# Nuclear genetic control of mitochondrial DNA segregation

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**Mammalian mitochondrial DNA (mtDNA) is a high copy-number, maternally inherited genome that codes for a small number of essential proteins involved in oxidative phosphorylation. Mutations in mtDNA are responsible for a broad spectrum of clinical disorders[1]. The segregation pattern of pathogenic mtDNA mutants is an important determinant of the nature and severity of mitochondrial disease, but it varies with the specific mutation, cell type and nuclear background and generally does not correlate well with mitochondrial dysfunction[2–11]. To identify nuclear genes that modify the segregation behavior of mtDNA, we used a heteroplasmic mouse model derived from two inbred strains (BALB/c and NZB; ref. 12), in which we had previously demonstrated tissue-specific and age-dependent directional selection for different mtDNA genotypes in the same mouse[13]. Here we show that this phenotype segregates in F2 mice from a genetic cross (BALB/c × CAST/Ei) and that it maps to at least three quantitative-trait loci (QTLs). Genome-wide scans showed linkage of the trait to loci on Chromosomes 2, 5 and 6, accounting for 16–35% of the variance in the trait, depending on the tissue and age of the mouse. This is the first genetic evidence for nuclear control of mammalian mtDNA segregation.**

Unlike nuclear DNA, the replication of mtDNA is not strictly linked to the cell cycle and there is no strict control of partitioning of mtDNAs at cytokinesis[14,15]. The rate of segregation of mtDNA sequence variants is thus a function of mtDNA copy number and turnover rate, and in the absence of selection, the process can be modeled as a random walk. Most pathogenic mtDNA mutations are heteroplasmic, and the segregation of mutated and wild-type mtDNAs often deviates from this random pattern[2,3,5,7,8,11,16], suggesting that the dysfunction caused by the mutation influences the process of segregation. The same mutation can also produce distinct clinical phenotypes in different pedigrees[17,18], a result of non-random distribution of mutated and wild-type mtDNAs[16,18], suggesting that nuclear background can influence mtDNA segregation.

We previously constructed heteroplasmic mice with two polymorphic mtDNA sequence variants to investigate the mechanisms that control mtDNA segregation behavior[12]. In this model, germ-line transmission of the two mtDNA variants (BALB and NZB) was random, but the pattern of mtDNA segregation postnatally depended on the tissue. In most tissues segregation was random during the mouse's lifetime, but four tissues showed directional selection for different mtDNA genotypes. Without exception, the proportion of NZB mtDNA increased by several times in the liver and kidney with age, whereas the proportion of BALB mtDNA increased similarly in the blood and spleen[13], suggesting a complex interaction between nuclear factors and mtDNA genotypes. To investigate the genetic basis for the tissue-specific segregation behavior of mtDNA, we first backcrossed heteroplasmic females (on a BALB/c nuclear background) with males from several common inbred mouse strains (DBA, 129Sv, NZB, C3H and C57BL/6J). The pattern of mtDNA segregation in the N2 progeny was, however, similar to that of the BALB parent in all crosses, showing the robust nature of the phenotype on similar nuclear backgrounds. We reasoned that differ-

