The American Journal of Human Genetics, Volume *90*

Supplemental Data

Mutations in *C12orf62*, a Factor that Couples COX I Synthesis with Cytochrome c Oxidase Assembly, Cause Fatal Neonatal Lactic Acidosis

Woranontee Weraarpachai, Florin Sasarman, Tamiko Nishimura, Hana Antonicka, Karine Auré, Agnès Rötig, Anne Lombès, and Eric A. Shoubridge

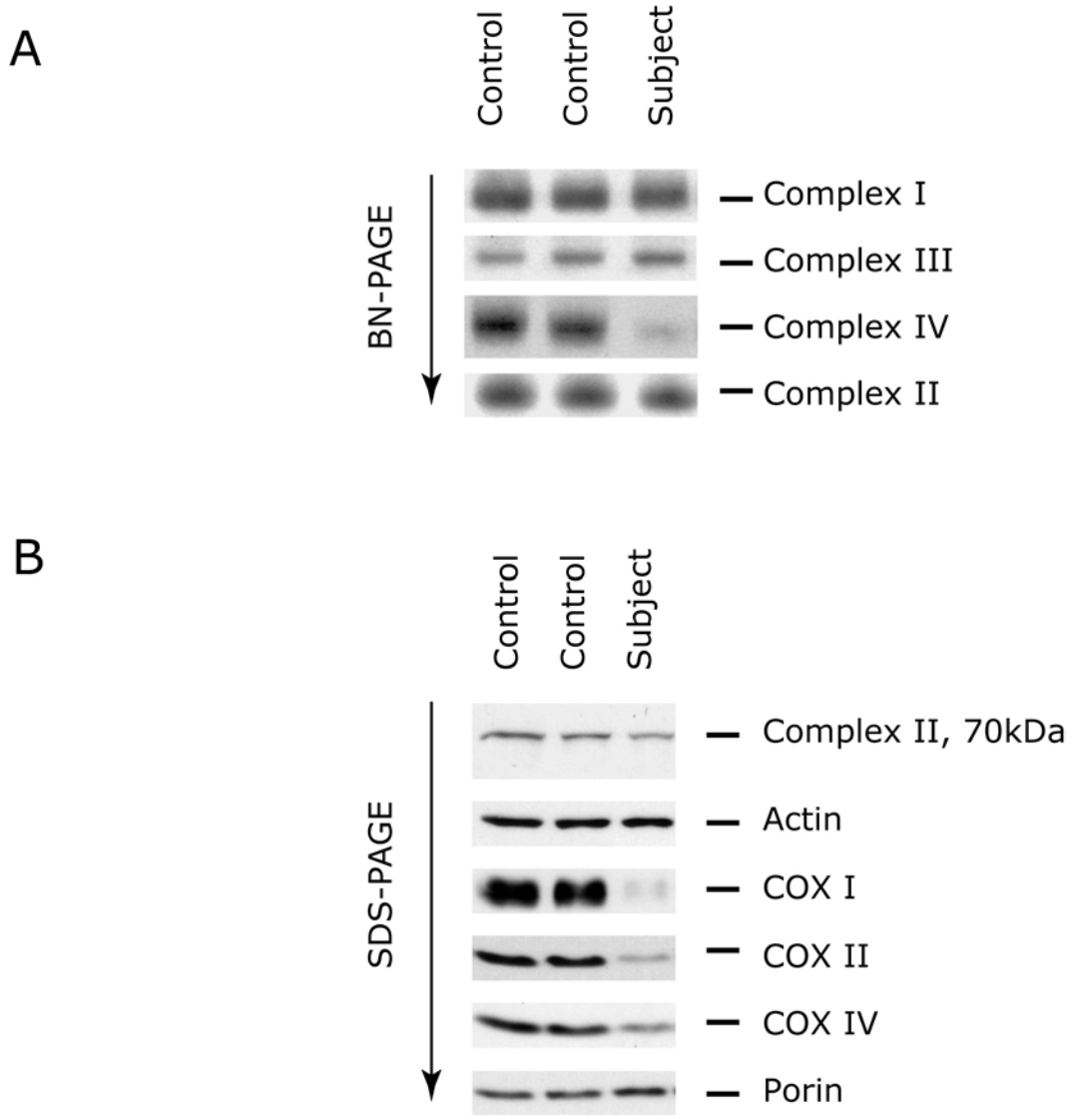

Figure S1. Characterization of the COX defect in subject fibroblasts

(A) BN-PAGE analysis of the OXPHOS complexes in the subject and two control fibroblast lines. Specific antibodies for subunits of the five OXPHOS complexes were used for immnoblotting. Complex II was used as a loading control.

