

Supplemental Data**FASTKD2 Nonsense Mutation in an Infantile****Mitochondrial Encephalomyopathy****Associated with Cytochrome C Oxidase Deficiency**

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Table S1. Respiratory chain activities of muscle homogenate and fibroblasts of Patient II-1 normalized to the activity of citrate synthase (CS).

Patient II-1 muscle*	enzyme/CS	Control mean (\pmSD)	%
Succinate-ubiquinone reductase	8.4	21.5 \pm 6.5	39
Succinate dehydrogenase	9.7	14 \pm 3.3	69
NADH-ubiquinone reductase	n.d.	18.5 \pm 5.5	-
DBH ₂ cytochrome reductase	42	127.5 \pm 39.5	33
Cytochrome c oxidase	23	170 \pm 50	14
Mg ATPase	273	155 \pm 66	176
Patient II-1 fibroblasts**			
Succinate-ubiquinone reductase	14.2	13.5 \pm 4.9	105
Succinate dehydrogenase	9.3	10.4 \pm 3.9	89
NADH-ubiquinone reductase	26.4	18.4 \pm 7.7	143
DBH ₂ cytochrome reductase	105	108.3 \pm 21.8	97
Cytochrome c oxidase	129	124.6 \pm 37,6	104
Mg ATPase	102	89.2 \pm 23.5	112

*Citrate synthase (CS) specific activity in muscle: 100 nmol/min/mg (normal control 145 \pm 65)

