The clinical features and genetic characteristics of *MT-ATP6*-related mitochondrial disease: a national, observational study

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Running head: Features of *MT-ATP6*-related mitochondrial disease

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KEY POINTS

Question: What are the key features of disease related to mutations in the mitochondrial *MT-ATP6* gene?

Finding: The clinical and follow-up data of 88 patients with mitochondrial disease due to *MT-ATP6* mutations (83% cases were ≥18 years of age) were analysed in a national (UK) cohort study . Five pathogenic variants account for 91% of the cases, and the core clinical features are cerebellar ataxia (84%), peripheral neuropathy (76%), and cognitive dysfunction (68%). Thirty six per cent of patients manifested with Leigh syndrome.

Meaning: Sequencing of the *MT-ATP6* gene should be considered in the diagnostic workup of patients with cerebellar ataxia and peripheral neuropathy. Prevalent cognitive difficulties merit attention in the clinical practice as they may result in challenges in social and functional performance. Our findings on the clinical heterogeneity and mutant heteroplasmy levels among the common *MT-ATP6* mutations have important implications in genetic counselling and reproductive options. Subacute brainstem dysfunction could occur in some adult patients without previously labelled to have 'Leigh syndrome' – prompt recognition and active supportive care are required to mitigate the risk of morbidity and mortality.

ABSTRACT

Importance: Mutations in the mitochondrial *MT-ATP6* gene are an important cause of mitochondrial disease. Phenotypes related to these mutations include Leigh syndrome (LS), and the syndrome of neuropathy, ataxia, and retinitis pigmentosa (NARP); however, there are also reports of other and intermediate phenotypes.

Objective: To characterise the phenotypic and genotypic spectrum of the *MT-ATP6* related mitochondrial disease in a large and well-defined mitochondrial disease cohort.

Design: A retrospective, observational cohort study.

Setting: Three national diagnostic and clinical centres for mitochondrial disorders in Newcastle upon Tyne, Oxford and London; patients were recruited to the Mitochondrial Disease Patient Cohort, UK.

Participants: Eighty-eight patients with clinically and genetically defined mitochondrial disease due to mutations in the *MT-ATP6* gene that were included in this study. Data from 36 clinically unaffected carriers of *MT-ATP6* mutations were also analysed.

Main Outcomes and Measures: All patients were interviewed and examined. All available medical notes and clinical, radiological, and genetic investigations were reviewed.

Results: We identified 88 clinically affected individuals and 36 asymptomatic family members from 60 pedigrees. Among the patients of whom the respective data were available, 57 of 69 (83%) had cerebellar ataxia, 42 of 57 (74%) had peripheral neuropathy, and 40 of 59 (68%) had some degree of cognitive dysfunction or learning disability. Thirty-two patients (36%) had a presentation compatible with LS, whereas just seven patients (8%) had the complete neuropathy, ataxia, and retinitis pigmentosa phenotype without LS. We observed meaningful differences between the clinical features associated with the common *MT-ATP6* mutations. Of interest, four adult patients developed unexpected, subacute brainstem

dysfunction akin to Leigh-like crisis, one patient had a stroke-like episode, and three patients exhibited symptoms similar to episodic ataxia.

Conclusions and Relevance: The majority of patients with genetic defects of *MT-ATP6* have cerebellar ataxia and peripheral neuropathy, and some degree of cognitive dysfunction seems relatively common among these patients. Moreover, some patients experience sudden exacerbations that are suggestive of a mitochondrial energy crisis and mandate active supportive treatment. These findings may be useful in the diagnostics and care of patients with *MT-ATP6* related mitochondrial disease.

INTRODUCTION

Mitochondrial disease, related to genetically determined defects of oxidative phosphorylation that result in impaired cellular energy production, is a common form of inherited multisystem disease.[1] Mutations in the *MT-ATP6* gene, encoding for the mitochondrial ATPase 6, are a recognized cause of mitochondrial DNA disease. Early established phenotypes of *MT-ATP6* related mitochondrial disease include Leigh syndrome (LS),[2] a severe, life-threatening multisystem disorder often presenting in infancy, and the syndrome of neuropathy, ataxia, and retinitis pigmentosa (NARP).[3] In addition to these established syndromes, other clinical presentations in association with *MT-ATP6* mutations have been reported, including Charcot-Marie-Tooth (CMT) disease like pure peripheral neuropathy[4] and spinocerebellar ataxia (SCA) with upper motor neuron signs.[5] The relative frequency of various presentations, or which clinical features are most suggestive of disease related to mutations in *MT-ATP6*, is however unclear.

Previous studies of patient cohorts with other types of mitochondrial disease have revealed considerable variation in associated clinical phenotypes and sometimes highlighted the relevance of previously under-appreciated features.[6-9] Improved understanding of the variable clinical features associated with different types of mitochondrial disease is useful for clinicians who diagnose and treat patients with these conditions. Moreover, better understanding of potential associations of phenotypes and different mutations may provide the basis for more informative clinical counselling of patients and their families. Previous studies have described features associated with a single pathogenic *MT-ATP6* mutation or mutations related to one particular phenotype. In order to elucidate the central clinical features of *MT-ATP6* related mitochondrial disease and the possible associations with the underlying mutations, we decided to investigate the clinical characteristics of patients with

mitochondrial disease due to *MT-ATP6* mutations in a well-characterized large mitochondrial disease patient cohort.

METHODS

Subjects

Subjects harbouring the pathogenic *MT-ATP6* variants were identified from the NHS Highly Specialised Service for Rare Mitochondrial Disorders (Newcastle, Oxford and London) and patients registered on the UK Mitochondrial Disease Patient Cohort (Mito Cohort) (REC:13/NE/0326) (date: Jan 2009 to June 2018). A standardised *pro forma* was used to capture clinical, radiological, neurophysiological and molecular genetics data from the case notes. The diagnostic criteria used for Leigh syndrome were described elsewhere.[10]

This study was approved and performed under the ethical guidelines issued by our institutions for clinical studies. Written informed consent for the genetic testing has been obtained from all participants.

Molecular genetics and measurement of mutant heteroplasmy

The *MT-ATP6* and *MT-ATP8* genes were screened by direct sequencing of PCR-amplified products[1111111111111111111]which are subsequently compared to the revised Cambridge reference sequence for human mtDNA (GenBank Accession number: NC_012920.1).[12]

The individual pathogenic *MT-ATP6* variant was screened by a pyrosequencing assay that permits the quantitation of mtDNA heteroplasmy at the relevant nucleotide, and can reliably detect to a level of >3% heteroplasmy; variants below this level may not be detected as

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normal controls can fall into this range. Accuracy of heteroplasmy is estimated to be ±2% based on in-house validation data, although this may deviate depending upon specific sequence context. Data analysis was performed using PyroMark Q24 software (v2.0.7).