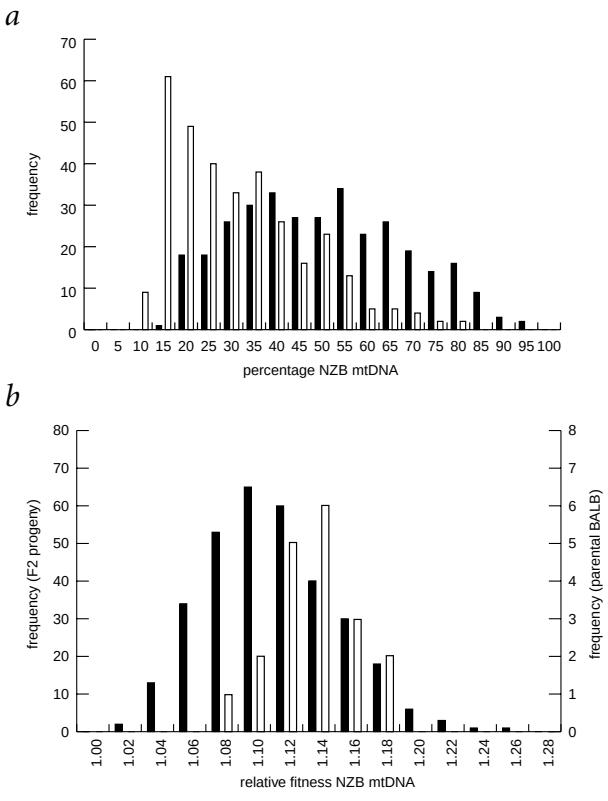


**Fig. 1** Frequency distributions of NZB mtDNA heteroplasmy and relative fitness in the liver of F2 mice. ***a***, Changes in the frequency distribution of the proportion of NZB mtDNA in the liver (dark bars) compared with skeletal muscle (light bars) from heteroplasmic F2 (BALB/c × CAST/Ei) mice at 3 months of age ($n$ = 326). The percentage of NZB mtDNA in the skeletal muscle of individual mice was relatively stable with age. The different proportions of NZB mtDNA in individual mice reflects random transmission of BALB and NZB mtDNA from heteroplasmic mothers. ***b***, Distribution of relative-fitness values for NZB mtDNA in the liver of heteroplasmic F2 (BALB/c × CAST/Ei) mice ($n$ = 326) at 3 months of age (dark bars) and in the parental BALB strain (light bars; $n$ = 19).

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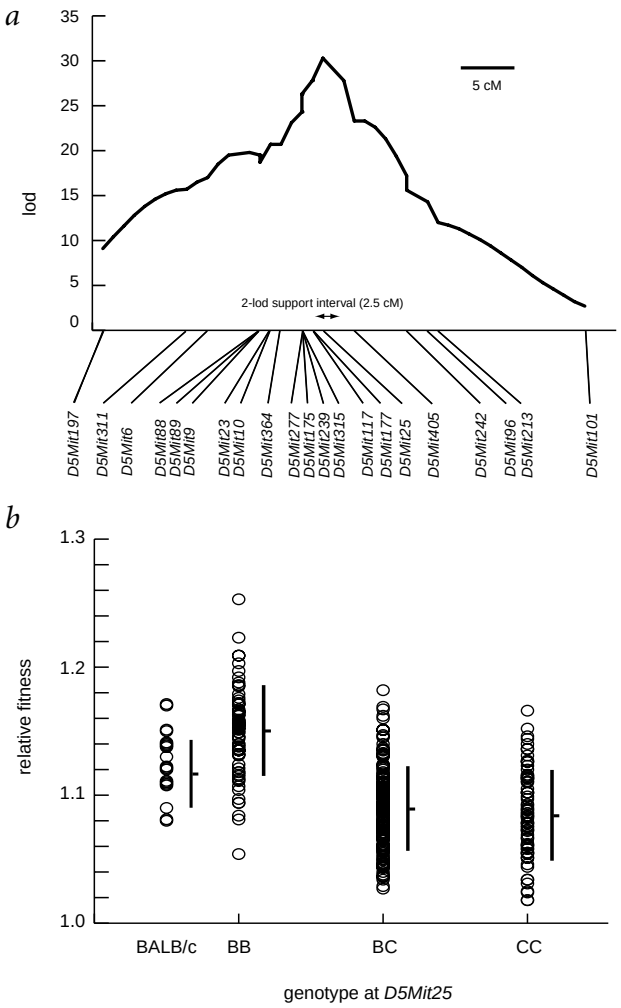


