

Supplementary Information for

Biparental Inheritance of Mitochondrial DNA in Humans

Shiyu Luo, C. Alexander Valencia, Jinglan Zhang, Ni-Chung Lee, Jesse Slone, Baoheng Gui, Xinjian Wang, Zhuo Li, Sarah Dell, Jenice Brown, Stella Maris Chen, Yin-Hsiu Chien, Wuh-Liang Hwu, Pi-Chuan Fan, Lee-Jun Wong, Paldeep S. Atwal, and Taosheng Huang

Corresponding author: Taosheng Huang

Email: Taosheng.Huang@cchmc.org

This PDF file includes:

Figs. S1 to S10 Captions for Datasets S1 to S3 Tables S1-S3

Other supplementary materials for this manuscript include the following:

Datasets S1 to S3

Sequence	PT17 Haplogroup	$\stackrel{\mathbb{A}}{=}$	Total Variants	Variants
9 II-40	U5b (U5b1d1c)		30	A73G, C1501 A263G, A750G, A1438G, A2179G, A2706G, T3197C A4769G, C5437T [A5556G] C70281, [A7768G] G7912A] A8860G, [G9477A, A11467G] G11719A, [A12308G [G12372A, T13617C, T14182C] C14766T, A15326G, [A15631G] [T15721C, C161921] [C16218T] C162701] C16320T] Submit New Variants to Mitomap

Fig. S1. The 30 SNPs in II-40's mtDNA (Family A) differing from the reference mtDNA haplogroup were predicted in Mitomaster. Haplogroup U5b (U5b1d1c)-related mtDNA variants are labeled in red rectangles.

Sequence alig	Inment		
Click a single sequence Show 10 reference entries	ence link below to show det	ails or click here to show	details for all Copy CSV Excel PDF Print
Sequence 🔺	PT17 Haplogroup	Total Variants	Variants \Leftrightarrow
© I-10	H1a (H1a1)	13	A73G A263G, A750G, A1438G, G3010A A4769G, T6365C A8860G, G14198A, A15326G, A16162G, T16209C T16519C Submit New Variants to Mitomap
Showing 1 to 1 of 1 entr	ies		Previous 1 Next

Fig. S2. The 13 SNPs in I-10's mtDNA (Family A) differing from the reference mtDNA haplogroup were predicted in Mitomaster. Haplogroup H1a (H1a1)-related mtDNA variants are labeled in red rectangles in addition to other defining SNPs (2706A, 7028C).

Show 10 ventries Copy CSV Excel Sequence PT17 Haplogroup Total Variants Variants 91-1 R0a (R0a1) 19 T58C. C64TI A263G, A750G [A827G] A1438G, T2442C] A2706G, T3847C] A G6182A, C7028T, A8860G, C13188T] C14766T, A15326G, T16126C] C1629 (T16362C] Submit New Variants to Mitomap Showing 1 to 1 of 1 entries	
R0a (R0a1) 19 T58C_C64TI A263G, A750G, A827GI A1438G, T2442CI A2706G, T3847CI A G6182A, C7028T, A8860G, C13188TI C14766T, A15326G, T16126CI C1629 T16362CI Submit New Variants to Mitomap	PDF
(R0a1) G6182A, C7028T, A8860G, C13188T C14766T, A15326G, T16126C C1629 T16362C Submit New Variants to Mitomap	
Showing 1 to 1 of 1 entries	
Previous	1 Next

Fig. S3. The 19 SNPs in I-1's mtDNA (Family A) differing from the reference mtDNA haplogroup were predicted in Mitomaster. Haplogroup R0a (R0a1)-related mtDNA variants are labeled in red rectangles.

Sequence	PT17 Haplogroup	\$ Total Variants	Variants
⊘ ∣₋1	K1b (K1b2a)	34	A73G, [146C, T195C, A263G, A750G, [1189C, A1438G, A1811G, A2706G, A3480G A4769G, [55913A] C7028T, A8860G, [59055A] A9058G, A9066C, [79698C][A10398G] A10550G, T11299C][A11467G] G11719A][A12308G, G12372A][T12738G, G12771A] C14157T] (L4176T, [1479BG] A15326G, [T16224C, T16311C] T16519C Submit New Variants to Mitomap]

Fig. S4. The 34 SNPs in I-1's mtDNA (Family B) differing from the reference mtDNA haplogroup were predicted in Mitomaster. Haplogroup K1b (K1b2a)-related mtDNA variants are labeled in red rectangles.

