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Appendix A: Supplemental material

Article: Biallelic variants in PYROXD2 cause a severe infantile metabolic disorder affecting mitochondrial function

Supplemental Table 1. Respiratory chain enzyme activities in skeletal muscle biopsy of *PYROXD2* patient.

	Activity		**Ref. Range**	**% Activity**	**% CS Ratio**	**% CII Ratio**
Complex I (NADH-coenzyme Q_1 oxidoreductase)	134	nmol/min/mg	(19 - 72)	327	121	132
Complex II (Succinate-coenzyme Q_1 oxidoreductase)	112	nmol/min/mg	(26 - 63)	248	91	
Complex II+III (Succinate cytochrome c reductase)	72	nmol/min/mg	(30 - 76)	157	59	64
Complex III (Decylbenzylquinol-cytochrome *c* oxidoreductase)	80.3	/min/mg	(13 - 51)	277	97	108
Complex IV (Cytochrome *c* oxidase)	10.84	/min/mg	(3.3 - 9.1)	164	61	67
Citrate Synthase	349	nmol/min/mg	(85 - 179)	271		

Note: All measurements are performed in duplicate and were only accepted when each of the duplicate values was within a 10% range of their average. Enzyme activities are shown as absolute values and as % residual activity relative to protein (% Activity), Citrate Synthase (% CS Ratio), and Complex II (% CII Ratio). % values ≤ 30 are shown in bold and represent major or minor criteria in the Bernier Diagnostic Scheme [1].

Supplemental Table 2. Respiratory chain enzyme activities in skin fibroblasts of *PYROXD2* patient.

	Activity		Ref. Range	% Activity	% CS Ratio	% CII Ratio
Complex I (NADH-coenzyme Q^1 oxidoreductase)	176	nmol/min/mg	(93 – 131)	150	85	90
Complex II (Succinate-coenzyme Q_1 oxidoreductase)	162	nmol/min/mg	(87 – 120)	164	95	
Complex II+III (Succinate cytochrome *c* reductase)	169	nmol/min/mg	(70 - 79)	223	128	133
Complex III (Decylbenzylquinol-cytochrome *c* oxidoreductase)	39.2	/min/mg	(21.2 - 26.2)	161	94	97
Complex IV (Cytochrome *c* oxidase)	7.53	/min/mg	(5.28 - 5.82)	136	78	81
Citrate Synthase	471	nmol/min/mg	(215 - 319)	169		

Note: All measurements are performed in duplicate and were only accepted when each of the duplicate values was within a 10% range of their average. Enzyme activities are shown as absolute values and as % residual activity relative to protein (% Activity), Citrate Synthase (% CS Ratio), and Complex II (% CII Ratio). % values ≤ 30 are shown in bold and represent major or minor criteria in the Bernier Diagnostic Scheme [1].

Supplemental Table 3. Proteomics data on *PYROXD2* patient, including data used to generate Volcano plots and profile plots of log2-transformed intensities for cellular proteins in patient and control fibroblasts.