**Fig. 2** Analysis of the QTL on Chromosome 5. ***a***, Plot of the lod curves under a dominant model for a QTL on Chromosome 5 (*Smdq1*) associated with directional selection of mtDNA in the liver at 3 months in heteroplasmic F2 (BALB/c × CAST/Ei) mice ($n = 326$). *D5Mit197* is at position 36 cM. ***b***, Relative-fitness distribution in the three genotypic classes at *Smdq1* (*D5Mit25*). Means ± s.d. are indicated by bars to the right of the distributions. We randomly jittered some of the data points for the parental BALB/c mice so that overlapping values could be distinguished.

ences in nuclear–mitochondrial interactions might be more probable in distantly related mouse strains, and to test this, we set up an intercross with the subspecies *Mus musculus castaneus* (CAST/Ei) and investigated the pattern of mtDNA segregation in the liver, kidney and spleen.

The trait (directional selection of mtDNA) segregated in the F2 progeny from the BALB/c × CAST/Ei cross in an age-dependent and tissue-specific manner. To quantify the rate of mtDNA selection in the liver, kidney and spleen, we measured the relative fitness (selective advantage) of either mtDNA genotype[19] in the different tissues of individual mice at 3 and 12 months of age. In contrast with the BALB parent, the relative fitness of NZB mtDNA in the liver and kidney and of BALB mtDNA in the spleen varied considerably among individual mice. In some mice, the fitness was near 1 (no selection for either mtDNA genotype) and in others, it was greater than had been observed in the BALB parent, indicating stronger selection. These differences cannot be attributed to a bias in the transmission of the two mtDNA genotypes from the F1 to F2 generation. The relative proportion of NZB and BALB mtDNA in an F1 mother was not significantly different than the mean proportion in her F2 litter, indicating that transmission was random as in the parental BALB strain (data not shown). Notably, we never observed a reversal in the direction of mtDNA selection with age (which would have produced a relative fitness of less than 1) in any tissue in the F2 mice. Tissues that were neutral with respect to mtDNA selection in the BALB parent (skeletal muscle, heart and brain) were also neutral in the F2 progeny.

The most pronounced segregation of the trait occurred in the liver (Fig. 1). At 3 months of age, the mean relative fitness of NZB mtDNA in F2 mice was 1.10 ± 0.04, whereas in the parental BALB mice it was 1.12 ± 0.027. Approximately 25% of the F2 mice had relative-fitness values less than had ever been observed in the parental strain, suggesting that a single strong locus might be responsible for mtDNA selection in the liver, and that mice homozygous with respect to the CAST allele might fall into this class. To test this hypothesis, we determined the relative-fitness distribution of NZB mtDNA in 50 F2 mice and then pooled DNA from mice at the extremes of this distribution, searching for excess homozygosity of the BALB allele in the pool from mice with high relative fitness and of the CAST allele in the pool from mice with low relative fitness. This analysis identified two candidate loci, of which only *D5Mit88* on Chromosome 5 showed significant linkage to the phenotype when genotyped in each of the 50 F2 mice. QTL mapping with a dense panel of 21 microsatellite markers across this region on Chromosome 5 in 326 F2 mice produced a lod score of 31.5 at *D5Mit25* and a 2-lod support interval of roughly 2.5 cM around *D5Mit25* (Fig. 2*a*). This QTL, which we designated *Smdq1* (for segregation of mitochondrial DNA QTL-#), accounted for 35% of the variance in the phenotype in the liver (Table 1). Linkage to *Smdq1* in the liver was not detected at 12 months because most mice were fixed for NZB mtDNA by that age, regardless of genotype. This suggests that the *Smdq1* locus affects the rate of segregation of the NZB mtDNA in the liver, and that the BALB genotype is associated with higher relative-fitness values than is the CAST genotype (Fig. 2*b* and Table 1).