Sequence alig	nment			
Click a single sequ	ence link below to show deta	ails or click here to show	details for all	
Show 10 entries	;		Сору	CSV Excel PDF Print
Sequence 🔺	PT17 Haplogroup	Total Variants	Variants	\$
Ø I-10	H (H)	9	A263G, A750G, A1438G, G1927A, G3531A, A4769G, A8860 Submit New Variants to Mitomap	G, A15326G, T16519C
Showing 1 to 1 of 1 entr	ies			
				Previous 1 Next

Fig. S5. The nine SNPs in I-10's mtDNA (Family B) differing from the reference mtDNA haplogroup were predicted in Mitomaster. Haplogroup H is defined by mtDNA genotype of 73A, 2706A, 7028C, 11719G and 14766C.

Sequence alig			debelle for all		
Show 10 entries	ence link below to show det	Total Variants	Variants	Copy CSV Excel PDF	Print
O II-30	T2a (T2a1a)	34	A73G, A263G, <u>G709A</u> A750G, A1438G, <u>G1888A</u> / A4917G, T7022C C7028T, <u>G8697A</u> A8860G, <u>[T102</u> A11812G, <u>G13368A</u> , T13965C, A14233G, A14687C A15326G, <u>C15452A</u> <u>A15607G</u> , <u>G15928A</u> <u>[T16126C</u> Submit New Variants to Mitomap]	463C A11251G G11719A, G C14766T, G14905A C15040T	

Previous 1 Next

Fig. S6. The 34 SNPs in II-30's mtDNA (Family B) differing from the reference mtDNA haplogroup were predicted in Mitomaster. Haplogroup T2a (T2a1a)-related mtDNA variants are labeled in red rectangle.

Sequence A	Predicted Haplogroup	≜ ▼	Total Variants	*	Variants 🔶	
F3-GF-mitomaster.dna	K2b (K2b1a1a)		34		A73G, T146C, A263G, A750G, A1438G, A1811G, C2217T A2706G, A3480G, A4769G, G5231A, C7028T, A8860G, G9055A, T9698C, T9716C, A10550G, T11299C, A11467G G11719A, C11869A, A12308G, G12372A, G13135A, A14037G, C14167T, C14766T, T14798C, A15326G, C16222T, T16224C, C16270T, T16311C, T16519C	Alignment Deta

Click a single sequence link below to show details or click here to show details for all.

Fig. S7. The 34 SNPs in I-1's mtDNA (Family C) differing from the reference mtDNA haplogroup were predicted in Mitomaster. Haplogroup K2b (K2b1a1a)-related mtDNA variants are labeled in red rectangle.

Sequence alignment

Click a single sequence link below to show details or click here to show details for all.

Sequence	Predicted Haplogroup	\$ Total Variants	\$ Variants		\$			
● F3-GM-mitomaster.dna	J2a (J2a1a1a2)	41	A73G, T146 T152C, [T19 A215G, A20 C295T, [31] T489C, G5 A750G, A14 T1850C, A2 A3447G, [T2 A3460G, C C7476T, G7 A8860G, A A10499G, <i>J</i> G13708A, <i>L</i> A11818G, <i>A</i> G13708A, <i>L</i> A14133G, G G15257A, <i>A</i> C15452A, G T16126C, G T16231C, G	95C 63G, 19C, 13A 438G, 2706G, 4216C 7028T, 7789A 103980 A11251 G11719 A12612 A13722 C14766 A15326 C16069 G16145	3, 16 26, 26 57, 56 54	Align	ment D	etaik

Fig. S8. The 41 SNPs in I-10's mtDNA (Family C) differing from the reference mtDNA haplogroup were predicted in Mitomaster. Haplogroup J2a (J2a1a1a2)-related mtDNA variants are labeled in red rectangle.