**CS specific activity in fibroblasts: 156 nmol/min/mg (normal control 150 \pm 50)

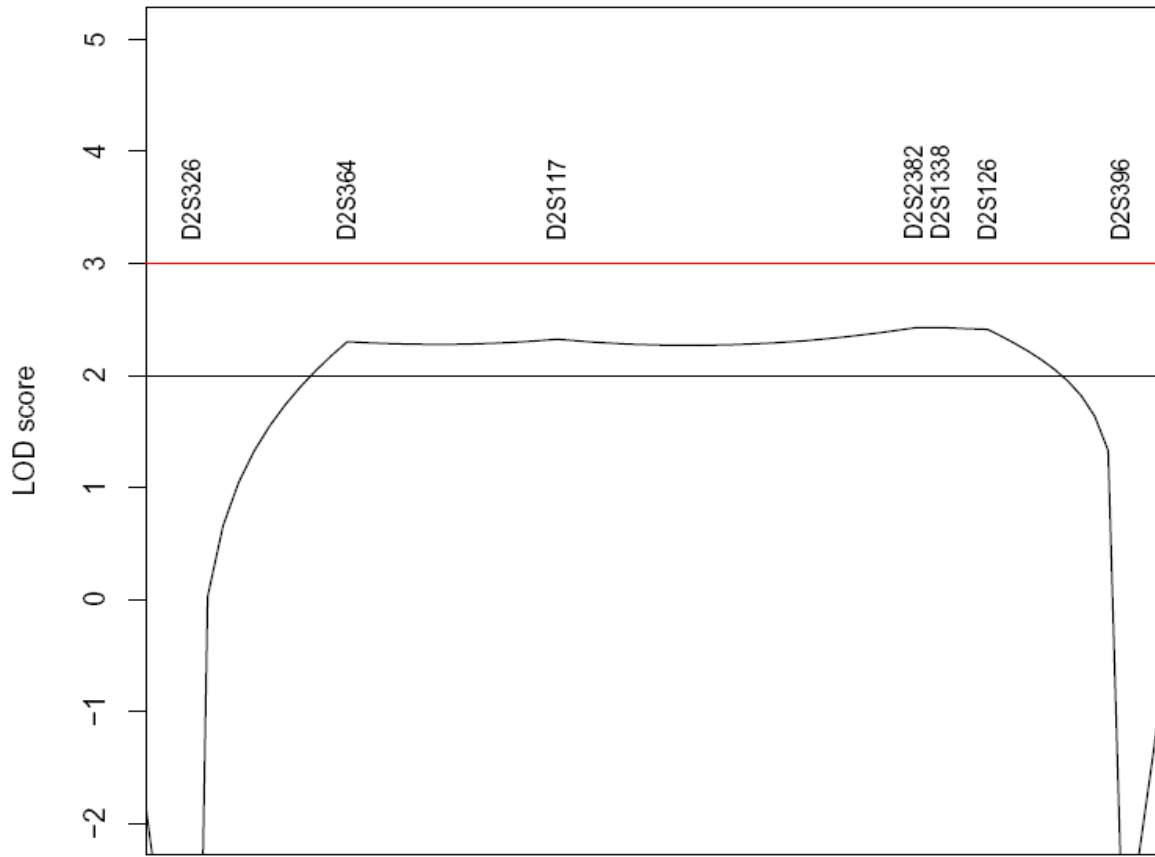
n.d.= not determined

Table S2. List of the genes in the disease locus analyzed by sequencing

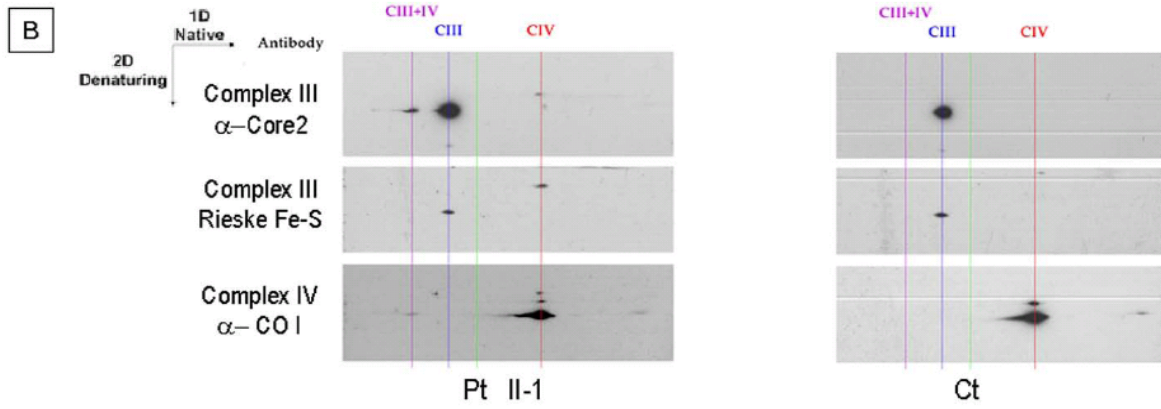
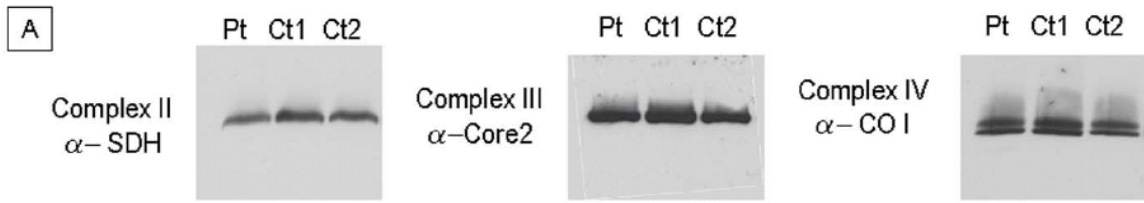
Symbol	HUGO nomenclature	Protein accession number
MT-ABC3	Mitochondrial ATP-binding Cassette 3	NP_005680
MARS	Mitochondrial methionyl-tRNA synthetase 2	NP_004981
MRPL44	Mitochondrial ribosomal protein L44	NP_075066
MTX2	Metaxin 2	NP_001006636
HSPD1	Heat shock 60kDa protein 1 (chaperonin)	NP_955472
HSPE1	Heat shock 10kDa protein 1 (chaperonin 10)	NP_002148
BCS1L	BCS1-like (yeast)	NP_004319
GLS	Glutaminase	NP_055720
NIF3L1	NIF3 NGG1 interacting factor 3-like 1 (S. pombe)	NP_068596
ADAM23	ADAM metallopeptidase domain 23	NP_003803
PNKD	Paroxysmal nonkinesigenic dyskinesia	NP_056303
OSGEPL1	O-sialoglycoprotein endopeptidase-like 1	NP_071748
C2orf47	Chromosome 2 open reading frame 47	NP_078796
COQ10B	Coenzyme Q10 ortholog B (S. cerevisiae)	NP_079423
FASTKD2	FAST kinase domain 2	NP_055744

Supplemental Figure 1

Chromosome 2



Supplemental Figure 2



Supplemental Figure 3

A.