**Table 1 • Linkage of multiple QTLs to tissue-specific mtDNA selection in heteroplasmic mice segregating NZB and BALB mtDNAs**

| Tissue | Age (mo) | Chromosome (locus) | Designation | lod[a] | %[b] | n | *P* value[c] | Model[d] | mtDNA genotype selection |
|---|---|---|---|---|---|---|---|---|---|
| Liver | 3 | 5 (*D5Mit25*) | *Smdq1* | 31.5 | 35 | 326 | – | D (CAST) | NZB |
| Kidney | 3 | 2 (*D2Mit480*) | *Smdq2* | 4.0 | 16 | 98 | $P = 0.0073$ | R (CAST) | NZB |
| Spleen | 12 | 6 (*D6Mit266*) | *Smdq3* | 4.4 | 20 | 89 | $P = 0.0063$ | free | BALB |

[a]Maximum lod calculated for each locus by interval mapping in QTL Cartographer. [b]Percentage of the variance in the quantitative trait that is explained by the particular locus. [c]Level of genome-wide significance for linkage determined by permutation analysis in QTL cartographer ($n = 10{,}000$). [d]D, dominant; R, recessive. Dominant or recessive allele is shown in parenthesis.

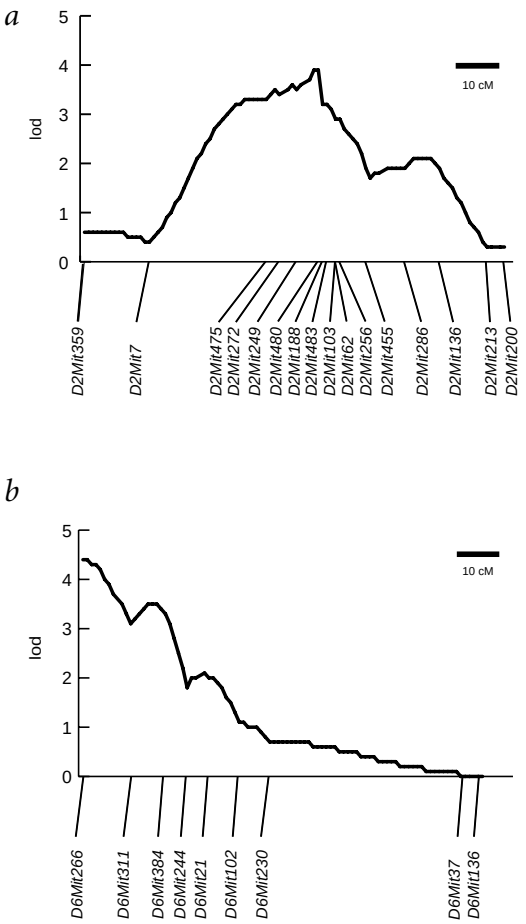


**Fig. 3** Plot of the lod curves under a constrained model for the QTL on Chromosome 2 and a free model for the QTL on Chromosome 6 associated with directional selection of mtDNA in heteroplasmic F2 (BALB/c × CAST/Ei) mice. ***a***, Kidney at 3 months (*Smdq2*); recessive model; *D2Mit359* is at 7.7 cM; $n = 98$. ***b***, Spleen at 12 months (*Smdq3*); free model; *D6Mit266* is at 3.3 cM; $n = 89$.

