

The flowchart illustrates the plasma fractionation protocol. It begins with a blood sample, which is centrifuged at 1,400 x g for 10 minutes at 4°C. The plasma is then stored at -80°C. The remaining cells are centrifuged at 15,000 x g for 30 minutes at 4°C. The supernatant is then centrifuged at 16,000 x g for 20 minutes at 4°C. The supernatant is then centrifuged at 120,000 x g for 2 hours at 4°C. The final fractions are labeled B, C, D, E, and F.

The figure consists of two bar charts. The left chart shows the median mtDNA copy number (HV-Short) for Controls and Cases across phases B (blue) and C (green). The right chart shows the median mtDNA copy number for Controls and Cases across phases D (orange), E (purple), and F (yellow). Both charts include error bars representing the 95% CI.

Median mtDNA Copy Number (HV-Short)

Group	Phase	Median mtDNA Copy Number (HV-Short)	95% CI
Controls	B	~500	~0 - 1,200
	C	~1,200	~800 - 2,000
Cases	B	~1,200	~200 - 2,400
	C	~9,000	~3,800 - 13,800

Median mtDNA Copy Number (HV-Short)

Group	Phase	Median mtDNA Copy Number (HV-Short)	95% CI
Controls	D	~50	~0 - 200
	E	~300	~100 - 600
	F	~2,200	~1,200 - 2,600
Cases	D	~400	~0 - 600
	E	~1,500	~800 - 2,500
	F	~3,600	~3,300 - 9,800

Error bars: 95% CI

Figure S2. Bar chart representation of mtDNA copy number variation of mitochondrial hypervariable region 1 (*HVI*) short gene among controls and cases comparing phases.

Table S1. Sequences of designed primers.

Region	Short Fragment (75–100 pb)	Long Fragment (175–200 pb)
<i>HBB</i>	F: 5'-GCATAAAAGTCAGGGCAGAGC-3'	F: 5'-ATTGCTTACATTGCTTCTGACAC-3'
	R: 5'-TGGTGTCTGTTGAGGTTGCTA-3'	R: 5'-TGGTCTCCTTAAACCTGTCTTGTA-3'
	84 pb	187 pb
<i>HV1</i>	F: 5'-AGTACATAAAAACCCAATCCACATC-3'	F: 5'-TACATTACTGCCAGCCACCA-3'
	R: 5'-GATGTGTGATAGTTGAGGGTTGAT-3'	R: 5'-GTTGGTATCCTAGTGGGTGAGG-3'
	85 pb	186 pb
<i>CYB</i>	F: 5'-CCACATCACTCGAGACGTAAAT-3'	F: 5'-CTAGCCATGCACTACTCACCAG-3'
	R: 5'-GGAAGAGGCAGATAAAGAATATTGA-3'	R: 5'-TTTCTGAGTAGAGAAATGATCCGTAA-3'
	86 pb	184 pb

CYB: Apocytochrome B; *HBB*: Hemoglobin subunit beta; *HV1*: Hypervariable región 1; F: forward primer; R: reverse primer.

Table S2. Ct mean analysis cases vs controls comparing genes and copy number.

