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Supplemental Data

Biallelic *IARS* Mutations Cause Growth Retardation with Prenatal Onset, Intellectual Disability, Muscular Hypotonia, and Infantile Hepatopathy

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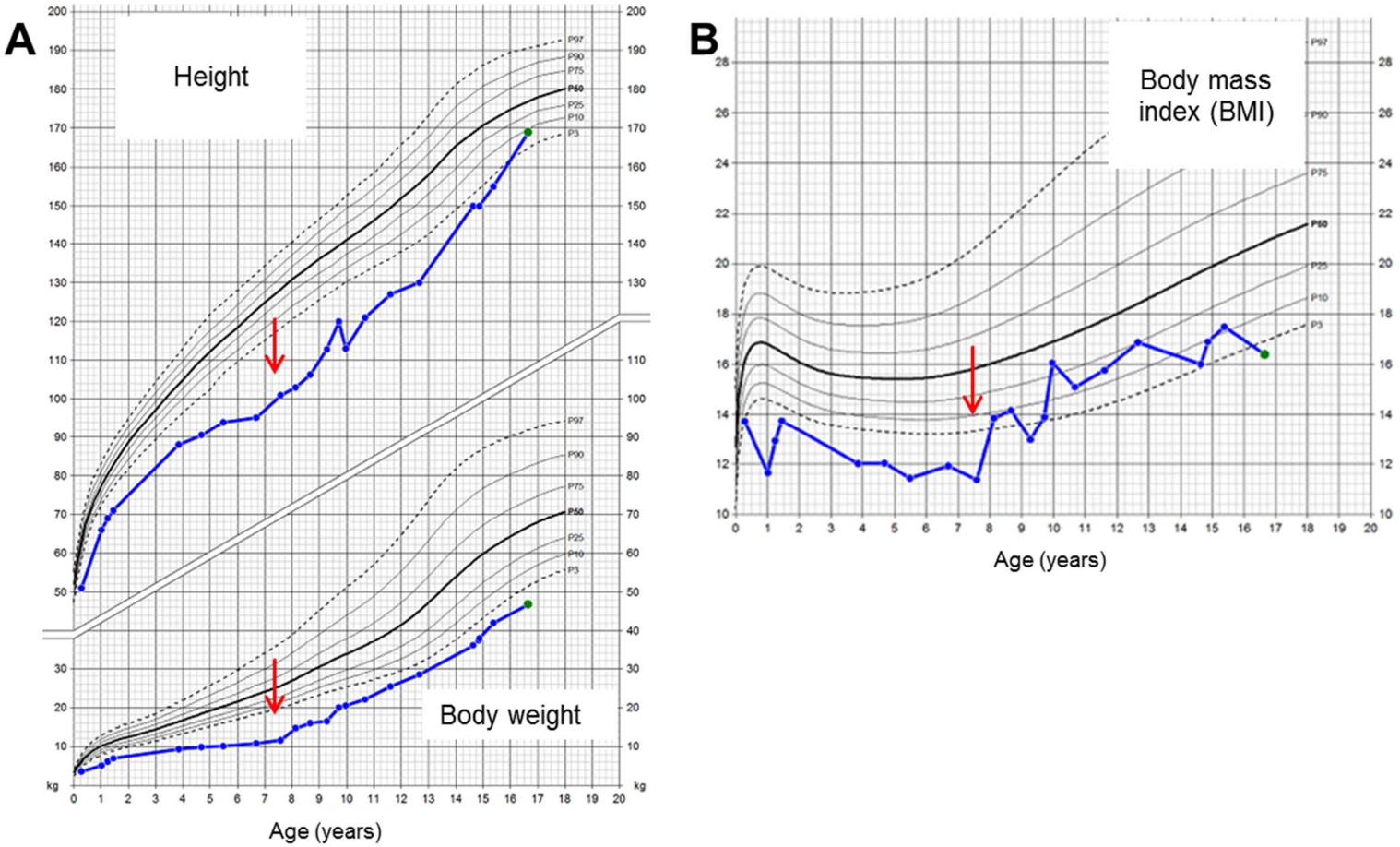
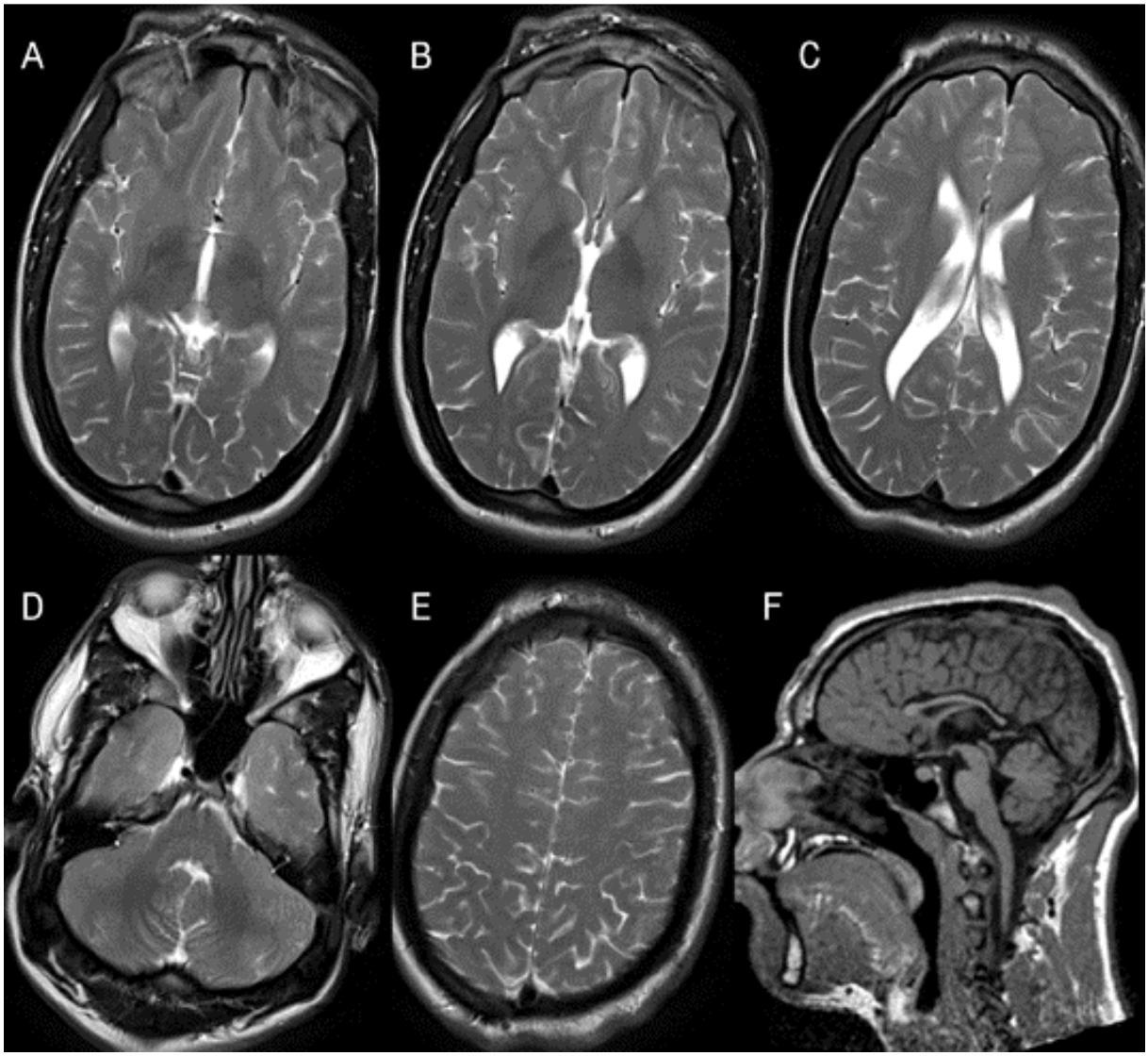
Figure S1. Growth curves, individual #65269 (DEU)



Curves of height (top) and body weight (bottom) (A) and body mass index (BMI) (B) of subject #65269 (DEU). Severe growth retardation and failure to thrive were at their worst at 7 years of age, when zinc deficiency was detected and supplementary treatment with 10mg zinc/day was initiated (0.9 mg/kg body weight/day; red arrow). After zinc supplementation began, the patient gained 3 kg in the first six months and BMI rose from 11.4 kg/m² (-4.2 SDS) to 13.9 kg/m² (-1.5 SDS).