Statistical analysis

Descriptive statistical analysis was performed using Minitab (V17.0), SPSS (V 23.0), and R (V3.5). Non-parametric data were summarised using median, range and interquartile range. Kruskal-Wallis test was performed to determine if there was any statistically significant difference between the different groups. The statistical significance was determined at ≤ 0.05. Chi-square test was performed to compare the proportion of variables between different categories, and adjusted p value was reported where appropriate based on the Bonferroni correction. Logistic progression model was used to evaluate the relationship of mutant blood heteroplasmy levels and individual risk of manifesting with disease, based on the methods previously described elsewhere (**Supplemental material**).[13,14]

RESULTS

Demographic description

We identified 88 clinically symptomatic patients (49 male) from 60 pedigrees (age of last follow up range 0.75–74 years, median 26.5 years, interquartile range (IQR): 33.3 years) harbouring *MT-ATP6* mutations. Overall, the median age of disease onset was 3.75 years (range: 0 - 71 years, IQR: 16.9 years). Patients with LS had a significantly lower median age of onset compared to those without LS (1.5 vs. 15 years, $p<0.001$). Earlier age of onset usually predicted a more severe disease course. The patients' general characteristics, genotypes, and mutation (blood) heteroplasmy levels are summarized in **Table 1**. In terms of

the survival status, 15 patients died (17%) and five patients were lost to follow up (**Supplemental Figure 1**).

We also identified 32 asymptomatic carriers (27 female) and their median age of last follow up was 40 years (range 10–84 years, IQR: 23 years), which was significantly higher than the clinically affected individuals ($p<0.001$).

Spectrum of clinical features

Among the patients of whom the respective data were available, the most common clinical examination finding was cerebellar ataxia (60/72; 83%), followed by peripheral neuropathy (43/58; 74%) and cognitive dysfunction/learning disability (40/62; 65%). Thirty six percent of patients (31/82) had a clinical phenotype compatible with LS, whereas just seven patients had the complete neuropathy, ataxia, and retinitis pigmentosa (NARP) phenotype without LS. Seizures or epilepsy were noted in 23% of patients (19/84).

Common bedside neurological findings were reported as follows: muscle weakness (42/65, 64%), lower limb spasticity (26/45, 58%), brisk reflexes (33/72, 46%), *pes cavus* (24/63, 38%), and areflexia (17/72, 24%). Dystonia was only documented in ten patients (12%), although we suspect it was under-reported among patients with LS. In particular, lower limb spasticity was a common clinical examination finding, which may be difficult to discern from fixed dystonia. Among the patients who had their muscle strength documented, distal weakness was the most common pattern (13/53, 25%), closely followed by proximal weakness (11/53, 21%) and mixed proximal and distal weakness (6/53, 11%). Mixed upper and lower motor neuron signs were identified in 54% of patients (34/63). Other types of movement disorders were identified in our *MT-ATP6* cohort: chorea-athethoid movement

(n=3), myoclonus (n=3), tremor (n=2), and parkinsonism (n=1). The prevalence of selected clinical features and findings in patients harbouring the five most common *MT-ATP6* mutations are presented in **Table 2**.

Acute metabolic and physical decompensation during inter-current illness was recognised in 45% of patients (27/60). Four adult patients experienced episodic, abrupt disease exacerbations in the form of a sudden Leigh-like crisis with worsening ataxia and brainstem symptoms like ophthalmoplegia, dysphagia, and dyspnoea, with corresponding T2-hyperintensities in brainstem, thalamus, and the cerebellum in brain MR imaging (**Figure 1**). Three of these patients harboured the m.8993T>C mutation, and one the m.9185T>C mutation.

The profile of clinical features was compared between patients with and without LS. A history of metabolic decompensation (91% vs 17%, $p<0.001$), presence of cognitive dysfunction/learning disability (95% vs 54%, $p=0.002$) and basal ganglia lesions (83% vs 17%, $p<0.001$) were significantly higher in patients with LS compared to those without LS. However, the defining features of NARP, namely neuropathy, ataxia, retinitis pigmentosa were common in both patient groups (**Supplemental Table 1**).

Other systematic features that are associated with adult mitochondrial disease[13] such as cardiac disease (8/53; 15%), diabetes mellitus (3/72; 4%), and sensorineural hearing loss (8/59; 14%), were relatively uncommon among patients with *MT-ATP6* disease.

Neuroimaging changes

Data for the cranial magnetic resonance (MR) imaging was available for analysis in 56 patients and nine of them had a normal scan (16%). Cerebellar atrophy, symmetrical basal ganglia lesions and brainstem abnormalities were present in 24 patients (43%), 24 patients (43%) and eight patients (14%), respectively. Seventeen of the 22 (77%) patients with basal ganglia lesions had clinical features and trajectory that were consistent with LS, three cases of LS without the basal ganglia lesions had the brainstem changes, and one patient had a normal scan before death under one year old. Some patients also presented with brain imaging changes usually considered atypical in *MT-ATP6* disease, such as bilateral occipital stroke like lesions in one patient and signal abnormalities in the cerebellar cortex (**Figure 1**).

Molecular genetics

We identified nine previously reported pathogenic variants in our cohort of patients. The frequencies of individual pathogenic variants are summarised in **Figure 2A**. The most common point mutation was m.8993T>C (27%), followed by m.8993T>G (25%), m.9185T>C (20%), m.9176T>C (13%) and m.9035T>C (9%). We were able to establish the maternal transmission of the pathogenic *MT-ATP6* variants in 68 patients (77%) and the mutation was likely arisen *de novo* in three patients (3%). The status of maternal transmission was not known in 17 patients (20%) and the most common reason was due to familial sample not available for screening.

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The age of onset and mutant heteroplasmy levels in blood were compared across different pathogenic variants, as shown in **Figure 2B** and **2C**. The variability in the mutant heteroplasmy level between different tissues (blood, urinary epithelial cells and buccal mucosal cells) was typically less than 10% in m.8851T>C, m.8993T>C, m.8993T>G,

m.9035T>C, m.9176T>C and m.9185T>C. Marked segregation was observed in m.8839G>C (26%, 76% and 58% in blood, urine and buccal samples respectively), m.9032T>C (25%, 59% and 96% in blood, urine and muscle respectively) and m.9134A>G (43% and 90% in blood and urine, respectively) (**Figure 2D**).

Risk of disease manifestation and heteroplasmy level

Blood heteroplasmy levels of five pathogenic variants (m.8993T>C, m.8993T>G, m.9035T>C, m.9176T>C and m.9185T>C) were pooled in the logistic regression model for calculating the risk of developing mitochondrial disease. The remaining rare pathogenic *MT-ATP6* variants were excluded because of the skewed tissue segregation of mutant heteroplasmy level. The risk of being clinically affected is low until the blood heteroplasmy level exceeds 50% (**Figure 3**). The mutation load for an estimated probability of 0.25 for being clinically affected is 60% (95% CI 39%-69%), with the probability increasing to 0.5, 0.75 and 0.95 at the heteroplasmy levels of 70% (95% CI 56%-77%), 80% (95% CI 72%-87%%) and 96% (95% CI 89%-100%), respectively. Such relationship cannot be established reliably for individual pathogenic variant because of the small sample size (**Supplemental Figures 1&2**).

