The American Journal of Human Genetics

Supplemental Data

Mutations in *NDUFB11*, Encoding a Complex I Component of the Mitochondrial Respiratory Chain, Cause Microphthalmia with Linear Skin Defects Syndrome

Vanessa A. van Rahden, Erika Fernandez-Vizarra, Malik Alawi, Kristina Brand, Florence Fellmann, Denise Horn, Massimo Zeviani, and Kerstin Kutsche

Supplemental Data

Figure S1

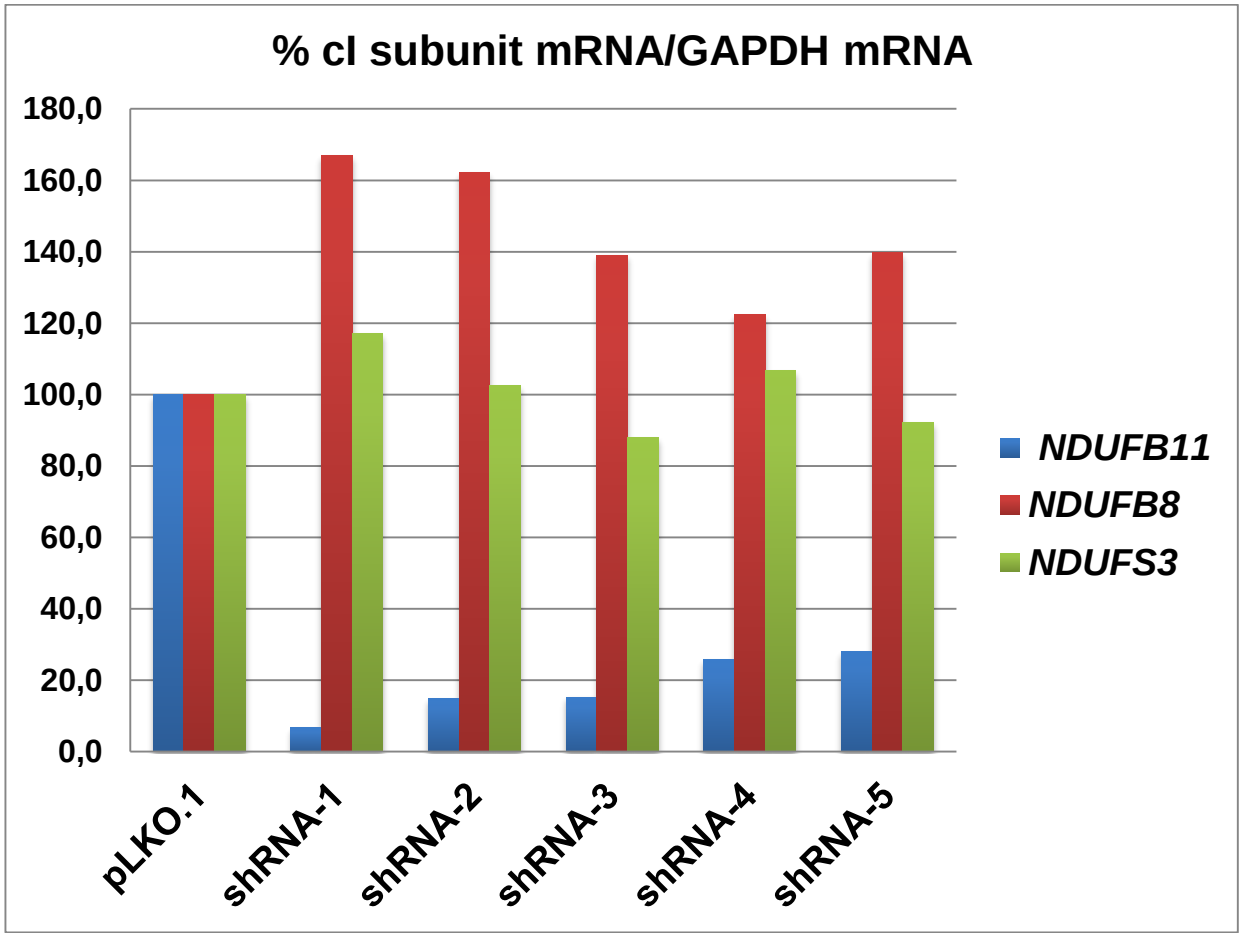

Figure S1: Quantitative PCR analysis of three complex I transcripts in *NDUFB11* knockdown and control HeLa cells