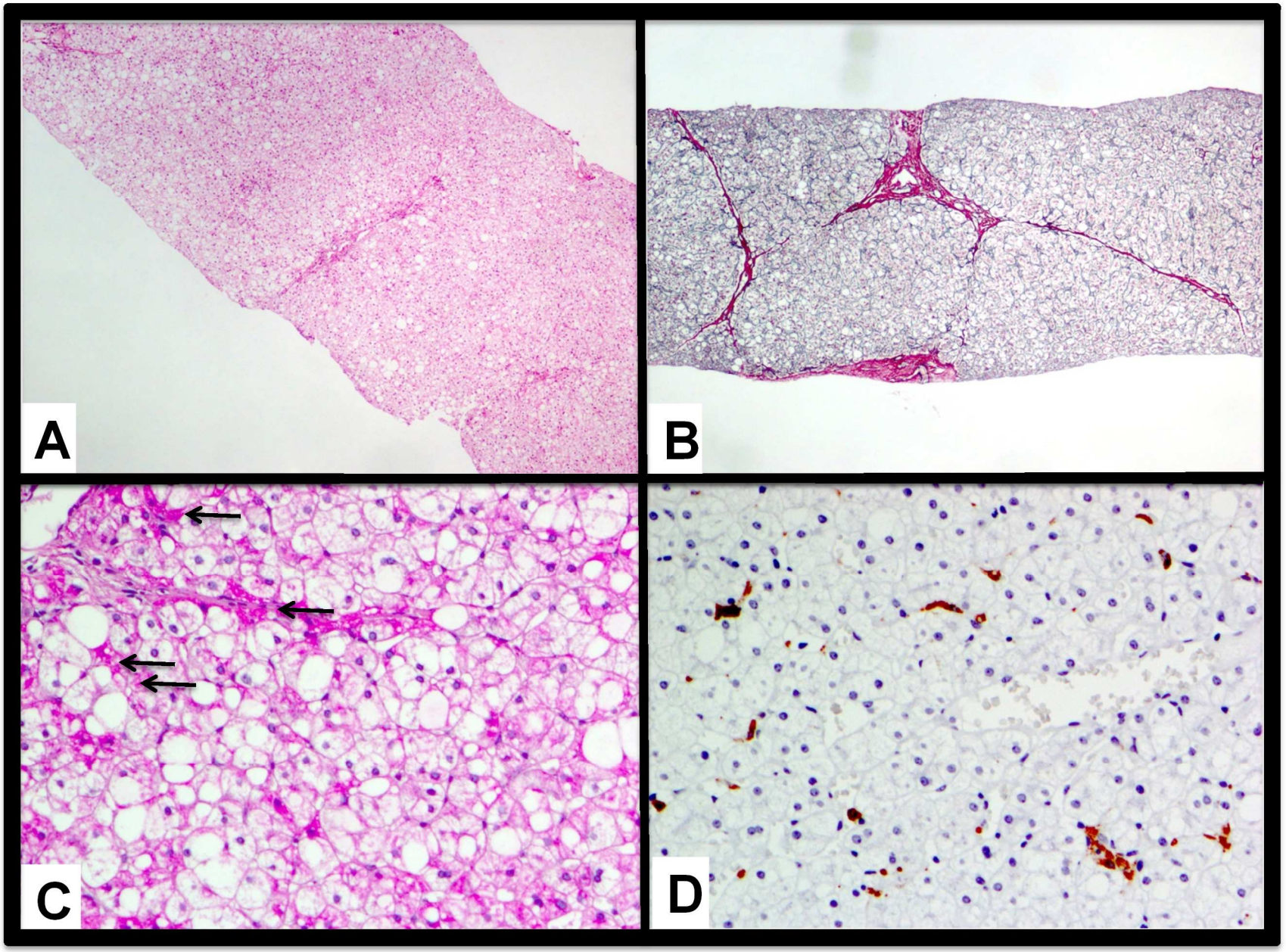
Figure S2. Brain magnetic-resonance images, individual #65269 (DEU), age 17 years