DISCUSSION

In this large, national cohort study, we observed a continuum of clinical phenotypes among the 88 individuals with *MT-ATP6* related mitochondrial disease. The age of onset was significantly lower in *MT-ATP6* mutations compared to other common mtDNA mutations[8,15,16], with the vast majority of patients manifesting before the age of 35 years. Cerebellar ataxia (83%) and peripheral neuropathy (74%) were the most common features among these patients, often associated with some degree of cognitive impairment or learning

disability (65%). We identified that patients with LS also exhibited overlapping features of NARP. Despite these common characteristics, we observed some emerging patterns associated with specific *MT-ATP6* mutations (**Table 2**). RP was most prevalent in m.8993T>G-related mitochondrial disease compared to other *MT-ATP6* variants; however, RP was only clinically identified in less than a third of all patients. Dystonia was an infrequent feature among all patients but when present, was predominantly generalised in nature. All patients with the m.9185T>C mutation manifested with a peripheral neuropathy; but none had RP, dystonia or seizures.

The peculiarities of mitochondrial genetics namely heteroplasmy and threshold effect are eloquently demonstrated in this cohort of patients.[17] Our results confirm that phenotypic manifestation of the m.8993T>G mutation appears to have the lowest threshold level compared to other *MT-ATP6* variants.. Leigh syndrome appears to manifest at a high threshold level over 90%, while other clinical phenotypes are associated with mutant load below 80%. One patient (mutant load 66%) was noted to have a progressive ataxia disorder at age 2 years, while another patient manifested with neuropathy (mutant load 45%) at age 30 years. However, in the m.8993T>C subgroup, patients clinically manifested at higher mutation load (~75%), with the threshold manifesting with LS similarly at mtDNA heteroplasmy level > 90% (**Figure 2C**). Our results confirm previous findings (56 pedigrees) that the threshold level for disease manifestation of m.8993T>G is lower than the m.8993T>C.[13] 1. White S, Collins V, R RW, Cleary M, Shanske S, DiMauro S, Dahl H, Thorburn D. Genetic Counseling and Prenatal Diagnosis for the Mitochondrial DNA Mutations at Nucleotide 8993. American Journal of Human Genetics. 1999;65:474-82

Several other *MT-ATP6* pathogenic variants including m.9035T>C, m.9176T>C and m.9185T>C are also associated a very high phenotypic threshold level (>90%). Perhaps most

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Our results confirm previous findings that the threshold level for disease manifestation of m.8993T>G is lower than the m.8993T>C but go beyond by putting them into the context of detailed study of phenotypes caused by a wider range of mutations in this region

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interesting are once such threshold levels are breached, it is not possible to predict the clinical phenotype and disease severity based solely on the mutant heteroplasmy level.

We also analysed genetic data of asymptomatic carriers of *MT-ATP6* mutations, obtained in the course of investigations of proband affected by the mitochondrial disease and pedigree tracing. While asymptomatic carriers harbour the mutant heteroplasmy level (median 43% in blood) that is generally lower than those who are severely affected with LS, the mutant loads of m.8993T>C, m.8993T>G and m.9185T>C overlap in some patients with non-syndromic neurological disease and currently asymptomatic individuals. These findings have important implications not only for presymptomatic carrier testing but also discussion around reproductive options, particularly given the expansion of choice in this area.[18] Given the neurodegenerative nature of the *MT-ATP6* related mitochondrial disorder, (currently) asymptomatic carriers harbouring mutant heteroplasmy levels >50% should be counselled about the lifetime risk of developing late-onset neurological features such as neurogenic weakness and cerebellar ataxia. In addition, our findings would also suggest testing for these mutations in late-onset ataxia cases in whom the usual causative genes have been proven negative.

Our certain syndromes identified in this cohort have been recognised (for example NARP syndrome in m.8839G>C,[19] progressive ataxia in m.9035T>C,[20] and cardiomyopathy in m.9134A>G),[21] we describe a constellation of novel clinical features. For example, a patient with the m.9134A>G mutation presented an expanded phenotype including LS, neuropathy, spastic ataxia, cognitive dysfunction, and hypertrophic cardiomyopathy.

Interestingly, forty-five per cent of patients experienced episodes of (sub-) acute deterioration of their functional status during a febrile illness. Whilst episodic developmental regression is a cardinal feature associated with LS (>95%), around 17% of patients without LS also experienced worsening ataxia or weakness. These episodes likely reflect the delicate energetic balance in the neuronal populations with the mitochondrial dysfunction, that is, a seemingly mild metabolic stressor frequently results in a dramatic deterioration in their neurological status. Perhaps of more concern in our cohort is that four adult patients without a pre-existing diagnosis of LS experienced severe metabolic decompensation manifesting with acute centrally-mediated respiratory failure, worsening dysphagia, generalised muscle weakness, with associated subacute changes in the brainstem and cervical cord on neuroimaging. These patients showed partial recovery in terms of central respiratory function, but often the associated ataxia and dysphagia fail to resolve. These serious neurological sequelae emphasise the need for early recognition of potential life-threatening complications and instigation of timely supportive care, irrespective of age of patient, genotype and prevailing initial clinical phenotype.

We found that more than half of patients had some degree of learning disability. Whilst severe cognitive dysfunction was mostly associated with LS, we also observed mild to moderate learning disability in many patients with less severe phenotypes. Increased awareness of learning disability associated with *MT-ATP6* related disease may particularly be important in both screening genetically undefined learning disability cases, but also in terms of sourcing appropriate education assistance for affected individuals. The cerebellum has a role not only in motor control but also in human cognition,[22,23] and it is tempting to speculate that, particularly outside the context of LS, the cognitive difficulties in patients with *MT-ATP6* related mitochondrial disease might be related to cerebellar dysfunction and its cortical

connections.[24] We suggest that the associations of cerebellar dysfunction and cognition in mitochondrial disease merit further study.

There are several diagnostic caveats associated with *MT-ATP6* related mitochondrial disease compared to the other common mtDNA mutations: 1) *MT-ATP6*-related mitochondrial disease predominantly involve both central and peripheral nervous systems, often with a combination of cerebellar ataxia and peripheral neuropathy, with muscle involvement such as chronic progressive external ophthalmoplegia and proximal myopathy significantly less prevalent compared to other mtDNA mutations[25]; 2) systemic features such as diabetes mellitus and cardiac involvement are uncommon in *MT-ATP6* mutations compared to other mtDNA mutations in which ataxia is prevalent[8,15]; 3) common muscle biopsy findings supportive of mitochondrial disease such as ragged-red fibers and COX-deficient ('blue fibers') are usually absent in *MT-ATP6* mutations. Moreover, conventional respiratory chain analysis is always normal in *MT-ATP6* mutations, imposing the diagnostic challenge of validating the pathogenicity of any novel variant in clinical practice. On the other hand, the clinical presentation of common pathogenic *MT-ATP6* variants may overlap with *POLG*-related mitochondrial disease[26] and other hereditary conditions such as CMT or SCA.[4,5] Therefore, our findings would suggest full sequencing of *MT-ATP6* in blood should be added to the diagnostic pipeline of patients presenting with cerebellar ataxia, neuropathy and learning disability, irrespective of apparent lack of'maternally-inherited history.

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We acknowledge potential limitations of this study. Firstly, the UK Mito Cohort is predominantly an adult cohort, and our results might not fully capture the spectrum of paediatric disease, particularly LS, associated with *MT-ATP6* mutations. Secondly, the retrospective nature of this study may present bias in relation to case identification. However,

we would suggest these findings hold significant clinical relevance in terms of diagnosis, management and genetic counselling in terms of future life time risk of disease development and/or progression, as well as potential reproductive options.