Values obtained from complex I transcripts specific to *NDUFB11*, *NDUFB8* and *NDUFS3* were normalized to the levels of *GAPDH* mRNA. The values obtained in *NDUFB11* shRNA-transduced (shRNA-1 to -5) cells are expressed as percentages relative to those obtained in cells transduced with the empty vector (pLKO.1). Each assay was performed in triplicate using TaqMan® Gene Expression Assays (Life Technologies) using cDNA generated from total RNA samples.

Figure S2

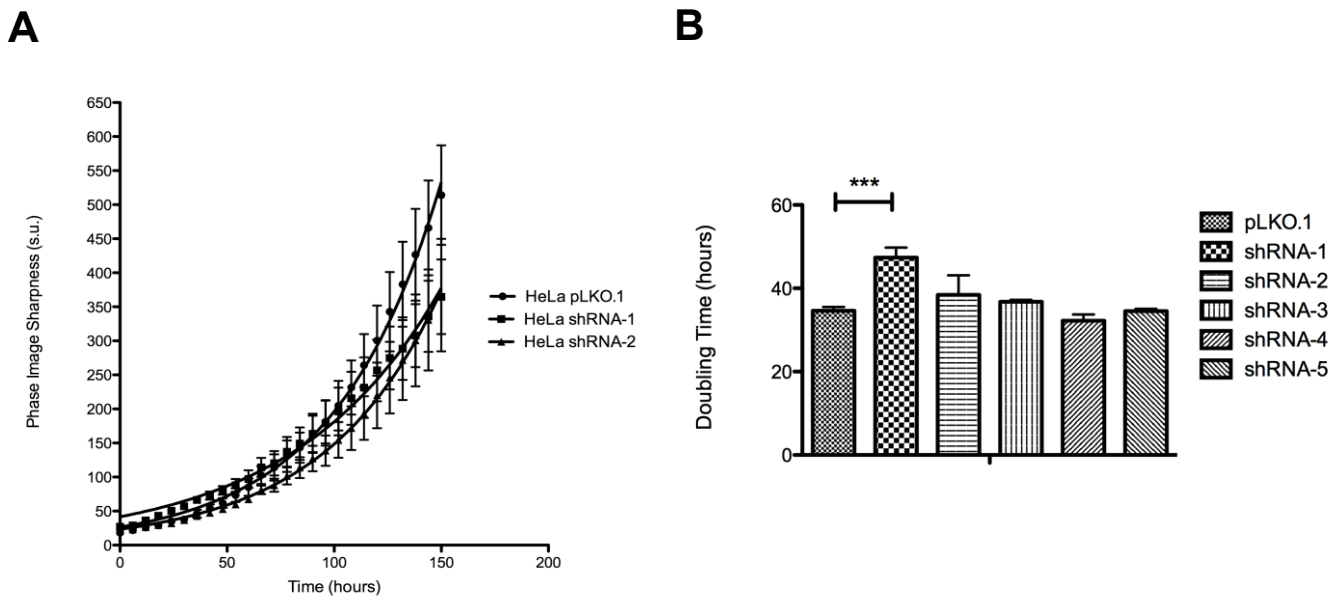

Figure S2: Growth curves of *NDUFB11* knockdown and control HeLa cells

(A) Growth curves of *NDUFB11* shRNA-transduced (shRNA-1 to -5) and control (pLKO.1) cells were obtained by IncuCyte ZOOM (Essen BioScience), which measured cell confluence every 6 hours for a period of 7 days. (B) Cell doubling time. For each HeLa cell line, four separate wells were measured. ***ANOVA $p<0.001$.