Phase	Gene	Adjusted <i>p</i> Value (*)	Adjusted <i>p</i> Value(cn)	Ct Mean Controls ± SD	Ct Mean Patients ± SD	Copies per µl Mean Controls ± SD	Copies per µl Mean Patients ± SD
B	<i>HV1-short</i>	0.084	0.064	24.79 ± 3.58	23.42 ± 3.57	1.09 ± 1.18	2.46 ± 3.06
	<i>HV1-long</i>	0.013	0.008	25.10 ± 4.46	22.73 ± 3.97	0.90 ± 1.18	2.62 ± 3.07
	<i>CYB-short</i>	0.043	0.007	26.43 ± 3.67	24.77 ± 3.69	0.69 ± 0.72	2.00 ± 2.43
	<i>CYB-long</i>	0.014	0.003	27.80 ± 4.91	25.11 ± 4.86	0.48 ± 0.65	1.67 ± 1.82
	<i>HBB-short</i>	0.512	0.245	32.47 ± 2.29	32.82 ± 2.51	0.62 ± 0.85	0.76 ± 1.32
	<i>HBB-long</i>	0.213	0.205	35.72 ± 3.02	34.80 ± 3.09	0.56 ± 1.91	0.58 ± 1.49
C	<i>HV1-short</i>	<0.001	0.001	21.62 ± 2.39	19.48 ± 2.32	5.93 ± 10.04	18.73 ± 32.15
	<i>HV1-long</i>	<0.001	0.001	20.41 ± 2.55	18.30 ± 2.43	7.81 ± 13.50	24.54 ± 45.83
	<i>CYB-short</i>	0.001	0.002	22.34 ± 2.79	20.28 ± 2.43	7.95 ± 12.76	17.82 ± 23.21
	<i>CYB-long</i>	0.001	0.002	21.69 ± 2.98	19.64 ± 2.52	8.29 ± 13.60	18.75 ± 27.54
	<i>HBB-short</i>	<0.001	<0.001	21.74 ± 1.73	20.13 ± 2.12	209.85 ± 229.09	677.88 ± 1019.3
	<i>HBB-long</i>	0.102	0.163	32.46 ± 2.42	31.58 ± 3.17	1.48 ± 3.26	12.77 ± 37.67
D	<i>HV1-short</i>	0.783	0.932	29.37 ± 2.64	29.20 ± 2.68	0.047 ± 0.06	0.046 ± 0.04
	<i>HV1-long</i>	0.024	0.003	29.33 ± 2.90	27.83 ± 3.06	0.08 ± 0.24	0.11 ± 0.18
	<i>CYB-short</i>	0.002	0.003	32.72 ± 3.46	30.38 ± 3.31	0.022 ± 0.04	0.07 ± 0.08
	<i>CYB-long</i>	0.001	0.001	33.91 ± 4.88	30.49 ± 3.69	0.043 ± 0.11	0.05 ± 0.07
	<i>HBB-short</i>	0.050	0.068	34.72 ± 2.83	33.39 ± 3.16	0.25 ± 0.44	0.66 ± 1.04
	<i>HBB-long</i>	0.209	0.254	35.22 ± 3.47	34.12 ± 3.72	0.69 ± 1.96	3.40 ± 10.99
E	<i>HV1-short</i>	0.068	0.026	19.82 ± 12.38	24.04 ± 7.54	0.39 ± 0.75	0.36 ± 0.47
	<i>HV1-long</i>	0.020	0.027	26.84 ± 3.24	25.34 ± 1.96	0.27 ± 0.64	0.27 ± 0.37
	<i>CYB-short</i>	0.002	0.003	27.96 ± 3.96	25.34 ± 2.45	0.28 ± 0.38	1.09 ± 1.64
	<i>CYB-long</i>	0.750	0.413	34.34 ± 3.36	34.70 ± 4.87	0.005 ± 0.009	0.05 ± 1.78
	<i>HBB-short</i>	0.721	0.564	31.58 ± 2.96	31.86 ± 3.44	0.92 ± 0.57	1.47 ± 2.02
	<i>HBB-long</i>	0.012	0.012	33.93 ± 3.17	35.91 ± 2.71	0.75 ± 1.76	0.09 ± 0.13
F	<i>HV1-short</i>	<0.001	<0.001	25.16 ± 1.29	23.79 ± 1.62	0.32 ± 0.29	0.87 ± 0.92
	<i>HV1-long</i>	<0.001	<0.001	25.82 ± 1.44	24.35 ± 1.84	0.15 ± 0.14	0.43 ± 0.50
	<i>CYB-short</i>	0.001	0.005	27.95 ± 1.41	26.80 ± 1.73	0.12 ± 0.11	0.29 ± 0.33
	<i>CYB-long</i>	<0.001	<0.001	27.49 ± 1.40	26.23 ± 1.66	0.11 ± 0.10	0.28 ± 0.35
	<i>HBB-short</i>	0.064	0.120	34.36 ± 2.13	33.46 ± 2.27	0.17 ± 0.13	0.36 ± 0.45
	<i>HBB-long</i>	0.098	0.107	33.44 ± 2.28	35.58 ± 1.96	0.39 ± 0.69	1.22 ± 3.24

Ct: cycle threshold; *CYB*: Apocytochrome B; *HBB*: Hemoglobin subunit beta; *HV1*: Hypervariable region 1; SD: Standard deviation. *HBB* as nuclear marker vs *HV1* and *CYB* as mitochondrial markers. (*) Just FDR *p* values comparing genes in cases vs controls by qPCR analysis. Adjusted *p* values (cn) represent values of comparisons cases vs controls in copy number by dPCR analysis. The italics indicates significant *p* values.