(B) SDS-PAGE analysis using antibodies against COX subunit1 (COX I), COX subunit2 (COX II) and COX subunit 4 (COX IV). Isolated mitochondria were extracted with 50 mM HEPES buffer, pH7.6, 150 mM NaCl, 1% taurodeoxy cholate, containing complete protease inhibitors (Roche), and 80 μg of protein were run on 12.5% polyacrylamide gels followed by wet transfer to a nitrocellulose membrane, and immunoblot analysis with the indicated antibodies. The 70-kDa subunit of complex II, porin and actin were used as loading controls.

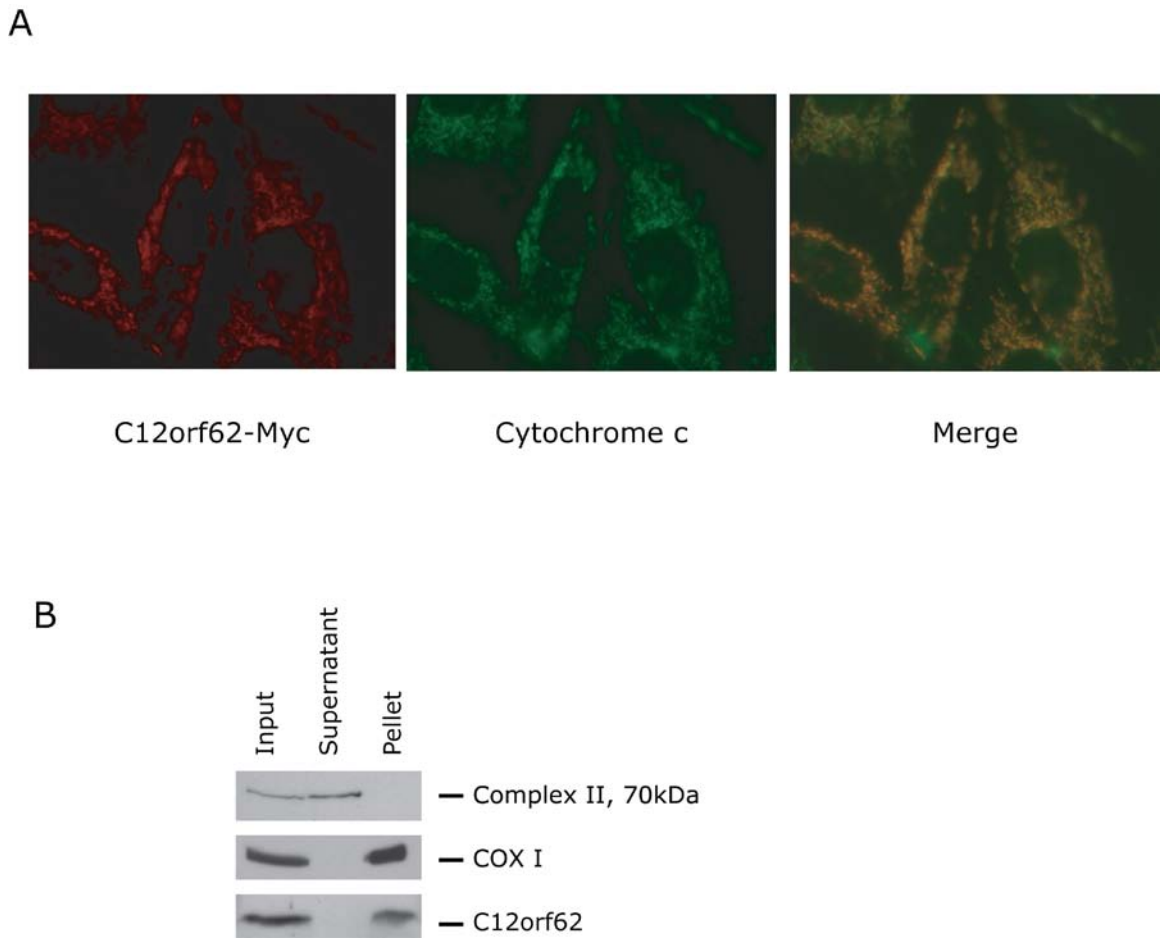

Figure S2. C12orf62 is a mitochondrial membrane protein

(A) Control fibroblasts overexpressing C12orf62-Myc were grown on coverslips for 24 hrs, fixed in paraformaldehyde, solubilized by Triton X-100, and incubated with anti-c-Myc (Sigma) and anti-cytochrome *c* (Santa Cruz Biotechnology) antibodies. Anti-mouse ALEXA Fluor 594 and anti-rabbit ALEXA Fluor 488 secondary antibodies (Invitrogen) were used for immunofluorescent detection.