Click a single sequence link below to show details or click here to show details for all.

how 10 ~ entries			Сору	CSV	Excel	PDF	Prir
Sequence	Predicted Haplogroup	Total Variants	Variants	$\stackrel{\wedge}{\forall}$			
₽F3-mother-mitomaster.dna	K2a (K2a7)	35	A73G, [T146C, T A263G, C315CC CA514d G709A A750G, A1438G A1811G A2706 A1811G A2706 A1811G A2706 G3480C, T45611 A4769G, C7028 G8292A, A8860 G9055A, T9698 T9716C, A10550 T11299C, A1146 G11719A, A1231 C12346T G123 C14167T C1476 T14798C, A1533 A16066G, [T1622 T16311C, T1651	2; 6; 6; 7; 6; 7; 0; 7; 0; 7; 0; 0; 0; 0; 0; 0; 0; 0; 0; 0	Align	ment D	eetai

Fig. S9. The 35 SNPs in II-30's mtDNA (Family C) differing from the reference mtDNA haplogroup were predicted in Mitomaster. Haplogroup K2a (K2a7)-related mtDNA variants are labeled in red rectangle.

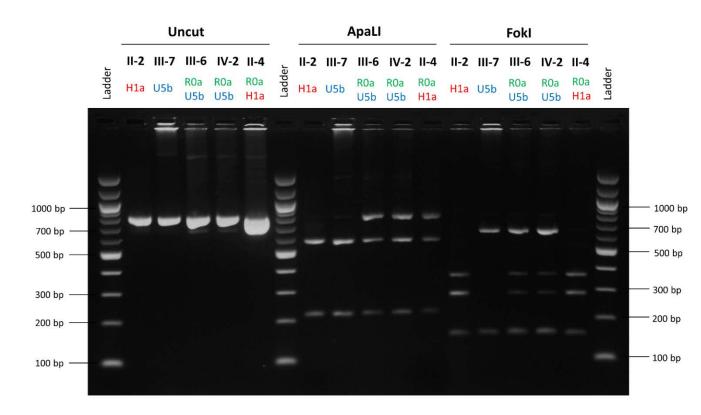


Fig. S10. RFLP analysis of selected members of Family A confirms the existence of the haplogroups detected by NGS. The haplogroups present in each individual are as indicated ("R0a" = R0a1, "H1a" = H1a1, and "U5b" = U5b1d1c). RFLP was performed on a 789 bp

PCR product covering positions 16426 to 626 in the human mitochondrial reference genome, which includes the hypervariable region HV2. The expected digest pattern for ApaLI is 789 bp for R0a1 and 572 bp and 217 bp for H1a1 and U5b1d1c. For FokI, the expected digest pattern is 632 bp and 157 bp for U5b1d1c and 355 bp, 277 bp, and 157 bp for R0a1 and H1a1.

Dataset S1. Comparison of mtDNA sequences of 11 family members in Family A. The mtDNA sequences were aligned with reference sequence- the human mitochondrial sequence reference NC_012920.1. SNPs were identified and submitted to MitoMaster for variant calls. All de novo SNPs were labeled with gray color and not included for analysis of mtDNA inheritance pattern in this family.

Dataset S2. Comparison of mtDNA sequences of five family members in Family B. The mtDNA sequences were aligned with reference sequence- the human mitochondrial sequence reference NC_012920.1. SNPs were identified and submitted to MitoMaster for variant calls.

Dataset S3. Comparison of mtDNA sequences of five family members in Family C. The mtDNA sequences were aligned with reference sequence- the human mitochondrial sequence reference NC_012920.1. SNPs were identified and submitted to MitoMaster for variant calls.

