Table S1. Donor demographics

Subject	Age(years)/ Sex/Ethnicity	DS Status	Cause of Death	Clinical History Highlights	mtDNA Haplogroup
1*	57/Male/White	Non-DS	Intracranial hemorrhage	Obesity	H
2	75/Male/White	Non-DS	Cardiac arrest	Vascular disease, cerebral palsy	TJ-group,
3	55/Female/ White	Non-DS	Cardiac arrest	Arthritis, diabetes	UK
4*	63/Female/Black	Non-DS	Cardiac arrest	Type 2 diabetes	[L0, L1, L2]
5*	61/Male/White	Non-DS	Cardiac arrest	Unavailable	TJ-group
6*	65/Female/ White	Non-DS	Unknown	Unavailable	H
7	74/Female/White	Non-DS	Ischemic stroke	Osteoarthritis, cerebrovascular accident	W
8	69/Female/Hispanic	Non-DS	Respiratory failure	Mitral valve disease, stroke	H
9	70/Male/White	Non-DS	Cardiac arrest	Mononeuritis	I
10	67/Female/White	Non-DS	Anoxia	Hyperparathyroidism	T
11	70/Female/White	Non-DS	Respiratory failure	Stroke, hypertension	TJ-group
12	80/Female/White	Non-DS	Respiratory failure	Pulmonary embolism	Uk
13	67/Female/White	Non-DS	Pulmonary embolism	Unavailable	T
14	80/Male/White	Non-DS	Respiratory failure	Pulmonary fibrosis	TJ-group
15*	72/Male/White	DS	Respiratory failure	Aspiration pneumonia	T
16	97/Female/Not reported	Non-DS	Unknown	Unavailable	TJ-group
17*	63/Male/White	DS	Respiratory arrest	Alzheimer's with dementia.	TJ-group
18*	58/Male/White	DS	Respiratory failure	Pneumonia	HV- group,
19*	39/Male/Not reported	DS	Cardiac arrest	Pneumonia	H
20	75/Male/White	Non-DS	Pneumonia	Melanoma	HV-group
21*	47/Female/White	Non-DS	Aortic aneurysm	Alcoholic cirrhosis	V
22*	59/Male/White	DS	Cardiac arrest	Chronic kidney disease	H
23	69/Male/Black	Non-DS	Aortic aneurysm	Unavailable	UK-group,
24	66/Female/Black	Non-DS	Aortic aneurysm	Distant myocardial infarction	[L0, L1, L2],
25	62/Female/White	Non-DS	Ischemic stroke	Von Willebrands disease	T
26	75/Female/Not reported	Non-DS	Unknown	Unavailable	TJ-group
27	86/Female/Black	Non-DS	Pneumonia	Chronic Bronchitis	[L0, L1, L2]

28	74/Female/Black	Non-DS	Unknown	Unavailable	[L0, L1, L2]
29	70/Male/Black	Non-DS	Deep Vein Thrombosis	Myocardial infarction	[L0, L1, L2]
30*	19/Female/White	Non-DS	Cardiac arrest	Type 1 diabetes	T
31*	40/Male/White	DS	Adenocarcinoma	Pneumonia	H
32	34/Male/White	Non-DS	Sepsis	Acute myeloid leukemia	V
33*	42/Male/White	Non-DS	Transplant rejection	End stage renal disease	Uk
34	68/Male/White	Non-DS	Renal failure	Congestive heart failure	H
35*	22/Male/Black	DS	Spontaneous	Unavailable	[L0, L1, L2]
36*	1/Male/Black	DS	Spontaneous	Unavailable	L3
37	1/Female/Black	DS	Respiratory failure	Upper respiratory infection	[L0, L1, L2]
38	10/Male/Black	DS	Cardiac arrest	Unavailable	H
39*	64/Male/White	DS	Respiratory failure	Ischemic stroke	Uk
40	82/Male/Not reported	Non-DS	Unknown	Unavailable	H
41	42/Male/White	Non-DS	Ventricular fibrillation	History of myocardial infarction	Uk
42*	0/Female/Black	Non-DS	Unknown	Expired shortly after birth	[L0, L1, L2]
43*	40/Male/White	Non-DS	Transplant rejection	Coronary artery disease	[L0, L1, L2]
44*	71/Female/White	Non-DS	Multi-organ failure	Congestive heart failure	H
45*	48/Female/White	DS	Spinal trauma	Unavailable	L3

*Samples used in age-matched analysis.

Table S2. Frequency of heteroplasmic mtDNA variants in cardiac mitochondrial genomes from age-matched donors with and without DS (n = 10 samples for both groups).