Table S1: Read depth for whole exome sequencing in subjects 1 and 2

	Subject 1	Subject 2	X chromosome (hg19)
Mean read depth across exome	97	99	
Mean read depth across *NDUFB11*	116	133	47,001,716 – 47,004,078
Mean read depth across *ARSH*	59	61	2,924,654 – 2,951,426
Mean read depth across *DGKK*	57	54	50,111,939 – 50,213,677
Mean read depth across *DCAF8L2*	442	459	27,765,013 – 27,766,908

Table S2: Private and rare X-linked sequence variants identified by whole exome sequencing in subjects 1 and 2

	Subject 1	**Subject 2**		
Chromosome	X	X	X	X
Position	47002089	47001806	2951233	50117973
Gene	*NDUFB11*	*NDUFB11*	*ARSH*	*DGKK*
Sequence variant	c.[=/262C>T]	c.402delG	c.1496A>G	c.3498G>T
Amino acid change	p.[=/Arg88*]	p.Arg134Serfs*3	p.Asn499Ser	p.Lys1166Asn
Variant type	nonsense	1 bp deletion (frameshift)	missense	missense
Sanger sequencing				
patient	+	+	+	+
mother	–	+	+	+
father	–	–	–	–
Pathogenicity prediction programs				
MutationTaster	disease causing	disease causing	polymorphism	na
SIFT	na	na	tolerated	tolerated
SNAP	na	na	neutral	neutral
PolyPhen-2	na	na	benign	benign
OMIM disorder	no	no	no	MIM 300856: major risk gene for hypospadias
1000 Genomes	absent	absent	absent	absent
EVS	absent	absent	absent	absent
ExAc	absent	absent	absent	MAF: 2.726e-05

X-chromosomal sequences were mapped to hg19 and positions of the sequence variants are given. Sequence variants were analyzed by the MutationTaster, SIFT, PolyPhen-2, and SNAP programs to predict a possible impact on protein function. When the program offered a choice of parameter settings, defaults were used. *Tolerated*, *polymorphism*, *benign* and *neutral* indicate that the change on protein level is unlikely to affect protein function. *Disease causing* indicates a likely impact of the amino acid alteration on protein function.
+ : variant present; – : variant absent; EVS: the National Heart, Lung, and Blood Institute (NHLBI) Exome Sequencing Project Exome Variant Server; ExAC: Exome Aggregation Consortium Browser; MAF: minor allele frequency; na: not applicable.

Table S3: Mitochondrial respiratory chain (MRC) activities in cultured skin fibroblasts of subject 1

	Activity normalized to citrate synthase (CS) activity	
MRC complex	Fibroblasts of subject 1	Fibroblast control range
cI (NADH:CoQ1 oxidoreductase)	15.6 ± 5.4	10.3 - 22
cII (Succinate dehydrogenase)	13.4 ± 7.6	5.5 - 10.9
cII (Succinate:CoQ1 oxidoreductase)	23.7 ± 10.4	8.8 - 16.9
cIII (Decylubiquinol:cytochrome *c* oxidoreductase)	12.9 ± 5.2	9.2 - 17.5
cIV (cytochrome *c* oxidase)	23.9 ± 7.8	23.5 - 26.4

Measures were performed in triplicate in each of two biological replicates.

Table S4: MRC activities in *NDUFB11* shRNA-1 cells in comparison with control cells (HeLa pLKO.1)

	Activity referred to citrate synthase (CS) activity	
MRC complex	*NDUFB11* shRNA-1 cells	HeLa pLKO.1 control range
cI (NADH:CoQ1 oxidoreductase)	1.4	10.5 - 12.5
cII (Succinate dehydrogenase)	8.1	2.3 - 7.8
cII (Succinate:CoQ1 oxidoreductase)	16.0	7.7 - 16.1
cIII (Decylubiquinol:cytochrome *c* oxidoreductase)	12.3	14.3 - 25.5
cIV (cytochrome *c* oxidase)	23.3	25.6 - 27.8

Measures in shRNA-1 HeLa cells were performed in triplicate in one sample. Measures in HeLa pLKO.1 cells were performed in triplicate in two different samples.