(B) SDS-PAGE analysis of alkaline sodium carbonate extracts of mitochondria from HEK 293 cells. HEK 293 cells were resuspended in ice-cold 250 mM sucrose/10 mM Tris-HCl/1 mM EDTA (pH 7.4) and homogenized with 10 passes through a pre-chilled, zero clearance homogenizer (Kimble/Kontes, Vineland, NJ). A post-nuclear supernatant

was obtained by two consecutive centrifugations for 10 min at 600*g*. Mitochondria were pelleted by centrifugation for 10 min at 10000*g*, and washed once in the same buffer. Mitochondria from HEK 293 cells (200 μg) were further extracted with 100 mM sodium carbonate pH 11.5 as previously described [2] and the relevant fractions were analyzed by SDS–PAGE. COX I was used as a marker of an integral membrane protein, and complex II -70 kDa subunit as a marker for an inner membrane-associated protein.

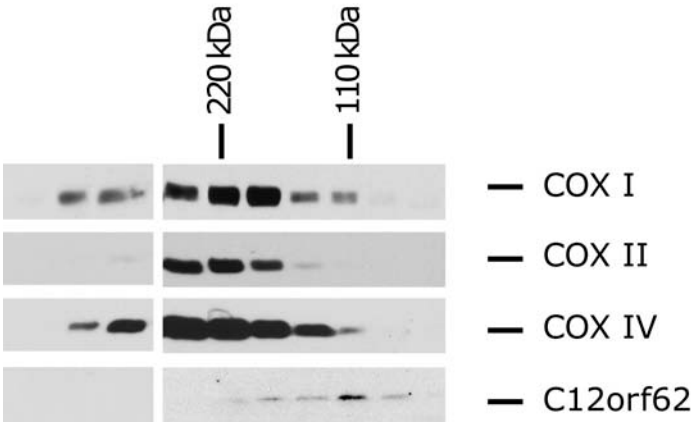

Figure S3. C12orf62 elutes in a high molecular weight complex of 110 kDa

Mitochondria from HEK 293 cells (800 μg) were extracted in 50 mM HEPES buffer, pH7.6, 150 mM NaCl, 1% taurodeoxy cholate and complete protease inhibitors (Roche). The extracted samples were separated on a Tricorn Superdex 200 10/30 HR column (GE Healthcare) as described [1] and each fraction was TCA-precipitated. Elution profiles of C12orf62 were determined by immunoblot analysis using antibodies against C12orf62.

Antibodies against COX I, COX II and COX IV were used to detect the elution pattern of the COX complex. The molecular weight of individual fractions was determined by the elution profile of a set of standards. The fully assembled COX (S4) appears at ~220 kDa.

Table S1. Clonal analysis of microcell-mediated transfer of all 22 autosomes into subject fibroblasts

Chromosome	Total Clones	Rescued clones (% total)
1	37	12 (32%)
2	36	4 (11%)
3	42	6 (14%)
4	13	2 (15%)
5	47	12 (26%)
6	48	1 (2%)
7	43	3 (7%)
8	40	2 (5%)
9	3	0
10	30	6 (20%)
11	6	0
12	24	4 (17%)
13	18	1 (6%)
14	12	0
15	18	1 (6%)
16	17	1 (6%)
17	5	0
18	12	0
19	38	4 (14%)
20	33	6 (18%)
21	17	2 (12%)
22	27	0

The total number of clones analyzed for each chromosome is indicated, as is the percentage of clones with greater than 60% of control COX activity.

Supplemental References

1. Kaufman BA, Durisic N, Mativetsky JM, Costantino S, Hancock MA, Grutter P, Shoubridge EA (2007) The mitochondrial transcription factor TFAM coordinates

the assembly of multiple DNA molecules into nucleoid-like structures. Mol Biol Cell 18:3225-3236

2. Yao J, Shoubridge EA (1999) Expression and functional analysis of SURF1 in Leigh syndrome patients with cytochrome c oxidase deficiency. Hum Mol Genet 8:2541-2549