A-E: T2-weighted axial images depicting hazy T2-hyperintensity of supratentorial white matter with sparing of the subcortical temporal (D) and frontal (A, B) white matter, which has the normal hypointense signal of myelinated white matter. The supratentorial corticospinal tract is involved from the Rolandic area (E) through the corona radiata and posterior limb of the internal capsule (B, C). A thin periventricular rim is spared (B, C). Microcephaly is present, with a neurocranium disproportionately small compared with the viscerocranium on the midsagittal T1-weighted image (F). There is a deficit of supratentorial white matter with widened lateral ventricles (C) and a thin corpus callosum (F).

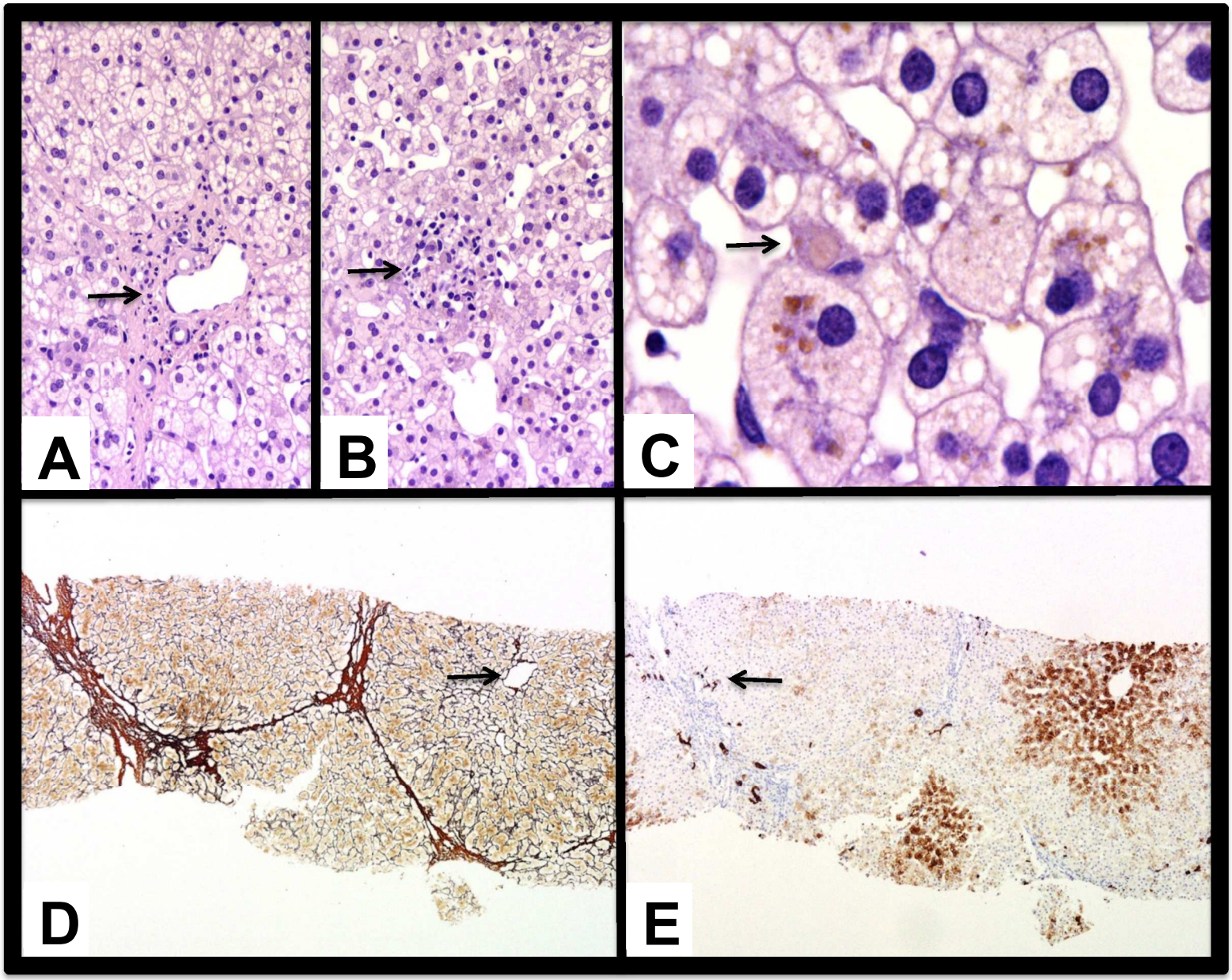
Brain images of #85880 (JPN) and #83921 (AUT) have been evaluated together with those of individual #65269 (DEU). They were normal at the respective ages of 16 and 2 years.