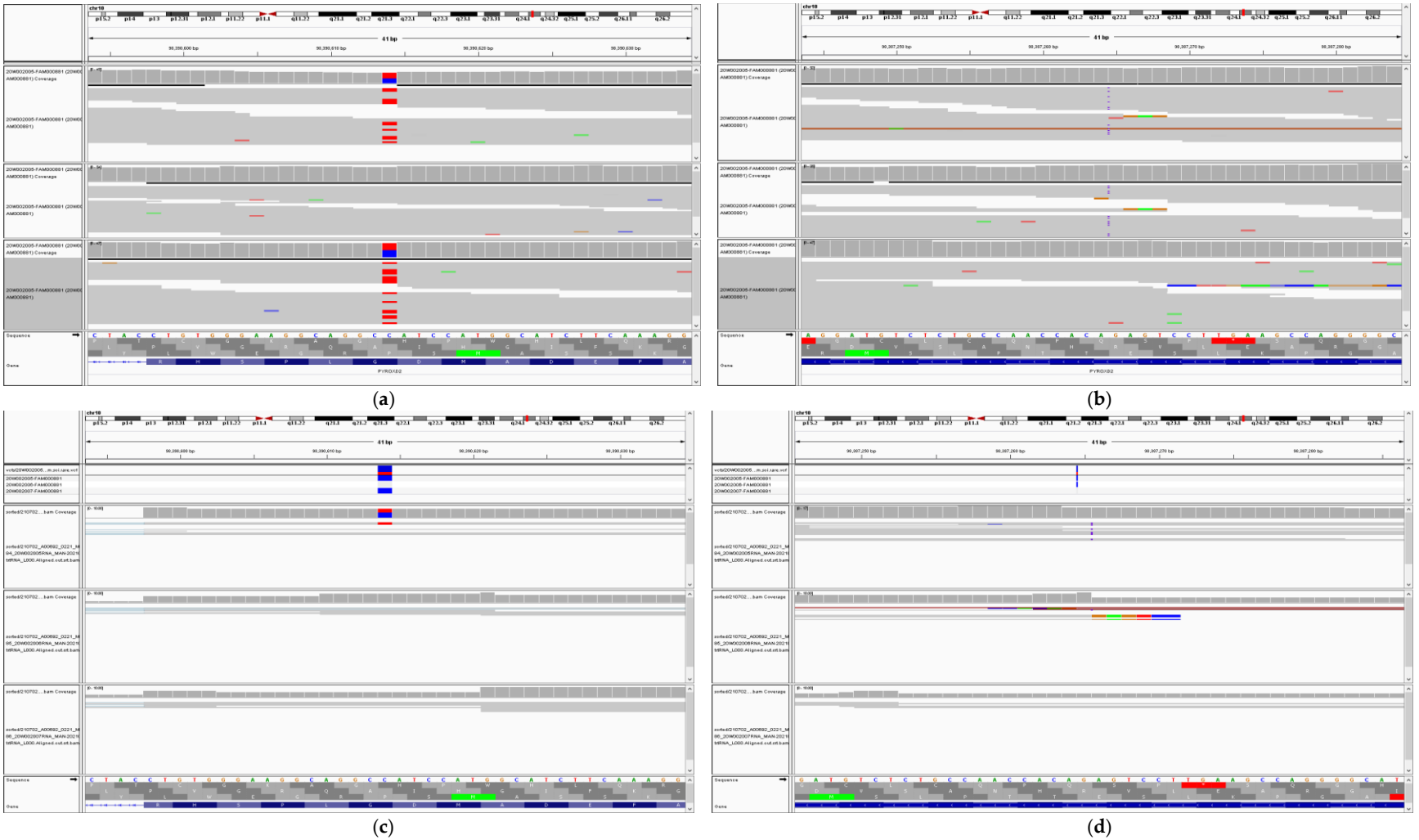
(SEE EXCEL SPREADSHEET)

Supplemental Table 4. GO terms enriched in proteins of lower level in patient fibroblasts compared to controls.

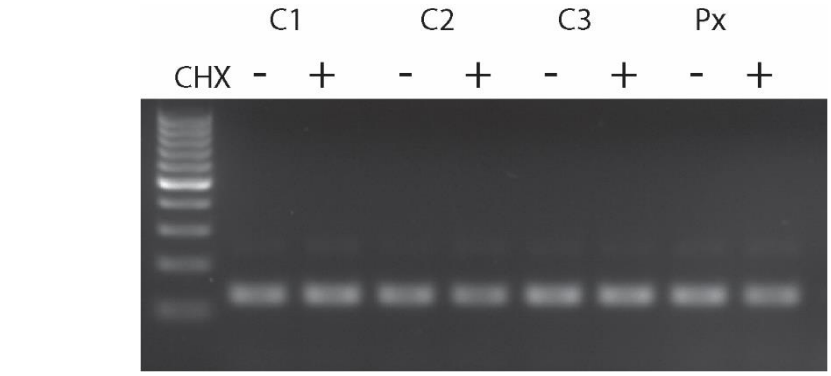
Reactome pathways	Homo sapiens - REFLIST (20595)	Uploaded (272)	Uploaded (expected)	Uploaded (over /under)	Uploaded fold Enrichment)	Uploaded (raw P-value)	uploaded (FDR)
Mitochondrial translation elongation (R-HSA-5389840)	88	16	1.16	+	13.77	4.25E-13	9.71E-10
Mitochondrial translation termination (R-HSA-5419276)	88	16	1.16	+	13.77	4.25E-13	4.85E-10
Mitochondrial translation initiation (R-HSA-5368286)	88	16	1.16	+	13.77	4.25E-13	3.24E-10
Mitochondrial translation (R-HSA-5368287)	94	16	1.24	+	12.89	1.04E-12	4.76E-10
Complex I biogenesis (R-HSA-6799198)	55	8	0.73	+	11.01	1.57E-06	5.96E-04
Striated Muscle Contraction (R-HSA-390522)	36	5	0.48	+	10.52	1.85E-04	3.84E-02
Smooth Muscle Contraction (R-HSA-445355)	39	5	0.52	+	9.71	2.60E-04	4.95E-02
Cargo recognition for clathrin-mediated endocytosis (R-HSA-8856825)	104	9	1.37	+	6.55	1.74E-05	4.98E-03
Translation (R-HSA-72766)	293	25	3.87	+	6.46	7.50E-13	4.28E-10
Respiratory electron transport (R-HSA-611105)	100	8	1.32	+	6.06	8.59E-05	2.18E-02
Clathrin-mediated endocytosis (R-HSA-8856828)	144	9	1.9	+	4.73	1.85E-04	4.23E-02
Metabolism of proteins (R-HSA-392499)	1977	51	26.11	+	1.95	4.23E-06	1.38E-03