In conclusion, we suggest that *MT-ATP6*-related mitochondrial disease is best conceptualized as a spectrum disorder that includes core clinical features of cerebellar ataxia, peripheral neuropathy, and learning disability, with or without a Leigh-like phenotype. Moreover, we would advocate the avoidance of over-emphasis on syndromic classification (i.e. NARP) as it is increasingly recognised in other forms of mtDNA disease as it is often unhelpful in these cases.[10] We also observe clinically relevant differences between different genotypes and threshold expression.

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AUTHOR CONTRIBUTIONS

Dr Martikainen and Dr Ng: Draft the manuscript.
Dr Martikainen, Dr Ng, Dr Gorman, Prof McFarland, Prof Taylor and Prof Turnbull: Conception and design, acquisition of data, analysis and interpretation of data.
Dr Bugiarni, Miss Apphia Jack, Dr Schaefer, Mr Sunil Sharma, Dr Imelda Hughes, Prof Horvath, Dr Blakely, Dr Alston, Dr Lim, Dr Christian DeGoede, Dr Meriel McEntagart, Dr

Stefan Spinty, Dr Roberts, Prof Chinnery, Prof Horvath, Dr Nesbitt, Dr Fratter, Prof Poulton, Prof Hanna, Dr Pitceathly: Acquisition and analysis of data.

All authors: Critical revision of the manuscript for intellectual content and approval of the final version submitted for publication.

CONFLICTS OF INTEREST

We authors report no conflicts of interest relevant to this manuscript.

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FIGURE LEGEND

Figure 1. MRI changes associated with the MT-ATP6 related mitochondrial disease. (A) Axial view of FLAIR sequence shows symmetrical, bilateral hyperintensities in striatum (caudate nuclei and putamina, arrows on the right), (B) Sagittal view of T2 sequence from the same patient shows marked cerebellar atrophy, thinning of corpus callosum and less severe cerebral atrophy. (C) T2 sequence of axial view shows peri-aqueductal hyperintensitiy in the midbrain and upper medulla (arrow) (D), which the location of medullary lesion (arrow) is better appreciated with the T2 sagittal view (E). Subacute changes in bilateral cerebellar hemisphere (F) and midbrain G), with corresponding restricted diffusion in DWI sequence (H, I). Asymmetrical hyperintensities in the occipital cortical and subcortical areas (more marked on the left, arrow) and cystic changes in the striatum (J).

Figure 2. (A) Proportions of patients with different *MT-ATP6* pathogenic variants in this study. (B) Age of disease onset in patients harbouring different *MT-ATP6* pathogenic variants. Each red circle represents the median blood heteroplasmy level for LS and each blue triangle indicates the median blood heteorplasmy level for non-LS. *$p<0.05$ (C) Variations in blood mutant heteroplasmy levels in different phenotypes and MT-ATP6 pathogenic variants. *$p<0.05$ **(D)** Difference in mutant heteroplasmy levels across different *MT-ATP6* variants. Grey circle represent individual patient data, blue circle denote the mean difference of heteroplasmy level that is below 10%, the blue triangle indicates the mean difference of heteroplasmy that is greater than 25%.

Figure 3. Estimated probability of being clinically affected (with 95% confidence interval) based on the blood heteroplasmy level for five *MT-ATP6* variants (m.8993T>C, m.8993T>G, m.9035T>C, m.9176T>C and m.9185T>C)

Table 1: Demographic characteristics, pathogenic variants, and blood heteroplasmy levels of patients with *MT-ATP6*-related mitochondrial disease (n=88).

Variant	N	No of pedigree	F/M	No of deceased	Median current age (y); (range, IQR)	Median onset age(y); (range, IQR)	Median blood het (%); (range, IQR)
m.8839G>C	1	1	1/0	0	61	28	26
m.8851T>C	2	1	1/1	0	49 (35-63)	35	99 (98-100)
m.8993T>C	24	20	10/14	4	27.5 (3-74, 38.8)	5.5 (0.5-71, 22.3)	93 (77-97, 12.5)
m.8993T>G	22	19	8/14	5	30 (0.75-59, 39)	2 (0-34, 11.1)	88 (45-98, 19)
m.9032T>C	1	1	1/0	0	53	n/a	25
m.9035T>C	8	3	5/3	1	24 (10-48, 23)	10 (3-19, 15.3)	95 (90-99, 4.5)
m.9134A>G	1	1	0/1	0	26	1	43
m.9176T>C	11	5	6/5	2	15.5 (2-49, 19.5)	1 (1-32, 3.9)	96 (87-100, 10.5)
m.9185T>C	18	9	7/11	3	25 (19-54, 29)	6 (2-15, 8)	99 (73-100, 5)
Total	88	60	39/49	15	-	-	-

F = female. IQR= interquartile range. M = male. n/a = not applicable. y= years.

Table 2. Clinical features and findings associated with five common mutations in the *MT-ATP6* gene.

Mutation	N	RP**	Ataxia	Neuropathy	*Pes cavus*	UMN sign	Cognitive dysfunction	Seizures	Dystonia	LS	Cerebellar atrophy	BG changes
m.8993T>C	24	3/18	20/22	15/17	9/22	9/20	14/18	6/22	3/24	8/23	9/14	8/14
m.8993T>G	22	12/13	10/11	4/6	1/12	10/14	6/8	9/20	3/20	11/17	7/13	8/13
m.9035T>C	8	2/7	8/8	3/7	2/3	4/8	5/7	0/8	1/8	2/8	4/7	1/7
m.9176T>C	11	1/9	6/10	5/10	4/11	6/10	5/9	3/8	3/10	6/11	1/8	3/8
m.9185T>C	15	0/13	12/17	14/14	7/12	10/16	9/16	0/18	0/17	3/18	5/10	3/10
Total	80	18/60	56/68	41/54	23/60	39/68	39/58	18/79	10/79	30/77	26/52	23/52

BG = basal ganglia. LS = Leigh syndrome. MRI = magnetic resonance imaging. N = number of patients. RP = retinitis pigmentosa. UMN = upper motor neuron sign defined as the presence of pathological brisk reflexes and/or positive Babinski sign. Denominator values vary due to partially incompletely available data. *Chi Square test with the Bonferroni correction ($p \leq 0.006$) showed that the proportion of patients with the m.8993T>G had retinitis pigmentosa was significantly higher than patients with the m.8993T>C (92% vs 17%, $p<0.001$) and m.9176T>C (92% vs 11%, $p=0.001$) mutations.