Figure S3. Liver biopsy, individual #85880 (JPN), age 2 1/12 years



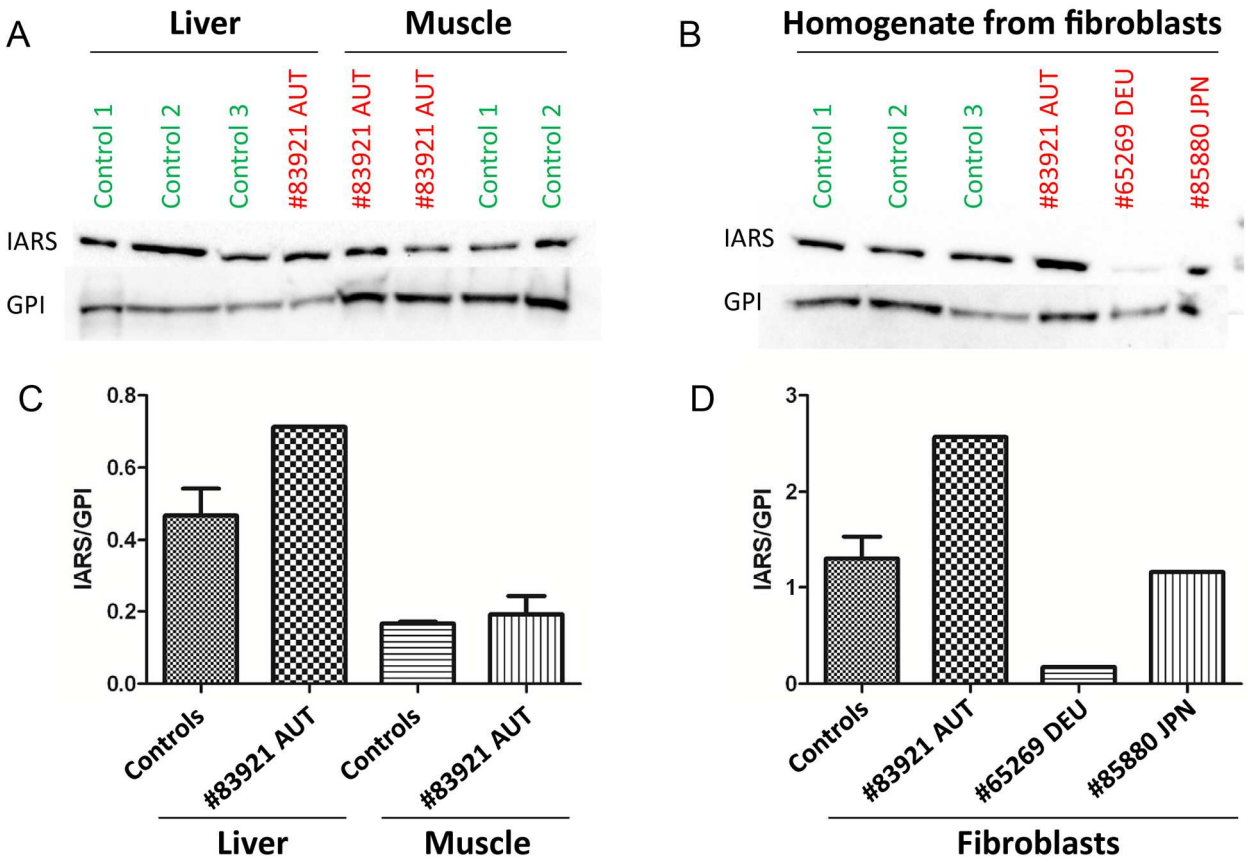
Steatosis and portal-tract fibrosis with evidence of accelerated hepatocyte turn-over in absence of other usual histopathologic features of hepatitis or steatohepatitis. Lymphoplasmacytic or granulocytic inflammation is not observed; ballooning and Mallory-Denk bodies are not seen. Cholestasis, copper accumulation, and siderosis are not found. (A) Hepatocyte pallor and delicate bridging fibrosis; inflammation is not a feature. Hematoxylin / eosin, original magnification 40x. (B) Fibrosis with bridging. Reticulin, original magnification 40x. (C) Small- and large-droplet steatosis displaces purplish glycogen within cytoplasm of hepatocytes. Purplish ceroid pigment is seen in some Kupffer cells and macrophages (arrows). Periodic acid - Schiff reaction / hematoxylin, original magnification 200x. (D) Clusters of dark brown Kupffer cells correspond to ceroid-laden cells in C, above; such accumulations mark sites of cell death and phagocytic response. Anti-macrosialin / -CD68 antibody – diaminobenzidine / hematoxylin, original magnification 200x.