Supplemental Table 5. GO terms enriched in proteins of higher level in patient fibroblasts compared to controls.

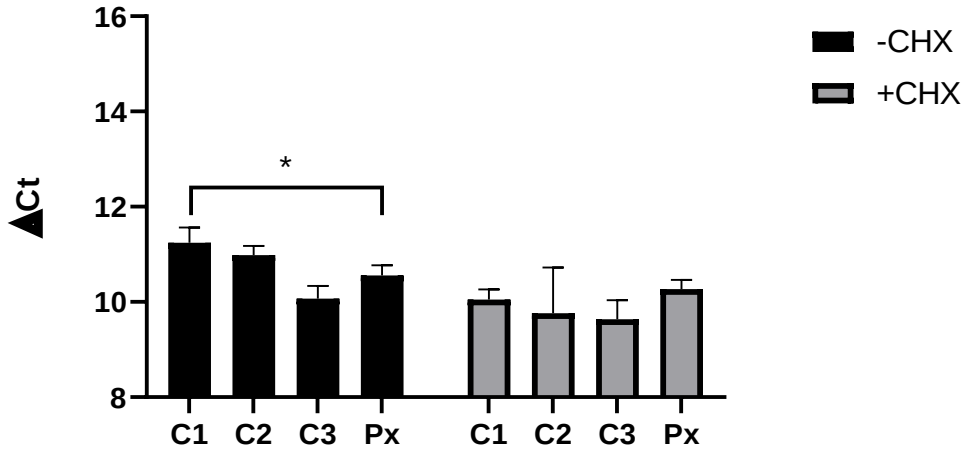
Reactome pathways	Homo sapiens - REFLIST (20595)	Uploaded (272)	Uploaded (expected)	Uploaded (over /under)	Uploaded fold Enrichment)	Uploaded (raw P-value)	uploaded (FDR)
Cholesterol biosynthesis via lathosterol (R-HSA-6807062)	4	2	0.02	+	> 100	2.96E-04	4.84E-02
Cholesterol biosynthesis via desmosterol (R-HSA-6807047)	4	2	0.02	+	> 100	2.96E-04	4.51E-02
Cholesterol biosynthesis (R-HSA-191273)	24	8	0.11	+	73.82	1.18E-12	2.70E-09
Other semaphorin interactions (R-HSA-416700)	19	3	0.09	+	34.97	1.27E-04	2.24E-02
Activation of Matrix Metalloproteinases (R-HSA-1592389)	33	5	0.15	+	33.55	7.35E-07	1.68E-04
Activation of gene expression by SREBF (SREBP) (R-HSA-2426168)	40	6	0.18	+	33.22	5.68E-08	3.24E-05
Regulation of cholesterol biosynthesis by SREBP (SREBF) (R-HSA-1655829)	53	6	0.24	+	25.07	2.61E-07	9.92E-05
Collagen degradation (R-HSA-1442490)	64	6	0.29	+	20.76	7.29E-07	1.85E-04
Metabolism of steroids (R-HSA-8957322)	148	10	0.67	+	14.96	2.38E-09	2.72E-06
Integrin cell surface interactions (R-HSA-216083)	84	5	0.38	+	13.18	5.08E-05	9.66E-03
Degradation of the extracellular matrix (R-HSA-1474228)	140	8	0.63	+	12.65	3.46E-07	9.87E-05
Extracellular matrix organization (R-HSA-1474244)	299	11	1.35	+	8.15	1.47E-07	6.70E-05
Metabolism of lipids (R-HSA-556833)	733	18	3.31	+	5.44	5.55E-09	4.23E-06
Metabolism (R-HSA-1430728)	2079	27	9.39	+	2.88	3.31E-07	1.08E-04
Unclassified (UNCLASSIFIED)	9941	23	44.89	-	0.51	5.29E-06	1.10E-03



