Can mitochondrial DNA abundance be used to predict disease severity in patients with 3243A>G mutation?

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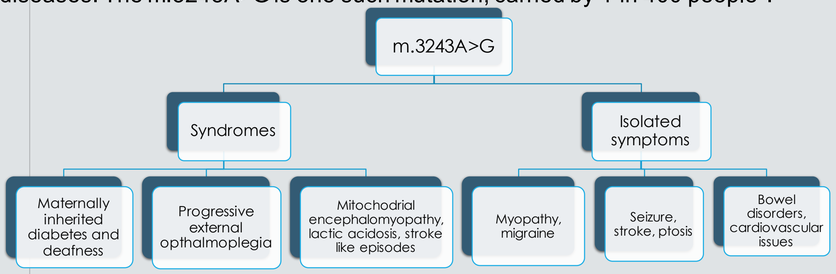
Aims

Investigating the association between mitochondrial DNA (mt-DNA) copy number and clinical disease severity in patients with the m.3243A>G mutation.

Introduction

Mitochondria are semi-autonomous organelles possessing their own DNA. Mutations in mitochondrial DNA (mt-DNA) leads to clinically heterogeneous diseases. The m.3243A>G is one such mutation, carried by 1 in 400 people[1].



Mitochondrial diseases are associated with a number of syndromes and phenotypes, as illustrated above. The exact cause of this phenotypic variability in the patients is yet to be understood. For a long time, it was believed that the proportion of mutated to normal mt-DNA (heteroplasmy) could explain the clinical heterogeneity and disease severity seen in patients. However, a study[3] suggests that the number of mitochondrial DNA molecules (copy number) is a much better predictor compared to heteroplasmy. My project investigated the significance of copy number as a predictor of disease severity and progression.

Methods

Copy number quantification assay

Real time PCR assay for copy number quantification[2]

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Analysis of skeletal muscle DNA from 50 patients with the mutation and 1 control

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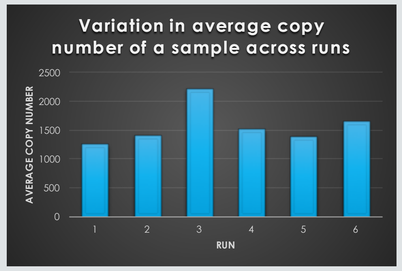
Copy number determined as the relative expression of a mitochondrial gene (ND1) to a nuclear gene (B2M)

We used standard primers and probes as defined for this singleplex Taqman assay to quantify the relative levels of mt-DNA copy number. Each sample was run in triplicates to minimize dilution error. From the cycle threshold (Ct) values obtained, we determined sample ND1 and B2M levels using the included standard curves.

Coping with variability in the assay

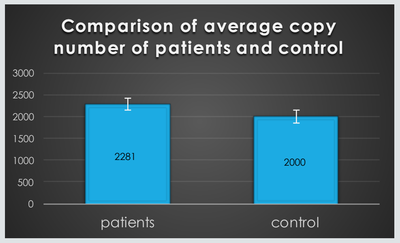


The average copy number, thus calculated, for each sample varied significantly across runs.

To account for this variability, we compared the average copy number for each sample to the average copy number of all samples per run.
This data was then incorporated with the pre-existing heteroplasmy data to calculate the total copy number, wild type copy number (wt-copy number) and mutant copy number (mut-copy number).

Results

Comparison of copy number of patients versus control



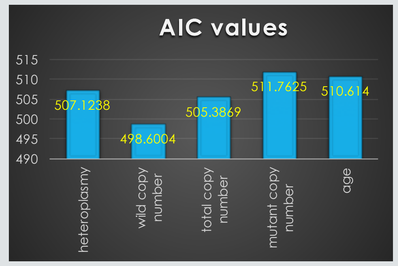
As seen previously[3], the average mt-DNA copy number per cell was higher in m.3243A>G patients compared to the control.

p- values obtained for major phenotypic features using ordinal logistic regression

Phenotype	Heteroplasmy	Wild type copy number	Mutant copy number	Total copy number
Exercise Intolerance	0.198	0.063	0.491	0.115
Migraine	0.448	0.406	0.978	0.726
Seizure	0.971	0.078	0.073	0.049
Stroke	0.923	0.032	0.024	0.014
Gastrointestinal Problems	0.748	0.114	0.198	0.114
Diabetes	0.062	0.833	0.033	0.300
Cardiovascular Problems	0.129	0.011	0.343	0.032
Ptosis	0.940	0.408	0.359	0.185
Myopathy	0.057	0.053	0.705	0.249

The table alongside depicts p-values for the major phenotypic characters. Values shaded blue indicate interesting findings.

Longitudinal modelling to compare heteroplasmy and copy number as predictors of disease progression



Model fit evaluation was done on the basis of Akaike information criterion (AIC), with the lowest AIC values showing the best fit. The parameter with the best model fit was wild type copy number (p=0.0001), whereas, heteroplasmy was a relatively poor predictor of disease progression (p=0.0146)

Conclusions

- We found that total copy number was more predictive of stroke and seizure (0.049) compared to heteroplasmy. Lower copy number was associated with greater frequency of seizures and strokes.
- Myopathy and cardiovascular symptoms were better predicted by wild type copy number compared to heteroplasmy.
- Diabetes was, however, better predicted by mutant copy number compared to heteroplasmy. A higher copy number was associated with a greater frequency of diabetes.
- From these findings, it seems probable that copy number (wild, mutant and total) could be used to predict particular phenotypic symptoms exhibited by patients. However, further research needs to be undertaken to fully understand the potential benefits of copy number over heteroplasmy as a predictive factor. If these findings are consolidated through future work, we might finally have an answer as to why different patients with the same mutation have different symptoms.

References

1.Nesbitt (2013), 'The UK Mitochondrial Disease Patient Cohort Study: clinical phenotypes associated with the m.3243A>G mutation-implications for diagnosis and management,Journal of neurology and neurosurgery, 84:936-938
2.Grady (2014), 'Accurate measurement of mitochondrial DNA deletion level and copy number differences in human skeletal muscle',Plos One, 9(12):e114462
3. Liu(2013), 'Wild type Mitochondrial DNA copy number in urinary cells as a useful marker for diagnosing severity of the mitochondrial disease', Plos One, 8(6):e67146

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