Gene or region[a]	**Down syndrome Mean frequency ± SD (number of variants)**	**Non-Down syndrome Mean frequency ± SD (number of variants)**
HCR	39.04±34.69 (15)	51.79±39.0 (12)
RCR	28.18±24.46 (57)	28.87±27.38 (75)
MT-TF	21.12±17.71 (2)	8.62 (1)
MT-RNR1	No variants detected	49.81±66.52 (2)
MT-TV	No variants detected	No variants detected
MT-RNR2	6.23±1.11 (4)	27.5±43.54 (4)
MT-TL1	No variants detected	No variants detected
MT-ND1	10.53±11.25 (12)	6.68±2.25 (16)
MT-TI	No variants detected	No variants detected
MT-TQ	No variants detected	No variants detected
MT-TM	No variants detected	No variants detected
MT-ND2	No variants detected	No variants detected
MT-TW	No variants detected	No variants detected
MT-TA	No variants detected	No variants detected
MT-TN	No variants detected	No variants detected
MT-TC	No variants detected	84.02 (1)
MT-TY	No variants detected	No variants detected
MT-CO1	97.19 (1)	54.48±60.42 (2)
MT-TS1	88.91 (1)	No variants detected
MT-TD	No variants detected	No variants detected
MT-CO2	No variants detected	No variants detected
MT-TK	No variants detected	No variants detected
MT-ATP8	No variants detected	No variants detected
MT-ATP6	97.38 (1)	87.93±16.11 (3)
MT-CO3	17.43±14.95 (2)	38.27±51.29 (3)
MT-TG	No variants detected	No variants detected
MT-ND3	No variants detected	No variants detected
MT-TR	No variants detected	No variants detected
MT-ND4L	2.07 (1)	No variants detected
MT-ND4	44.34 (1)	5.84 (1)
MT-TH	No variants detected	No variants detected
MT-TS2	No variants detected	No variants detected
MT-TL2	No variants detected	No variants detected
MT-ND5	3.96±1.81 (5)	3.37±0.27 (6)

MT-ND6	No variants detected	No variants detected
MT-TE	No variants detected	No variants detected
MT-CYTB	No variants detected	27.17 (1)
MT-TT	93.00 (1)	93.80 (1)
MT-TP	No variants detected	No variants detected

[a] RefSeq NC_012920.1

Table S3. Comparison of mtDNA variant number and frequency in RCR loci in age-matched donors with and without DS (n = 10 samples for both groups).

Locus	Nucleotide span	Number of variants Down syndrome (Mean ± SD)	Number of variants non Down syndrome (Mean ± SD)	*p*-Value (variant number comparison)[a]	Variant frequency (freq%) Down syndrome (Mean ± SD)	Variant frequency (freq%) non Down syndrome (Mean ± SD)	*p*-value (variant frequency comparison)[a]
CSBI	213-235	0.00 ± 0.00	0.00 ± 0.00	-	0.00 ± 0.00	0.00 ± 0.00	-
TFX	233-260	0.00 ± 0.00	0.10 ± 0.00	-	0.00 ± 0.00	8.90 ± 0.00	-
TFY	276-303	1.40 ± 0.70	1.60 ± 0.70	0.46	25.97 ± 18.72	27.21 ± 15.02	0.89
CSBII	299-315	3.50 ± 0.85	4.00 ± 0.94	0.17	38.91 ± 10.77	35.26 ± 5.71	0.39
CSBIII	346-363	0.00 ± 0.00	0.00 ± 0.00	-	0.00 ± 0.00	0.00 ± 0.00	-
mt4H	371-391	0.00 ± 0.00	0.00 ± 0.00	-	0.00 ± 0.00	0.00 ± 0.00	-
LSP	392-445	0.00 ± 0.00	0.00 ± 0.00	-	0.00 ± 0.00	0.00 ± 0.00	-
TFL	418-445	0.00 ± 0.00	0.00 ± 0.00	-	0.00 ± 0.00	0.00 ± 0.00	-
TFH	523-550	0.70 ± 0.82	0.80 ± 0.79	0.80	9.93 ± 10.84	9.18 ± 10.96	0.97
HSP1	545-567	0.90 ± 1.20	0.90 ± 0.88	1.00	8.56 ± 9.31	10.55 ± 11.29	0.55

[a] Student's t-test used for comparisons of normally distributed data, Mann-Whitney U test used for non-normally distributed data.

Table S4. Power calculations for comparing heteroplasmic frequency of age-matched sample groups with and without DS (n = 10 samples for each group).

Expected difference between mean heteroplasmic frequency (%)	**Standard Deviation of mean heteroplasmic frequency (%)**	**Power (α=0.050)[a]**
30.00	20.00	1.00
30.00	40.00	0.92
30.00	60.00	0.61
30.00	80.00	0.39
40.00	20.00	1.00
40.00	40.00	0.99
40.00	60.00	0.85
40.00	80.00	0.61
50.00	20.00	1.00
50.00	40.00	1.00
50.00	60.00	0.96
50.00	80.00	0.80
60.00	20.00	1.00
60.00	40.00	1.00
60.00	60.00	0.99
60.00	80.00	0.92

[a]one-sample Z-test, two tailed