Supplemental Figure 1. GS and RNAseq analysis of *PYROXD2* in patient fibroblasts. (A) The paternally inherited PYROXD2 missense variant (NM_32709.2: c.1276G>A; p.(Gly426Ser); and **(B)** the maternally inherited frameshift variant (NM_32709.2: c.1490dupC, p.(Val498Cysfs*79) from GS data. **(C)** RNAseq analysis confirmed the missense variant was expressed in the proband in approximately 50% of the observed reads; and **(D)** the frameshift variant was observed in the RNASeq data in both the proband and the mother in approximately 50% of the reads.

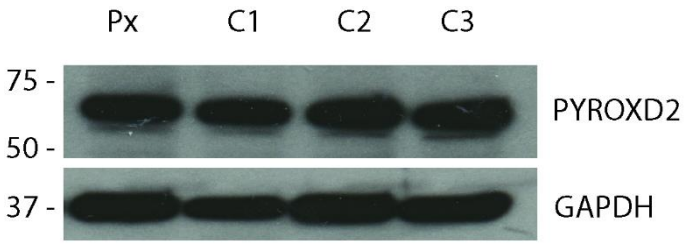


(**a**)

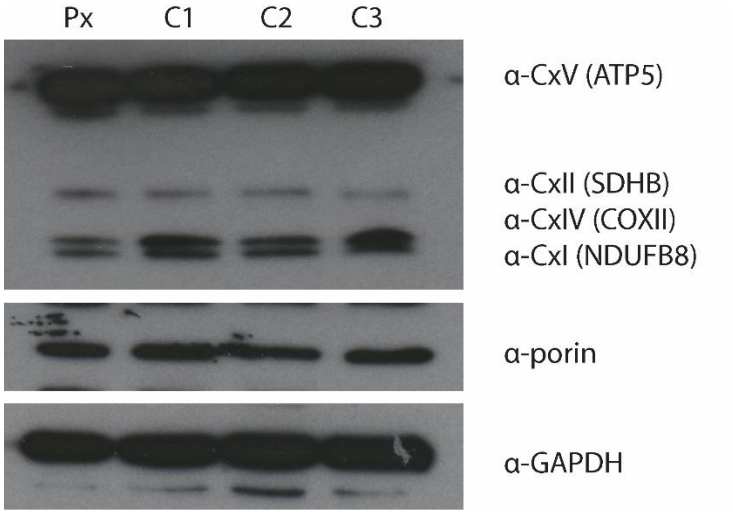


(**b**)

Supplemental Figure 2. RT-PCR and qPCR of *PYROXD2* in patient fibroblasts. (A) RT-PCR analysis shows similar levels of PYROXD2 transcript in patient and controls, with (+) or without (-) cycloheximide. **(B)** qPCR of PYROXD2 show no difference in PYROXD2 levels between two of the three controls (C1 - C3) and patient (Px) fibroblasts. * p <= 0.05.



Supplemental Figure 3. Western blot of PYROXD2 in patient fibroblasts. Western blot of PYROXD2 in patient fibroblasts (Px) and three controls (C1 - C3) showing normal levels of PYROXD2 protein.



Supplemental Figure 4: Western blot of OXPHOS complex subunits in patient fibroblasts. Western blot of a representative subunit of each OXPHOS complex including; cytochrome c oxidase subunit 2 [COX2], cytochrome b-c1 complex subunit 2 [UQCRC2], succinate dehydrogenase [ubiquinone] flavoprotein subunit B [SDHB], NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 8 [NDUFB8] and ATP synthase subunit alpha [ATP5A] in patient fibroblasts (Px) and three controls (C1 - C3) showing normal levels of OXPHOS subunit proteins.

References

1. Bernier, F.P.; Boneh, A.; Dennett, X.; Chow, C.W.; Cleary, M.A.; Thorburn, D.R. Diagnostic criteria for respiratory chain disorders in adults and children. *Neurology* **2002**, *59*, 1406-1411, doi:10.1212/01.wnl.0000033795.17156.00.