Homo	MNNKAGSFFWNLRFQSTLVSTSRMTMLCCLGLCKPKIVHSNWN----ILNNFHNRMQSTD	56
Pongo	MNNKAGSFFWNLRFQSTLVPTSRIMRLYRLGLCKPKIVHSNWN----ILNNFHNMRSTD	56
Bos	MTNRAGSFLWNLRLQSTLVPTGRVRLYLPLAFCRPNLAYSNWNRN-LLNLTFGNKMHS-	58
Mus	MNSKARSLLTWIRRFSTLLPRSALRIDPLGTCRPEVIHSKWNPRNHLNLFVDEGLQPS-	59
Gallus	MGYLLNAVRY-LRRCSVPRPSAPTSRHHVVTGRHRDCLDNTNCRITFLNAPPSLRGSP-	58
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Homo	IIRYLFQDAFIFKSD-VGFQTKGISTLTALRIERLLYAKRFFDSKQSLVPVDKSDDELK	115
Pongo	IIRYLFQDAFIFKSD-VGFQTKGISTLTARRIERLLYARRLFFDSKQSLVPVDKSDDLGK	115
Bos	-TRYLFQDALIFKSEGDGCQTGINTATVFTVDKLLCPRRSFDKHFVSVN----ELK	113
Mus	-VRYLFQDIFIKSVDCIQTKGISHSVAVFKPDRLLCPRRSFDKHSFVSDGTSDDLK	118
Gallus	-LRFLSHKADAFVGVDEVQEK-----SREAAVSEQAPQSSLE	95
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Homo	KVNLNHEVSNEDVLTKETKPNRISSRKLSEECNSLSDVLDAFSKAPTFPSSNYFTAMWTI	175
Pongo	KVNLNHEVSNEDVLTKETKPNRISSRKLSEECNSLSDVLDAFSKAPTFPSSNYFTAMWTI	175
Bos	KS-FHQEASDEDVLTKRKPKALISSTKLSQECNSLSDVLDIFSKAPTFPSSNYFSAMWTI	172
Mus	KINFHHTSS-EDVFTKVRPTPVNYKKLAQECNSLSDVLDTFKAPTFPGSNYFLAMWII	177
Gallus	LEKLEGDAQSFVKAALDENEQFFNRLHTCACPSDVLDLASESAVSIKQFTNCLTTIWKL	155
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Homo	AKRLSDDQKRFKRLMFSHPAFNQLCEHMMREAKIMQYKYLFLSLHAIVKLGIPQNTILV	235
Pongo	AKRLSDDQKRFKRLMFSHPAFNQLCEHMMREAKIMQYKYLFLSLYSVMVKLGIPQNTILV	235
Bos	AKRMSDDQKRFKRLMFSHPAFNQLCEHMMREAKIMYFGDLLFSLHAIVKLGIPQNTLLV	232
Mus	AKRISDDQKRFKRLMFSHPAFNQLCEHMMREAKIMHYDHLFLSLNAIVKLGIPQNTLMV	237
Gallus	LRSMDDQRRYKRLVFEHPAFVRLCQQLLRDSRRMTRGDLVFLHAVVNLGVPQNTLLV	215
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Homo	QTLRVTQERINECDEICLSVLSTVLEAMEPCKNVHVLRTGFRILVDQQVWKIEDVFTLQ	295
Pongo	QTLRVTQERINECDEICLSVLSTVLEAMEPCKNVHVLQMGFRILVDQQVWKIEDVFTLQ	295
Bos	QTLRVTQERINECDEICLSVLSTVLEAMEPCKNVHVLQMGFRILVDQQVWKIEDVFTLQ	292
Mus	QTLRVTQERINECDEICLSVLSTVLEAMEPCKNVHVLQMGFRILVDQQVWKIEDVFTLQ	297
Gallus	QTLRVCQEKLNQLDNRCMSVLAATLAGMDEKNSALQAGLQLLVEQRLPSIRDFILQ	275
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Homo	VVMKCIGKDAPIALKRKLEMKALRELDRFVLSNSQHMFEVLAAMNHRSLILLDECSKVVL	355
Pongo	VVMKCIGKDAPIALKRKLEMKALRELDRFVLSNSQHMFEVLAAMNHRSLILLDECSKVVL	355
Bos	TVMRSIGKDAPIALKRKLEMKALRELDRFVLSNSQHMFEVLAAMNHRSLILLDECSKVVL	352
Mus	TVMKCIGKDAPIALKRKLEMKALRELDRFVLSNSQHMFEVLAAMNHRSLILLDECSKVVL	357
Gallus	NLMKCMGKDAPVFLKLLKEMAVLREIDHLYPNAHRVFLGLVAMNYCSPVPLNACSKKIQ	335
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Homo	DNIHGCPRLRIMINILQSCKDLQYHNLDFKGLADYVAATFDIWKFRKVLFIILILFENLGF	415
Pongo	DNIHGCPRLRIMINILQSCKDLQYHNLDFKGLADYVAATFDIWKFRKVLFIILILFENLGF	415
Bos	SNIHGCPKILINILQSCRDLYQYHNLDFKGLADYVAATFDIWKFRKVLFIILILFENLGF	412
Mus	DNVHGCPKVLISILQSCRDLYQYHNLDFKGLADYVAATFDIWKFRKVLFIILILFENLGF	417
Gallus	ENIHDPYWLILILEACHSLQYRNKLFSAVADYVNSIVCLLDKRIILFLSAFETLGF	395
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Homo	RPVGLMDLFMKRIVEDPELSNMKNILSLHTYSSLNHVYKQNKQFVEVMASALTGYLH	475
Pongo	RPVGLMDLFMKRIVEDPELSNMKNILSLHTYSSLNHVYKQNKQFVEVMASALTGYLH	475
Bos	RPVGLMDLFIKKADEPGFLNVKSLVGLNHYSSLNHVYKQNKQFVEVMASALTGYLH	471
Mus	RPPGLMDKLMKVVQEPGSLNVKNIVSILHVSSSLNHVHKIHN-REFLEALASALTGYLH	476
Gallus	QPSELMGVLAEKVTEDEFLDLKSLFLIVLRVYSRLNYPVPGQH-LLFYETLHSCLNKYLP	454
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Homo	TISSENLLDAVYSFCLMNYFPLAPFNQLLQKDIISELLTSDDMK--NAYKLHTLDTCLKL	533
Pongo	TISSENLLHAVYSFCLMNYFPLAPFNQLLQKDVISELLTSDDMK--NVYKLHMLDTCLKL	533
Bos	HISSENLLNAVCSFCWMNCFPLALINQLLQKDIIDLLTSGDVER-NVHRLHVATCLKL	530
Mus	HISSESLNNAVHVSFCMNYFPLAPINQLIKENIINELLTSGDTEK-NIHKLHVNTCLKL	535
Gallus	QISNAELLKAVYSLCILGYLPHLALNQLLKKDSFEELMSGDLYKEKREMMHLCVRCMEL	514
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Homo	DDTVYLRDIALSLPQLPRELPPSHNAKVAEVLSSLLGGEGHFSKDVHLPNHYHIDFEIR	593
Pongo	DDTVYLRDIALSLPQLPRELPPSHNAKVAEVLSTDRS-PHNFPKAVAAATNSTHYIFEIR	592
Bos	DDAPCHKDLVLVLPQLPMTFCQPQ--KVAAVLSDLLG-EGCFSSQSVQLPHNYHIDFEIR	587
Mus	DESTY-KSVHIPLPQLP--LSASQPNKLAEVLSSLLGGEGHFSRNVPLPHNYHIDFEIR	592