rCRS Position	Reference	Variant	I-1 ^(D)	I-10 ^(D) /II-2/III-3		II-40/III-7	III-6/IV-1/IV-2/IV-3
58	Т	С	<u>C</u>	Т	с/ _Т	Т	с/ _Т
64	С	Т	<u>T</u>	С	^T C	С	т <mark>с</mark>
73	А	G	Α	<u>G</u>	^A ∕G	G	[∧] G
150	С	Т	С	С	С	<u>T</u>	c/T
263	Α	G	G	G	G	G	G
750	Α	G	G	G	G	G	G
827	А	G	G	Α	G/A	А	G/A
1438	Α	G	G	G	G	G	G
2179	Α	G	А	Α	Α	G	[∧] /G
2442	Т	С	<u>C</u>	Т	c/T	Т	с/ _Т
2706	А	G	G	Α	G/A	G	G
3010	G	Α	G	<u>A</u>	G/A	G	G
3197	Т	С	Т	T	Т	<u>C</u>	тс
3847	Т	С	<u>C</u>	Т	c/T	T	c/T
4769	Α	G	G	G	G	G	G
5437	С	Т	С	С	С	T	с/ _Т
5656	Α	G	Α	Α	Α	G	^{A/} G
6182	G	A	Α	G	A/G	G	A/G
6365	Т	С	Т	<u>C</u>	T ^V C	Т	T
7028	С	Т	Т	C	тс	Т	Т
7768	Α	G	Α	Α	A	<u>G</u>	A/G
7912	G	A	G	G	G	A	G/A
8860	A	G	G	G	G	G	G
9477	G	A	G	G	G	<u>A</u>	G/A
11467	A	G	A	A	A	G	^A G
11719	G	A	G	G	G	Ā	G/A
12308	A	G	A	Α	Α	G	A/G
12300	G	A	G	G	G	Ā	G/A
13188	C	T	<u>T</u>	C	T ^V C	C	Ϋ́C
13617	T	C	T	T	T	<u><u>C</u></u>	Ϋ́C
14182	T	C	T	T	T	<u>C</u>	Ϋ́C
14198	G	A	G	A	G/A	G	G
14766	C	T	T	C	T ^V C	T	T
15326	A	G	G	G	G	G	G
15631	A	G	A	A	A	G	A/G
15721	T	G C	T	T	T	<u>C</u>	Ψ _C
16126	T	C	<u><u>C</u></u>	T	C/T	T	C/T
16162	A	G	A	<u>G</u>	A/G	A	A
16192	C	T	C	C	C	<u>T</u>	C/T
16192	Т	C I	T	<u><u> </u></u>	T ^V C	T	T
16209	C I	T	C	<u> </u>	C	T T	C/T
	C	T	C	C	C	<u>T</u>	C/T
16270			T	C	T'C	C	T ^T C
16291	C	Т	C	C	C C		C/T
16320	С	T		Т	C C/T	<u>T</u> T	C/T
16362	T	C	<u>С</u> Т	C	T ^V C	T	Т
16519	T	С					
Heteroplasmy %			100	100	^{40/} 60 R0a1/ <u>H1a1</u>	100 <u>U5b1d1c</u>	^{40/} 60 <u>R0a1/U5b1d1c</u>

Table S1. Alignment of a total of 46 differential mtDNA variants in family A

Entries labeled (D) represent deduced mtDNA genotypes. Haplogroup related nucleotides have been underlined in the table.