Supplemental Table 1. Comparison of the profile of clinical features betweens patients with and without LS. * unadjusted p value <=0.05, ** adjusted p value < 0.003 (Bonferroni correction for multiple comparisons)

	LS	Non LS	p value
Clinical features			
Retinitis pigmentosa	33.3%	25.6%	0.738
Ataxia	85.7%	80.0%	0.738
Neuropathy	73.3%	73.8%	1
Muscle weakness	73.7%	56.1%	0.258
Spasticity	70.0%	50.0%	0.305
Babinski sign	21.7%	24.4%	1
Pes cavus	24.0%	45.9%	0.11
Dystonia	29.2%	6.1%	0.041*
Cognitive dysfunction	94.7%	53.8%	0.002**
Seizures	32.3%	12.2%	0.044*
Bulbar symptoms	84.6%	57.1%	0.156
Acute metabolic decompensation	91.3%	16.7%	<0.001**
Neuroimaging			
BG changes	82.6%	16.7%	<0.001**
Brainstem	26.1%	6.7%	0.065
Cerebellar atrophy	26.1%	56.7%	0.049*

Figure 1

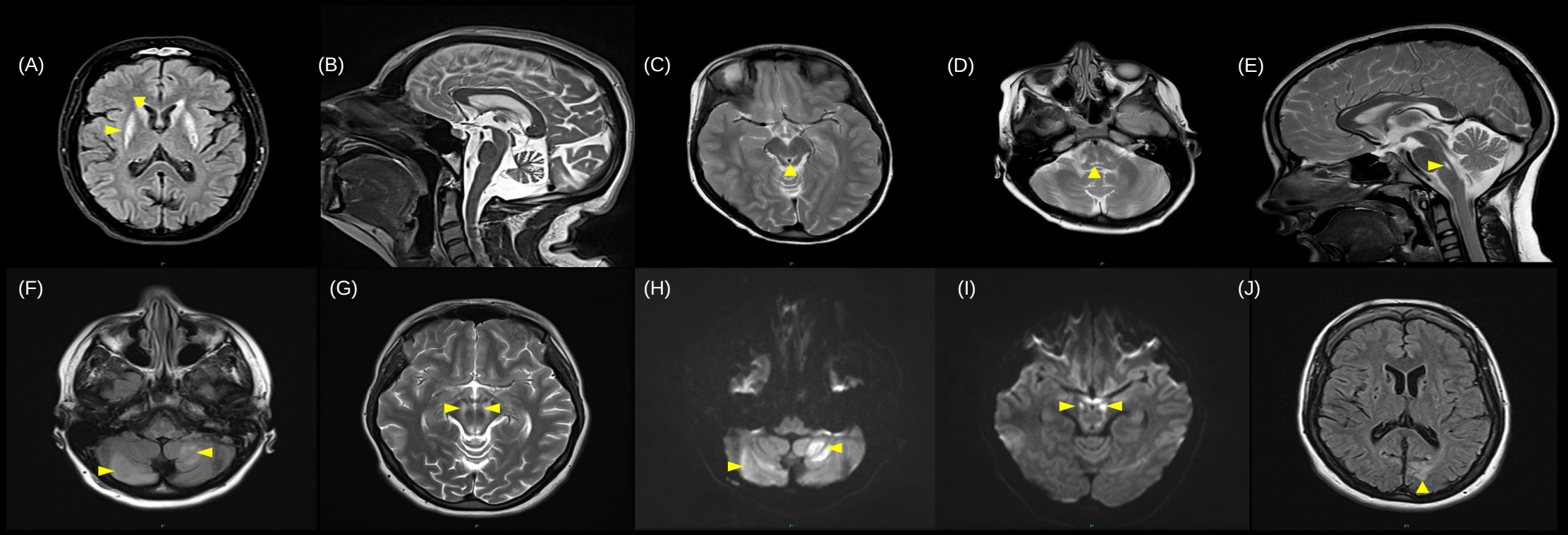


Figure 2A

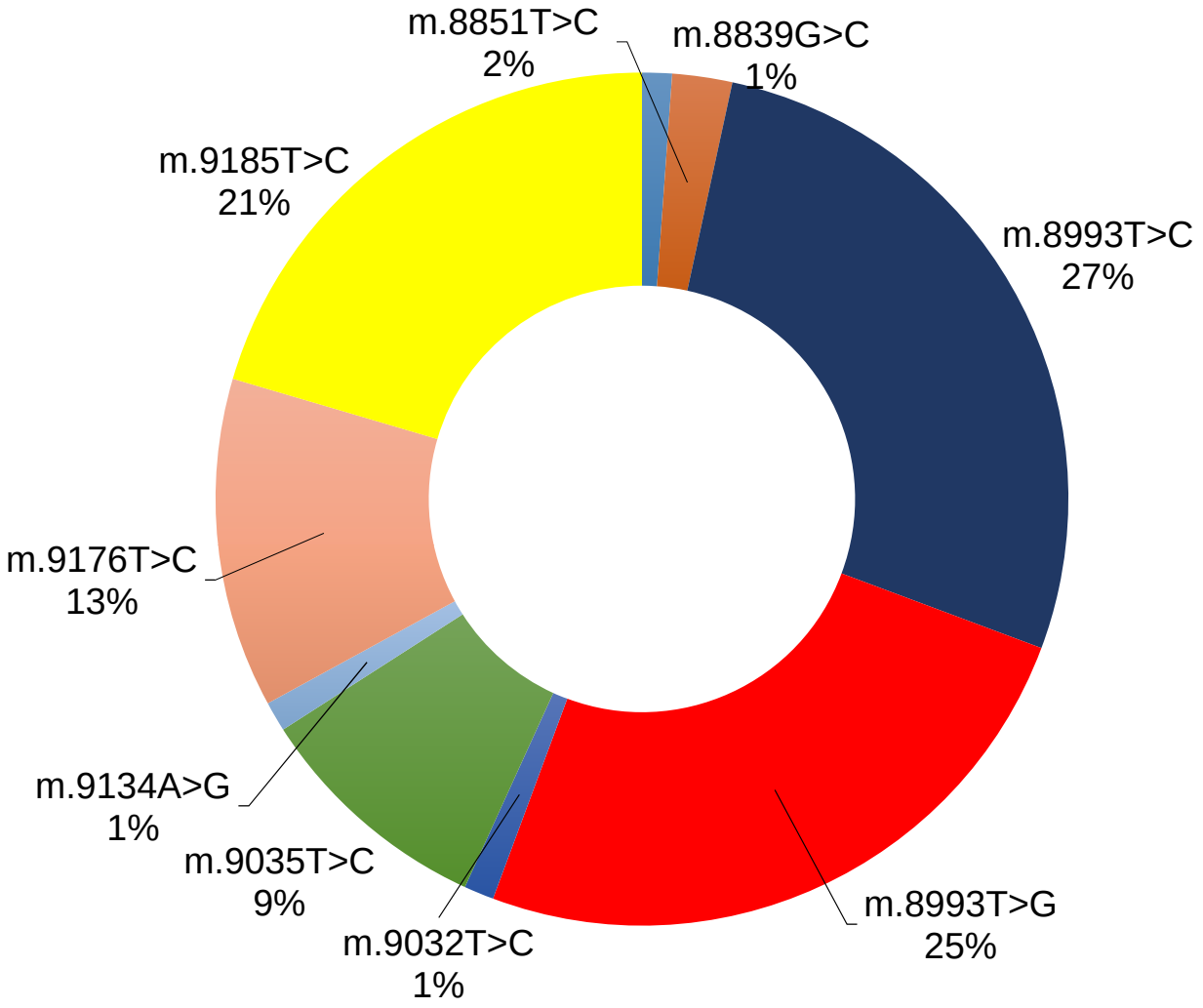

m.8851T>C
2%
m.8839G>C
1%
m.8993T>C
27%
m.9185T>C
21%
m.9176T>C
13%
m.9134A>G
1%
m.9035T>C
9%
m.9032T>C
1%
m.8993T>G
25%