Figure S4. Liver biopsy, individual #83921 (AUT), age 1 2/12 years



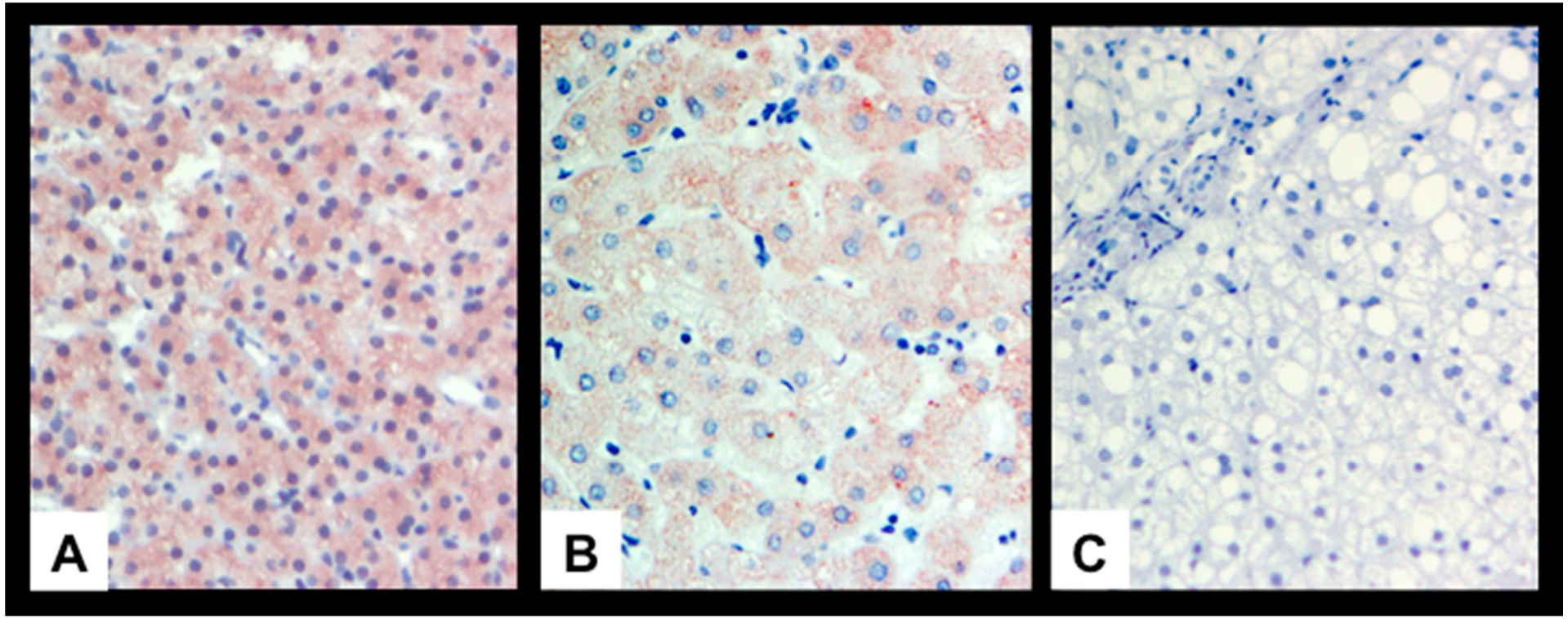
Cholestasis, steatosis with steatohepatitis, portal-tract fibrosis with bridging in biliary rather than post-necrotic pattern, and evidence of accelerated hepatocyte turn-over. Lymphoplasmacytic or granulocytic inflammation is not observed; ballooning and Mallory-Denk bodies are not seen. Copper accumulation and siderosis are not found. (A) and (B) Hepatocyte pallor and vacuolation. A portal tract (arrow, A) is free of inflammation, but a focus of mononuclear (phagocytic) response to necrosis (arrow, B) lies near a draining venule. Hematoxylin / eosin (H&E), original magnification 200x. (C) Hepatocellular steatosis; a Kupffer cell containing ceroid pigment is noted (arrow). Hepatocyte cytoplasm contains flecks of bile pigment. H&E, original magnification 1,000x. (D) Moderate and mild portal-tract fibrosis with extensive portal-portal bridging fibrosis. A draining venule (arrow) is unremarkable. Reticulin, original magnification 40x. (E) Dark brown neocholangioles (“ductular reaction”) highlighted as expressing cytokeratin (CK) 7 are seen at margins of damaged portal tracts (arrow). Heterotopic expression of CK7 by hepatocytes, a feature of chronic cholestasis, is widespread. Anti-CK7 antibody – diaminobenzidine / hematoxylin, original magnification 40x.

Figure S5. Immunoblotting analysis of liver and muscle (individual #83921, AUT) and fibroblasts (individual #83921, AUT; individual #65269, DEU; individual #85880, JPN)



Western blot analysis with 600 g supernatants from (A) liver and muscle and (B) homogenates from fibroblasts was performed with antibodies against IARS (Anti-IARS, Rabbit; Biomol, Cat.-No. A304-748A-T) and GPI (glucose-6-phosphate isomerase) as loading control. Quantitative analysis (C, D) revealed a normal relative amount of IARS/ GPI in all samples of individuals #83921 (AUT) and #85880 (JPN), while a decreased amount of IARS protein was found in fibroblasts of #65269 (DEU).

Figure S6. Immunostaining of liver-biopsy materials from individual #83921 (AUT) and individual #85880 (JPN) for IARS



(A) AUT. Hepatocyte cytoplasm marks diffusely. (B) A liver control. Marking is apparent in both A and B; variation in intensity is apparent, and is likely multifactorial. (C) JPN. No marking for IARS is seen. Anti-IARS / hematoxylin, original magnification 200x, all images.

