNA-Phe m.622G>A (7), was detected in the dataset. The heteroplasmy level was 1.45%, well below levels (>60%) that associate with disease (1, 8).

The impact of potentially mildly deleterious mtDNA variants on the course of disease

A summary of cohort attributes can be seen in Table 1, with a summary of the statistical results being shown in table 2 with detailed information concerning mtDNA variant load scores for each cohort member being shown in Supplemental Table 1. The correlation of mtDNA variant load calculated from MutPred scores of > 0.5 with clinical outcomes was investigated using Pearson correlation giving the following results : age of symptom onset (p=0.88), age of disease progression (p=0.31), age at death (p=0.19), time from symptom onset to needing a wheelchair (p=0.05), time between symptom onset and death (p=0.13), time between symptom onset and the onset of the secondary progressive phase (p=0.69), and time from the onset of the secondary progressive phase to death (p=0.15). Thus, there was only one result of significance from all the correlations conducted between clinical phenotypes and mtDNA variant load. This being the time from symptom

onset to the time that the patient was required to use a wheelchair (p=0.05) this result was no longer significant after correction for multiple testing. The number of relapses patients experienced in the first two years of disease was also considered, as more than two relapses being considered a poor prognostic indicator (9). This analysis was conducted using a Student's *t*-test, with the variant loads of those with greater than two relapses being compared to those with fewer; again, no significant difference was seen (p=0.89). Similar non-significant effects was seen when variant load was calculated using only amino acid changes with a MutPred value >0.7 (p=0.8).

Post-mortem whole brain weight was also correlated with mtDNA variant load using a Pearson's Correlation with a non-significant relationship between brain weight and mtDNA variant load (p=0.91). A qualitative post-mortem measure of inflammation, the degree of inflammation was considered in the context of the mtVLM using a Spearman's Rho; and was not significant (p=0.39) (10).
(10).

Discussion

Prior studies on the role of population mtDNA variation in MS considered whether common mtDNA population variation acts as a susceptibility factor; however, such reports revealed inconsistent results (2, 3). Here, for the first time, we present a detailed investigation concerning the putative role of mtDNA population variation in the clinical course of MS by using a new association model namely the VLM. The VLM focuses on mtDNA variants in the protein encoding genes predicted by computational methods to be mildly deleterious, which could potentially contribute to the disease process in considers the action of these variants in synergy (4, 5). We applied the VLM by correlating the calculated variant load of each patient with various central disease outcomes, as per the patient records. This analysis did not produce any significant correlations. Thus, by using a large, clinically well-described sample set of MS patients with complete mtDNA sequence data, our data refutes a strong association between mtDNA variation and several distinct measures of clinical progression in MS. However, we did find borderline significant associations with the length of disease and its impact on wheelchair use, prior to correction for multiple comparisons, implying a larger study may offer further

sights. The advantage of the UKMSTB dataset is that we have a full history with pathological outcome but this level of detail results in smaller numbers that is a major a limitation in genetic studies.

Additionally, results do not eliminate the possibility of more moderate associations, nor the possibility of significant associations in subsets of MS patients, e.g. patients with primary progressive MS. The current results also do not completely exclude the possibility of detecting an association between mtDNA variation and other quantified variables such as those obtained from scoring histopathologically-stained patient brain specimens.

Acknowledgements: We thank the MS Brain Bank technical staff for assisting in preparing the samples used in this study. This work was supported by a PhD studentship awarded by the Saudi Government (to RM) and by a grant awarded by the MS Society of Great Britain and Northern Ireland (to RR, RN; 910/09)

Author contributions: ISP provided technical expertise, conducted analysis/experiments and commented on various draft versions of the paper. RM conducted experimental work and analysis. MRG and RC conducted analysis. RR con-ceived areas of the project provided the samples and commented on drafts. RN contributed clinical expertise and commented on drafts. JLE conceived the data analysis, conducted such analysis and wrote the paper. All authors approved the final version of the paper.