Gallus DSPSFMKPAFVPTEIFSSLSVVTLR--KAREALLELLGDENMFRQNVQLPYRIDFEIW 572
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Homo MDTNRNQVLPLSDVDTTSATDIQRVAVLCVSRSAYCLGSSHPRGFLAMKMRHLNAMGFHV 653
Pongo MDTNRNQVLPLSDVDTTSATDIQRVAVLCVSRSAYCLGSSHPRGFLAMKMRHLNAMGFHV 652
Bos MDANRSQVLPFSDVDVVTSTDIQRVAVLCVPKSAYCLDSTHPKGYLAMKMRHLKIMGFHV 647
Mus MDTNRTQVFSFSDVDASSATNMQRVAVLCVPKSVYCLNSCHPRGLMAMKIRHLNMMGFHV 652
Gallus MDSDTKKVLPITATDSYADRSVQRLAFLVPPSAFCLGTTHPQGKLAMKKRHLSKLGYHV 632
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Homo ILVNNWEMDKLEMEDAVTFLKTKIYSVEALPVAAVNVQSTQ 694
Pongo ILVNNWEMDKLEMEDAVTFLKTKIYSVEALPVAAVNVQST- 692
Bos ILVNNWEVEKLEMKDAVFLKTKIYSPKALSSADIHLQSTC 688
Mus ILIHNWELKLMEDAVTFVRKKIYSDEVLPTADTTV---- 689
Gallus IPVLNKKFQELTNEGAIEFLKGKIYSENVSPFSEVNLCDNN 673
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B.