Figure 2B

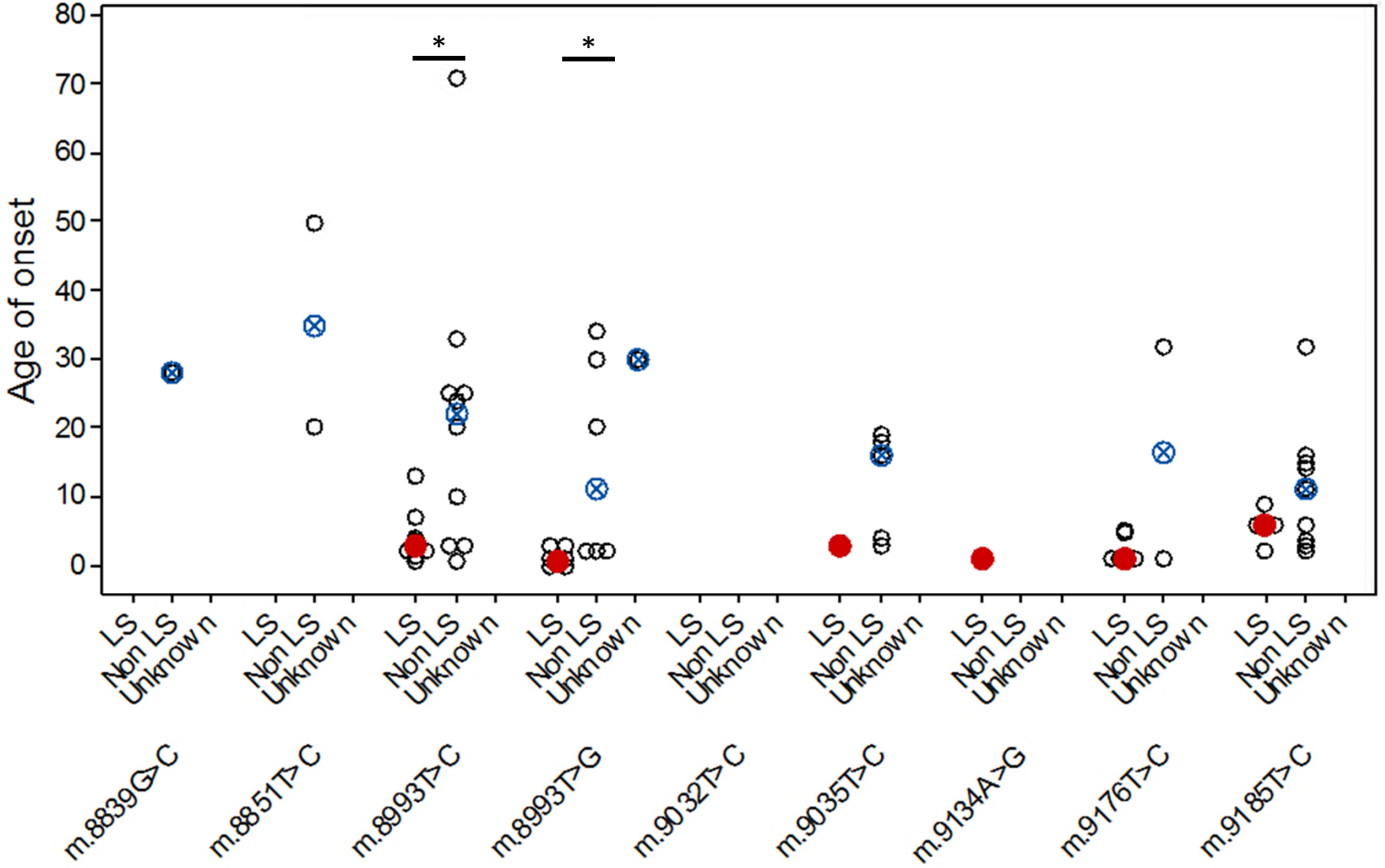

Age of onset
80
70
60
50
40
30
20
10
0
*
*
LS
Non LS
Unknown
m.8839G>C
m.8851T>C
m.8993T>C
m.8993T>G
m.9032T>C
m.9035T>C
m.9134A>G
m.9176T>C
m.9185T>C