rCRS Position	Reference	Variant	I-1 ^(D)	I-10 ^(D)	II-3	II-30/III-1/III-5	III-2
73	Α	G	G	<u>A</u>	G/A	G	G
146	Т	С	<u>C</u>	Т	C/T	Т	C/T
195	Т	С	C	Т	C/T	Т	C/T
263	Α	G	G	G	G	G	G
709	G	A	G	G	G	<u>A</u>	G/A
750	A	G	G	G	G	G	G
1189	T	C	<u>C</u>	T	C/T	T	с/ _Т
1438	A	G	G	G	G	G	G
	A	G			G/A	A	G G/A
1811			G	A			
1888	G	A	G	G	G	A	G/A
1927	G	A	G	A	G/A	G	G
2706	A	G	G	<u>A</u>	G/A	G	G
2850	Т	С	Т	Т	T	<u>C</u>	T ^V C
3480	Α	G	<u>G</u>	Α	G/A	Α	G/A
3531	G	A	G	A	G/A	G	G
4216	Т	С	Т	Т	Т	<u>C</u>	тс
4769	Α	G	G	G	G	G	G
4917	Α	G	Α	A	Α	G	^{A/} G
5913	G	Α	<u>A</u>	G	A/G	G	A/G
7022	Т	С	Т	Т	Т	<u>C</u>	туС
7028	С	Т	Т	<u>C</u>	T/C	T	Т
8697	G	A	G	G	G	A	G/A
8860	A	G	G	G	G	G	G
9055	G	A	A	G	A/G	G	A/G
9058	A	G	G	A	G/A	A	G/A
9066	A	C	C	A	C/A	A	C/A
9698	T A	C	<u><u>c</u></u>	T	C/T	T	A C/T
					G/A		G/A
10398	A	G	G	A		A	
10463	T	C	T	T	T C/	<u><u>C</u></u>	
10550	A	G	G	A	G/A	A	G/A
11251	Α	G	A	A	A	G	A/G
11299	Т	С	<u>C</u>	Т	C/T	Т	<u>с/</u> Т
11467	Α	G	<u>G</u>	Α	G/A	Α	G/A
11719	G	A	Α	<u>G</u>	^{A/} G	Α	Α
11812	Α	G	Α	Α	Α	<u>G</u>	^{A/} G
12308	Α	G	<u>G</u>	Α	G/A	Α	G/A
12372	G	A	<u>A</u>	G	A/G	G	^{A/} G
12738	Т	G	<u>G</u>	Т	G/T	Т	G/T
12771	G	A	A	G	A/G	G	^{A/} G
13368	G	A	G	G	G	<u>A</u>	G/A
13965	Т	С	Т	Т	Т	C	тс
14167	С	Т	T	С	T/C	C	^T C
14233	A	G	Ā	Α	A	G	^{A/} G
14687	A	G	Α	Α	Α	G	A/G
14766	C	T	Т	C	T/C	T	T
14798	T	C	<u><u>C</u></u>	T	C/T	T	C/T
14905	G	A	G	G	G	<u>A</u>	G/A
15040	C	T	C	C	C	T	C/T
15326	A	G	G	G	G	G	G
15320	A C	A	C	C	C		C/A
	A	A G	A	A	A	<u>A</u> <u>G</u>	•··A •··G
15607				G A	G		
15928	G	A	G			<u>A</u>	G/A
16126	T	C	T	T	T C/-	<u><u>C</u></u>	TC C/-
16224	T	C	<u>C</u>	T	C/T	Т	C/T
16294	С	Т	С	С	С	<u>T</u>	C/T
16296	С	Т	С	С	C	<u><u>T</u></u>	C/T
16311	Т	С	<u>C</u>	Т	c/T	Т	c/T
16519	Т	С	С	С	С	С	С
Heteroplasmy %			100	100	^{28/} 72	100	^{51/} 49
Haplogroup			<u>K1b2a</u>	H	K1b2a/H	<u>T2a1a</u>	K1b2a/T2a1a

Table S2. Alignment of a total of 58 differential mtDNA variants in family B

Entries labeled (D) represent deduced mtDNA genotypes. Haplogroup related nucleotides have been underlined in the table.