Homo MLTTLKPFGSVSVESKMNNKAGSFFWNLRQFSTLVSTSRTMRLCCLGLCKPKIVHSSNWN- 43
Pan ILTTLKPFGSVSVESKMNNKAGSFFWNLRQFSTLVSTSRTMRLCCLGLCKPKIVHSSNWN- 43
Pongo ILTTLKPFGSVSVESKMNNKAGSFFWNLRQFSTLVPTSRTMRLYRLGLCKPKIVHSSNWN- 43
Bos LXGIRKISRVAQFAGMTNRAGSFLWNLRLSTLVPTGRTVRLYPLAFCRPNIAYSSNWN 44
Mus LTYKRIRNTSPDAPLEMNSKARSLLWTIRRFSTLLPRSRALRIDPLGTCRPEVIHSKWNP 44
Gallus YVLSPGLFPMVGFPQIMGYLLNAVRY-LRRCSPVPRSPAPTSRHVVWTGRHRDCLDNTNC 43
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Supplemental data

Figure S1. Multipoint linkage analysis

The graph represents the region between markers D2S326 and D2S396 with a location score ≥ 2 . A pairwise lodmax score of 2.428 was on D2S1338.

Figure S2. BNGE of patient and control muscle

Panel A. One-dimension BNGE of muscle homogenates from patient II-1 (Pt) and 2 controls (Ct1 and Ct2). An antibody against the SDH 30 kDa subunit was used to detect complex II; an antibody against Core2 was used for complex III; and an antibody against COX-I was used for complex IV.

Panel B. Second-dimension BNGE of patient II-1 (Pt) and control (Ct) muscle homogenates. Antibodies against Core2 and Rieske Fe-S protein were used to detect complex III; an antibody against COX-I was used for complex IV.

Figure S3. Comparative Protein Sequence analysis of FASTKD2

Panel A. Interspecies alignment of full-length FASTKD2 protein sequences, obtained by ClustalW software online.

Accession numbers: *Pongo pygmaeus*: ENSPPYG00000013112; *Bos Taurus*: NP_001095587; *Mus musculus*: NP_766010; *Gallus gallus*: XP_421951; *Pan troglodytes*: ENSPTRG00000012848.

Panel B. Predicted sequences corresponding to the translatable ORF of human FASTKD2 protein sequence from the first predicted methionine (M1) to the second methionine (M17). The M1 is consistently absent in non-human species, suggesting that the M1-M17 sequence is not translated and therefore that in humans as in other species the FASTKD2 protein starts from the second potential ATG initiation codon (M17).