Figure 2C

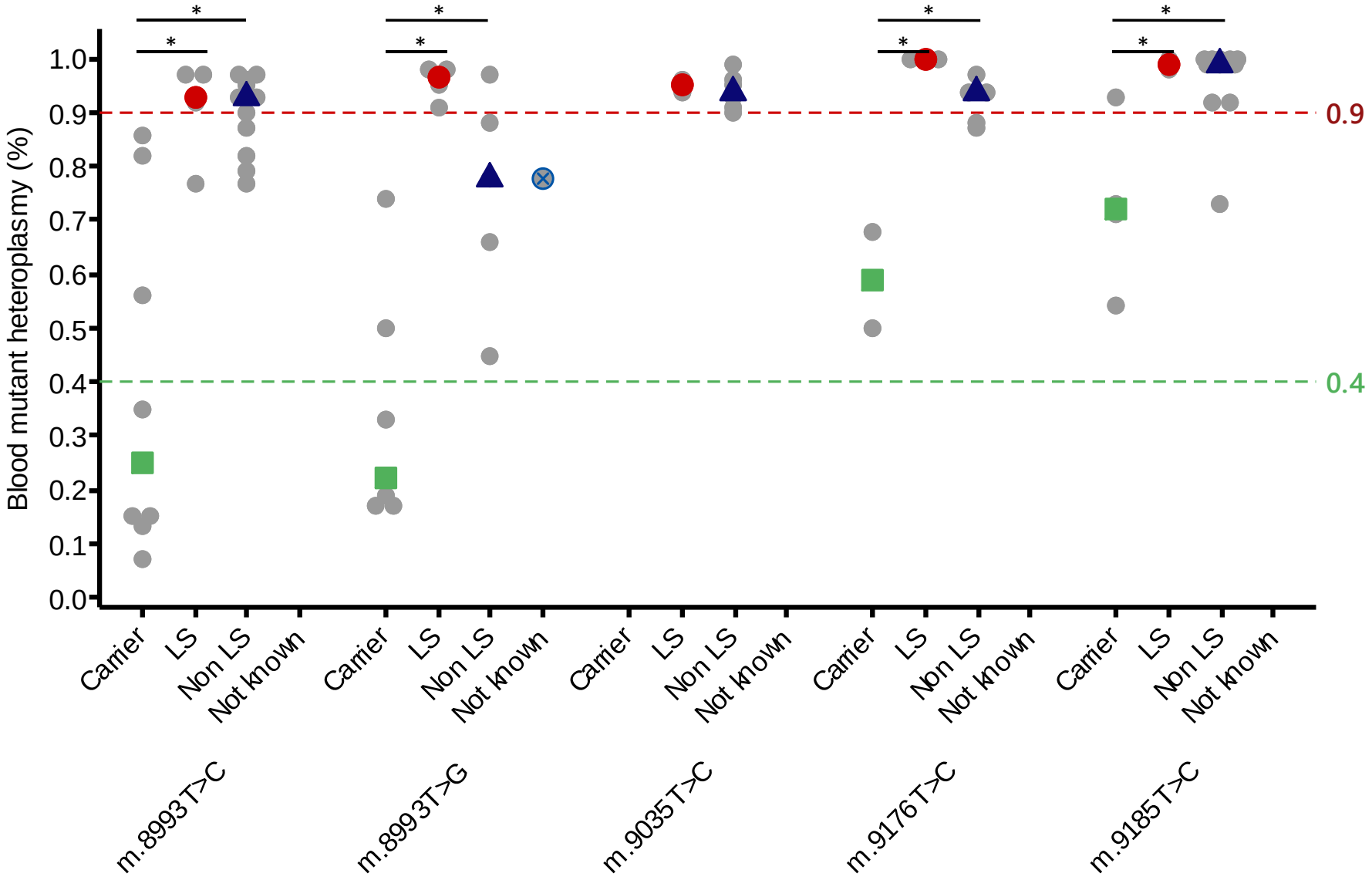

Blood mutant heteroplasmy (%)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.9
0.4
*
Carrier
LS
Non LS
Not known
m.8993T>C
m.8993T>G
m.9035T>C
m.9176T>C
m.9185T>C

Figure 2D

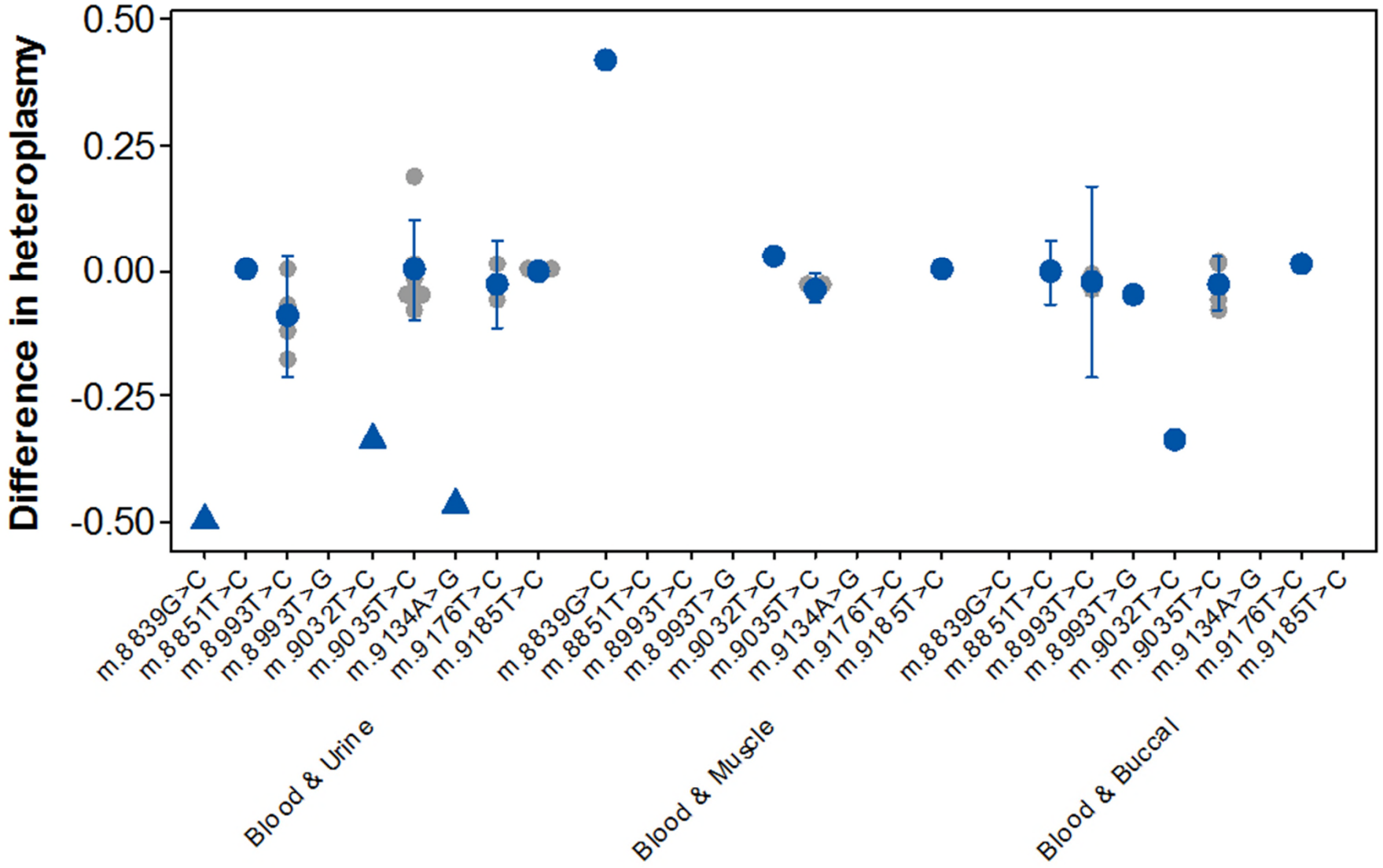
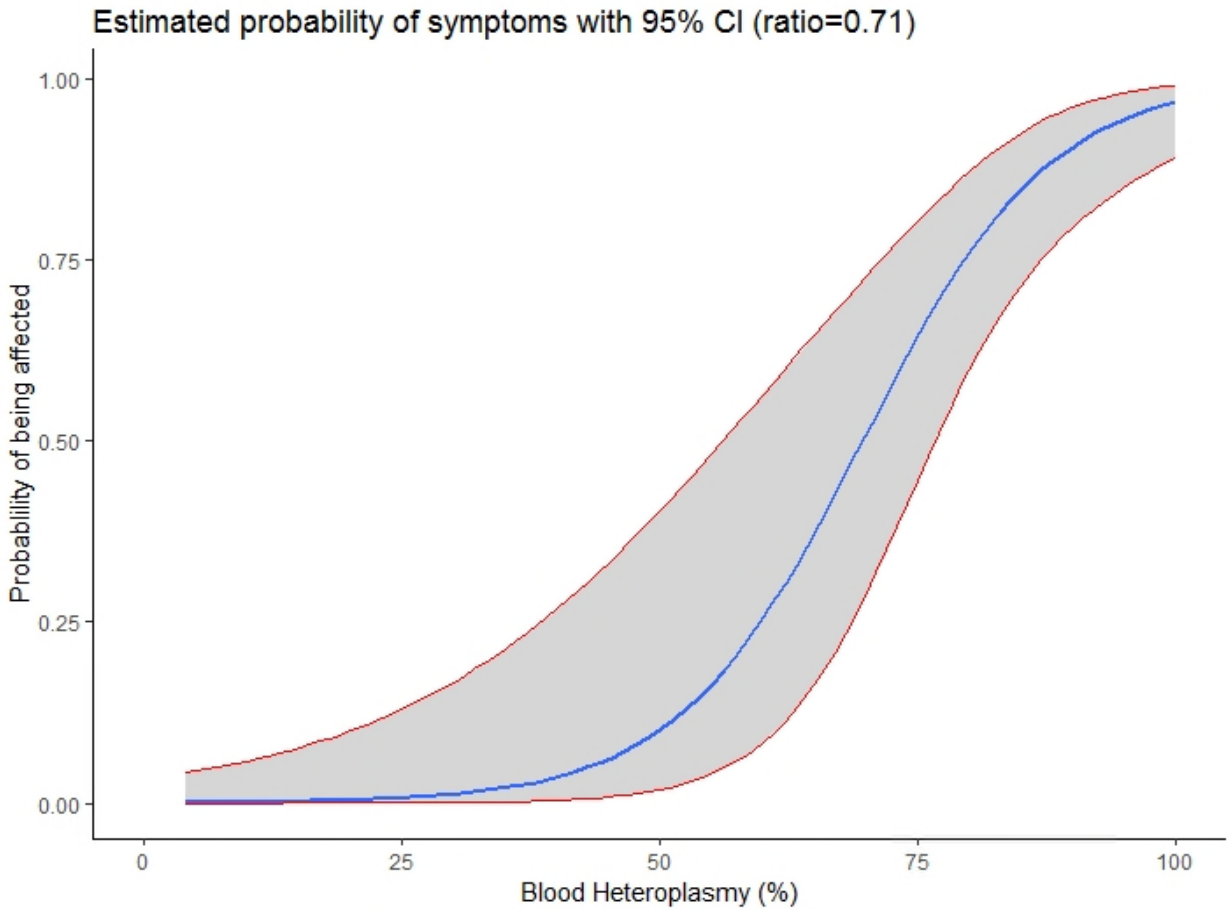
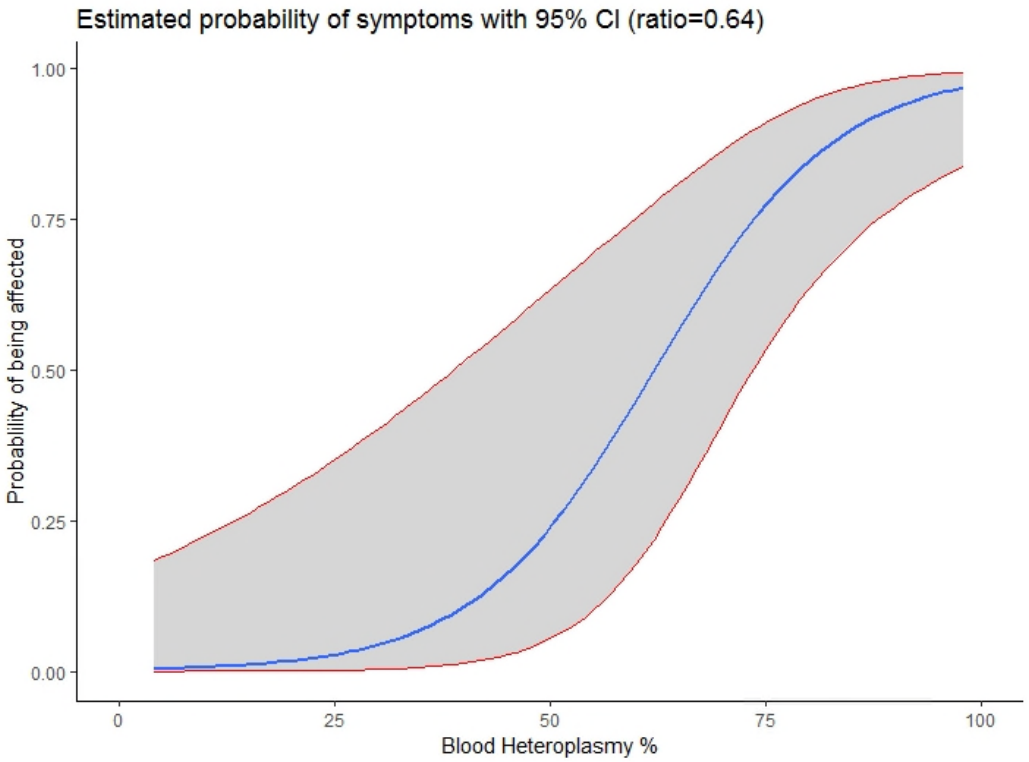