Segregation of the relative fitness of NZB or BALB mtDNA in the kidney and spleen of F2 mice was not as marked as in the liver. The mean relative-fitness values in these tissues were $1.03 \pm 0.02$ (in the kidney at 3 months), $1.03 \pm 0.01$ (in the kidney at 12 months) and $1.01 \pm 0.01$ (in the spleen at 12 months). The trait did not link to Chromosome 5 in either tissue in either 3-month-old or 12-month-old mice. To test whether other QTLs could account for mtDNA selection in these tissues, we carried out genome-wide scans in a random sample of 3-month-old ($n = 50$) and 12-month-old ($n = 60$) F2 mice. Loci that showed suggestive linkage, accounting for at least 15% of the variance in the trait, were genotyped with denser panels of microsatellite markers in additional F2 mice (total of 98 mice at 3 months and 89 mice at 12 months) to confirm linkage. This analysis uncovered two other loci on Chromosomes 2 and 6 (Fig. 3 and Table 1). In the kidney, a recessive QTL (*Smdq2*) on Chromosome 2 (lod 4.0, $P = 0.0073$) accounted for 16% of the variation in the phenotype in 3-month-old mice, and in the spleen, we identified a QTL on Chromosome 6 (*Smdq3*) in 12-month-old mice (lod 4.4, $P = 0.0063$) that accounted for 20% of the variation in the phenotype. A weaker suggestive QTL on Chromosome 6 (lod 3.3, $P = 0.0866$) in the kidney of 12-month-old mice accounted for 16% of the variation in the phenotype and mapped to the same locus (*Smdq3*). As none of the F2 mice in this experiment had fixed for either mtDNA genotype, these data suggest an age-dependent shift in genetic control of mtDNA segregation in this tissue. It is notable that the Chromosome 6 QTL, though only suggestive in the kidney, was associated with opposite effects on mtDNA selection in the spleen versus the kidney.

Cloning of QTLs has proven to be difficult and is often fraught with genetic complexities[20]. Although we cannot be sure that *Smdq1* corresponds to a single gene, it is an attractive target for further investigation because of the magnitude of the effect of the locus. We are in the process of generating interval-specific congenic mice that should help us further refine this interval. The 2-lod support interval from the current data is roughly 2.5 cM and contains approximately 94 known or predicted genes. Several of these code for mitochondrially targeted proteins including uracil DNA glycosylase (DNA repair), POP5 (subunit of MRP RNase), COX6AL (structural subunit of cytochrome c oxidase) and Glu-tRNAGln amidotransferase (tRNA biosynthesis). Sequencing of the cDNAs from these candidates did not detect any sequence variants that would predict polymorphisms in the amino-acid sequence in either the BALB or CAST alleles.

The mechanism underlying the tissue-specific segregation behavior of different mtDNA genotypes is yet unknown. In the four tissues that show directional selection, the rate of selection is independent of initial mtDNA genotype frequency and is constant with time[13,21], arguing against a mechanism that depends directly on function. Consistent with this, oxidative phosphorylation function in the liver, as assayed by maximal respiratory chain activity and cell growth on a non-fermentable carbon source *in vitro*, is independent of mtDNA genotype[21]. In addition, the rate of replication of the two different mtDNAs in the liver is indistinguishable, suggesting that selection for NZB mtDNA in this tissue depends on some aspect of the maintenance or turnover of the mitochondrial genome itself[21].

How could allelic variants that do not directly affect function determine patterns of mtDNA segregation? One possibility is that this allelic variation translates into structural differences that form the basis of molecular recognition by the proteins that are associated with mtDNA, or perhaps by the enzymatic machinery that recognizes mtDNA damage and repairs it. MtDNA is organized into a protein–DNA complex, the mitochondrial nucleoid, which, in yeast, is crucial for mtDNA maintenance and segregation. The protein composition of the yeast mitochondrial nucleoid comprises elements of the DNA replication machinery and several metabolic enzymes also found in the mitochondrial matrix, which appear to be dual-function proteins[22,23]. Of the 11 proteins so far identified in the yeast nucleoid, none of their mouse orthologs map to the QTLs reported in this study.

This study has uncovered several tissue-specific genetic elements underlying the segregation of polymorphic mtDNA sequence variants in mammals. The tissue specificity, age dependence and different modes of action of the QTLs suggest that the organization and maintenance of mtDNA might not only be cell-type specific but also may change with age. The identification of the genes at these loci should provide some insight into the basic mechanisms responsible for mtDNA maintenance in mammals and may help explain, or provide avenues to modify, the diverse patterns of segregation seen in pathogenic mtDNAs in human disease.