SUPPLEMENTAL TABLES

Table S1. Anthropometrical data

			at birth			at one year			at six years			at current age			
ID	sex	gestational age at birth (weeks)	length (cm/SDS)	weight (g/SDS)	head circumference (cm/SDS)	height (cm/SDS)	weight (kg/SDS)	head circumference (cm/SDS)	height (cm/SDS)	weight (kg/SDS)	head circumference (cm/SDS)	age	height (cm/SDS)	weight (kg/SDS)	head circumference (cm/SDS)
#65269 (DEU)	m	38	45/-2.7	2020 / -3.0	29 / -4.2	66 / -4.4	5.1 / -4.7	38 / -8.1	95.2 / -5.9	10.8 / -8.0	42 / -7.4	18 y	169 / -1.6	50.0 / -2.8	48.5 / -5.1
#85880 (JPN)	f	38	42/ -2.7	1564 / -4.1	29 / -3.5	58 / -6.2	4.5 / -4.2	38 / -4.1	100.0 / -2.8	16.0 / -1.2	n.a.	19 y	155 / -0.6	46.2 / -0.9	“normal”
#83921 (AUT)	m	38+4	48/-1.6	2700 / -1.6	30 / -3.8	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	3y	82 / -5.0	11.5 / -2.0	47 / -3.3

f, female; m, male; SDS, standard deviation score; n.a., not available

Table S2. OXPHOS activities

ID	RCC	% of lowest control / absolute values / reference range
#65269 (DEU)	**Muscle**	
	I	**77%** / 65 mU/U CS / 84-273
	II	n.a.
	II+III	normal / 9.7 mU/mg protein / 4.2-29
	IV	**52%** / 270 mU/U CS / 520-2080
	PDH	**64%** / 18.7 mU/U CS / 29-89
	Liver	not done
	Fibroblasts	all normal
#85880 (JPN)	**Muscle**	not done
	Liver	not done
	Fibroblasts	
	I	**73%** / 196 mU/U CS / 267-792
	II	normal / 423 mU/U CS / 299-1162
	II+III	normal / 381 mU/U CS / 346-1281
	III	normal / 144 mU/U CS / 22-281
	IV	normal / 14.0 mU/U CS / 14-64
	PDH	n.a.
#83921 (AUT)	**Muscle**	all normal, including PDH
	Liver	
	I	**46%** / 70 mU/U CS / 150–370
	II	normal / 970 mU/U CS / 780-1172
	I+III	normal / 540 mU/U CS / 260-610
	III	normal / 6950 mU/U CS / 1670-5170
	IV	normal / 3210 mU/U CS / 880-1580
	V	normal / 1810 mU/U CS / 350-1340
	PDH	n.a.
	Fibroblasts	not done
weak calf syndrome (n=6)	**Muscle**	
	I	normal / 190 (153-274) mU/U CS / 77-512
	II	normal / 146 (66-233) mU/U CS / 66-450
	II+III	normal / 239 (103-233) mU/U CS / 88-584
	III	normal / 50 (24-79) mU/U CS / 15-100
	IV	normal / 41 (32-51) mU/U CS / 11-72
	PDH	n.a.
	Liver	
	I	normal / 1134 (665-1598) mU/U CS / 299-1494
	II	normal / 2232 (1634-2678) mU/U CS / 465-2324
	III	normal / 326 (202-555) mU/U CS / 133-332
	IV	normal / 258 (166-359) mU/U CS / 26-130
	PDH	n.a.
	Fibroblasts	
	I	normal / 342 (240-449) mU/U CS / 109-544
	II	normal / 477 (141-954) mU/U CS / 127-638
	II+III	normal / 532 (450-84) mU/U CS / 339-1696
	III	normal* / 43 (13-75) mU/U CS / 19-94
	IV	normal / 57 (49-69) mU/U CS / 19-94
	PDH	n.a.
		* In one IARS calf complex III activity was low (13 mU/U CS)

Table S3. Zinc levels, weak calf syndrome and controls

Age	Plasma zinc in µg/dL - affected calves	n	Plasma zinc in µg/dL - healthy calves	n
0-3 months	139.3 (SD 60.7)	4	116.6 (SD 21.4)	10
4-9 months	93.6 (SD 19.5)	7	112.2 (SD 20.7)	10
>12 months	109.4 (SD 10.6)	5		
Age unknown	107.0 (SD 24.0)	2		