Difference in heteroplasmy
0.50
0.25
0.00
-0.25
-0.50
m.8839G>C
m.8851T>C
m.8993T>C
m.8993T>G
m.9032T>C
m.9035T>C
m.9134A>G
m.9176T>C
m.9185T>C
Blood & Urine
Blood & Muscle
Blood & Buccal

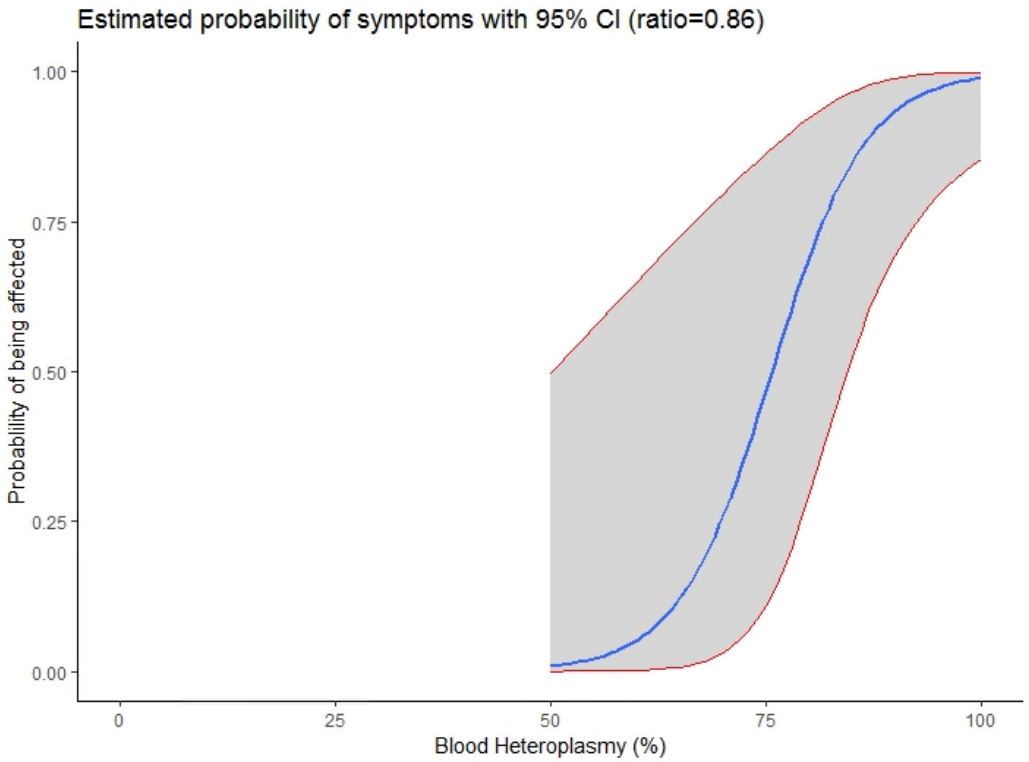
Figure 3


Estimated probability of symptoms with 95% CI (ratio=0.71)
Probabilility of being affected
1.00
0.75
0.50
0.25
0.00
0
25
50
75
100
Blood Heteroplasmy (%)

Supplemental Figure



A) m.8993T>C and m.8993T>G



B) m.9035T>C, m.9176T>C and m.9185T>C