Declaration of conflicting interests: The author(s) declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

References

1. Tuppen HA, Blakely EL, Turnbull DM, Taylor RW. Mitochondrial DNA mutations and human disease. Biochim Biophys Acta. 2010;1797(2):113-28. doi: 10.1016/j.bbabio.2009.09.005. Epub Sep 15.
2. Ban M, Elson J, Walton A, Turnbull D, Compston A, Chinnery P, et al. Investigation of the Role of Mitochondrial DNA in Multiple Sclerosis Susceptibility. Plos One. 2008;3(8).
3. Yu X, Koczan D, Sulonen AM, Akkad DA, Kroner A, Comabella M, et al. mtDNA nt13708A variant increases the risk of multiple sclerosis. PLoS One. 2008;3(2):e1530. doi: 10.371/journal.pone.0001530.
4. Pienaar IS, Howell N, Elson JL. MutPred mutational load analysis shows mildly deleterious mitochondrial DNA variants are not more prevalent in Alzheimer's patients, but may be under-represented in healthy older individuals. Mitochondrion. 2017;34:141-6.
5. Venter M, Tomas C, Pienaar IS, Strassheim V, Erasmus E, Ng WF, et al. MtDNA population variation in Myalgic encephalomyelitis/Chronic fatigue syndrome in two populations: a study of mildly deleterious variants. Sci Rep. 2019;9(1):2914.
6. Weissensteiner H, Forer L, Fuchsberger C, Schopf B, Kloss-Brandstatter A, Specht G, et al. mtDNA-Server: next-generation sequencing data analysis of human mitochondrial DNA in the cloud. Nucleic acids research. 2016;44(W1):W64-9.
7. Lott MT, Leipzig JN, Derbeneva O, Xie HM, Chalkia D, Sarmady M, et al. mtDNA Variation and Analysis Using MITOMAP and MITOMASTER. Current protocols in bioinformatics / editoral board, Andreas D Baxevanis [et al]. 2013;1(123):1.23.1-1..6.
8. Yarham JW, Al-Dosary M, Blakely EL, Alston CL, Taylor RW, Elson JL, et al. A Comparative Analysis Approach to Determining the Pathogenicity of Mitochondrial tRNA Mutations. Human Mutation. 2011;32(11):1319-25.
9. Scalfari A, Neuhaus A, Degenhardt A, Rice GP, Muraro PA, Daumer M, et al. The natural history of multiple sclerosis: a geographically based study 10: relapses and long-term disability. Brain. 2010;133(Pt 7):1914-29.
10. Reynolds R, Roncaroli F, Nicholas R, Radotra B, Gveric D, Howell O. The neuropathological basis of clinical progression in multiple sclerosis. Acta neuropathologica. 2011;122(2):155-70.

Indicator of MS Progression	Minimum	Maximum	Mean	SD Deviation
Variant load using only the scores > 0.5	0	3.41	0.42	0.6
Variant load using only the scores > 0.7	0	2.12	0.04	0.22
Post-mortem delay before brain tissue collection	5.00	48.00	18.38	7.67
Patient age at disease onset	9.00	60.00	30.70	8.99
Age of disease progression	20.00	74.00	42.86	10.36
Wheelchair use commenced	18.00	77.00	46.98	11.94
Patient age at death	34.00	92.00	59.03	12.25

Table 1: Attributes of the clinical cohort used in the current study.

Variant load using only the scores > 0.5: Average variants loads of cohort members considering only variants thought to be at least mildly deleterious after assessment with http://mutpred.mutdb.org
Variant load using only the scores > 0.7: Average variants loads of cohort members considering only variants thought to be pathogenic after assessment with http://mutpred.mutdb.org
Patient age at disease onset: Age at first symptoms nowadays the first episode is considered the start of MS
Age of disease progression: The age at which the patient entered the secondary progressive phase
Wheelchair use commenced Age at which the patient required the use of a wheelchair
Patient age at death: The age of the patient at death

Phenotype	Test applied	Uncorrected p-value
Age of symptom onset	Pearson's correlation	p=0.88
Age of disease progression	Pearson's correlation	p=0.31
Age at death	Pearson's correlation	p=0.19
Time from symptom onset to needing a wheelchair	Pearson's correlation	p=0.05*
Time between symptom onset and death	Pearson's correlation	p=0.13
Time between symptom onset and the onset of the secondary progressive phase	Pearson's correlation	p=0.69
Time from the onset of the secondary progressive phase to death	Pearson's correlation	p=0.15
Number of relapses patients experienced in the first two years of disease, 2 vs 2+	t-test	p=0.89
Post-mortem whole brain weight	Pearson's correlation	p=0.91
Qualitative post-mortem measure of inflammation	Spearman’s Rho	p=0.39

Table 2: Phenotypes considered in comparisons to mtDNA variant load. Note there is a result of borderline significance indicated with an * this is no longer significant after correction for multiple comparisons.