## Methods

**Mouse breeding and phenotyping.** We outcrossed female BALB/c mice heteroplasmic for NZB and BALB mtDNA[12] with male *Mus musculus castaneus* (CAST/Ei; Jackson Labs) to produce F1 pups and then intercrossed the F1 mice to produce F2 progeny (BALB/c × CAST/Ei). We collected tissues from the F2 mice at 3 and 12 months of age and extracted DNA by conventional methods or with a Qiagen DNAeasy kit. These studies were approved by the McGill University Animal Care Committee.

We phenotyped the mice by measuring the level of NZB heteroplasmy[21] in the liver, kidney, spleen, skeletal muscle and heart to calculate the relative-fitness level of the NZB (liver and kidney) and the BALB (spleen) mtDNAs. To calculate the relative-fitness level, we used the equation $p_n/q_n = (w/w')^n (p_0/q_0)$, where $p$ and $q$ represent the proportion of NZB and BALB mtDNAs, respectively, $n$ represents the number of generations and $w/w'$ represents the relative fitness[19] . We assumed a half-life for mtDNA of 9.4 d in the liver and 10.7 d in the kidney[24] to calculate the number of generations. In the spleen, we assumed that the half-life of mtDNA is determined by cell half-life and that this is 5 d in most cells[25]. We estimated the initial level of heteroplasmy as the average of that in the skeletal muscle and heart, two tissues that were neutral with respect to mtDNA segregation in our heteroplasmic mice[13] and in the F2 progeny. For QTL mapping, we included only those mice with an initial heteroplasmy level above 10% NZB mtDNA in the genome scan (85% of all F2 progeny generated). We imposed this arbitrary threshold to eliminate potential biases in relative-fitness values that can accompany small changes in heteroplasmy.

**Genome scan and statistical analysis.** To search for QTLs in the liver, we determined the relative-fitness distribution for NZB mtDNA from a random sample of 50 F2 mice (3 months of age) that had greater than 10% NZB mtDNA. We pooled mice at the extremes of this relative-fitness distribution and genotyped the pooled samples with 54 microsatellite markers (Research Genetics) spaced approximately every 30 cM. To fine-map the Chromosome 5 QTL in the liver, we generated 422 F2 mice with heteroplasmy for NZB exceeding the 10% threshold. When we examined the genotype distribution of markers in the fine-mapped region, we noticed a statistically significant distortion from the expected mendelian ratios (under-representation of BALB homozygotes) in pups from about one third of the F1 mothers ($n = 5$). The biological basis for this phenomenon is not known at the present time, but it did not correlate with the level of NZB mtDNA heteroplasmy in the mothers, and the distribution of relative-fitness values in F2 mice from these mothers was indistinguishable from that observed in F2 mice derived from litters with no genotype distortion. Inclusion of these mice did not affect the QTL analysis, but because conventional QTL analysis assumes mendelian ratios in the F2 mice, we excluded from the analysis those mice that came from the distorted litters, leaving 326 F2 mice.

To search for QTLs in the kidney and spleen, we individually genotyped 50 randomly chosen F2 mice at 3 months and 60 F2 mice at 12 months with the same 54 microsatellite markers as above, and then genotyped loci that showed suggestive linkage with a denser panel of markers in a total of 98 (3 months) or 89 mice (12 months). We genotyped the markers using standard procedures and analyzed them on 3–4% agarose (MetaPhor) gels stained with ethidium bromide. We carried out the QTL linkage analysis using both MapManager QTb29 and QTL Cartographer[26,27]. We confirmed the genome-wide significance level of a likelihood ratio statistic generated by interval mapping by 10,000 permutation tests in QTL Cartographer[28,29]. To calculate lod scores, we divided the likelihood ratio statistic values by 4.61 (ref. 30).

**URL.** QTL Cartographer is available at http://statgen.ncsu.edu/qtlcart.


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## Competing interests statement

*The authors declare that they have no competing financial interests.*

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