rCRS Position	Reference	Variant	I-1 ^(D)	I-10 ^(D)	П-3	II-30	III-6/III-7/IV-1	rCRS Position	Reference	Variant	I-1 ^(D)	I-10 ^(D)	II-3	II-30	III-6/III-7/IV-1
73	А	G	G	G	G	G	G	10550	Α	G	<u>G</u>	Α	G/A	<u>G</u>	G
146	Т	С	<u>C</u>	С	С	<u>C</u>	С	11251	Α	G	Α	<u>G</u>	A/G	Α	Α
150	С	Т	С	<u>T</u>	c/T	С	С	11299	Т	С	<u>C</u>	Т	C/T	<u>C</u>	С
152	Т	С	Т	<u>C</u>	т _с	<u>C</u>	тс	11377	G	Α	G	<u>A</u>	G/A	G	G
195	Т	С	Т	<u>C</u>	т _с	Т	Т	11467	Α	G	G	A	G/A	G	G
215	А	G	A	G	^{A/} G	A	Α	11719	G	Α	Α	А	Α	Α	Α
263	А	G	G	G	G	G	G	11818	А	G	Α	G	^{A/} G	Α	Α
295	С	Т	С	<u>T</u>	c/T	С	С	11869	С	Α	<u>A</u>	С	A/C	С	^{A/} C
319	Т	С	Т	<u>C</u>	^T C	Т	Т	12308	A	G	<u>G</u>	A	G/A	<u>G</u>	G
489	Т	С	Т	<u>C</u>	тс	Т	Т	12346	С	Т	С	С	С	T	C/T
513	G	Α	G	<u>A</u>	G/A	G	G	12372	G	Α	<u>A</u>	G	A/G	<u>A</u>	Α
709	G	Α	G	G	G	A	G/A	12612	Α	G	Α	G	A/G	Α	Α
750	А	G	G	G	G	G	G	13135	G	Α	A	G	A/G	G	^A ∕G
1438	Α	G	G	G	G	G	G	13708	G	Α	G	<u>A</u>	G/A	G	G
1811	А	G	G	А	G/A	<u>G</u>	G	13722	Α	G	Α	<u>G</u>	^{A/} G	Α	Α
1850	Т	С	Т	<u>C</u>	т _с	Т	Т	14037	A	G	<u>G</u>	A	G/A	A	G/A
2217	С	Т	<u>T</u>	С	т _с	С	^T C	14133	A	G	Α	<u>G</u>	^{A/} G	Α	Α
2706	А	G	G	G	G	G	G	14167	С	Т	<u>T</u>	С	T'C	T	Т
3447	А	G	A	G	^{A/} G	A	Α	14766	С	Т	Т	Т	Т	Т	Т
3480	Α	G	G	Α	G/A	G	G	14798	Т	С	<u>C</u>	Т	C/T	<u>C</u>	С
4216	Т	С	Т	<u>C</u>	т _с	Т	Т	15257	G	Α	G	<u>A</u>	G/A	G	G
4561	Т	С	Т	Т	Т	<u>C</u>	^T C	15326	Α	G	G	G	G	G	G
4769	Α	G	G	G	G	G	G	15452	С	Α	С	<u>A</u>	C/A	С	С
5231	G	Α	<u>A</u>	G	^{A/} G	G	A/G	16066	A	G	Α	А	Α	G	^A ∕G
7028	С	Т	Т	Т	Т	Т	Т	16069	С	Т	С	<u>T</u>	C/T	С	С
7476	С	Т	С	T	c/T	С	С	16126	Т	С	Т	<u>C</u>	T'C	Т	Т
7789	G	Α	G	A	G/A	G	G	16145	G	Α	G	A	G/A	G	G
8292	G	Α	G	G	G	A	G/A	16222	С	Т	T	С	тс	С	тс
8860	Α	G	G	G	G	G	G	16224	Т	С	<u>C</u>	Т	C/T	<u>C</u>	С
9055	G	Α	<u>A</u>	G	^{A/} G	A	Α	16231	Т	С	Т	<u>C</u>	T'C	Т	Т
9698	Т	С	<u>C</u>	Т	c/T	<u>C</u>	С	16261	С	Т	С	<u>T</u>	C/ T	С	С
9716	Т	С	<u>C</u>	Т	c/T	<u>C</u>	С	16270	С	Т	<u>T</u>	С	тс	С	^T C
10398	А	G	A	G	^{A/} G	A	Α	16311	Т	С	<u>C</u>	Т	C/ T	<u>C</u>	С
10499	А	G	A	G	^{A/} G	A	Α	16519	Т	С	С	Т	C/T	С	С
Heteroplasmy %			100	100	^{70/} 30	100	^{76/} 24	Heteroplasmy %			100	100	^{70/} 30	100	^{76/} 24
Haplogroup			K2b1a1a	<u>J2a1a1a2</u>	K2b1a1a/J2a1a1a2	<u>K2a7</u>	K2b1a1a/K2a7	Haplogroup			K2b1a1a	J2a1a1a2	K2b1a1a/J2a1a1a2	<u>K2a7</u>	K2b1a1a/K2a7

Table S3. Alignment of a total of 68 differential mtDNA variants in family C

Entries labeled (D) represent deduced mtDNA genotypes. Haplogroup related nucleotides have been underlined in the table.