The American Journal of Human Genetics, Volume *100*

Supplemental Data

Mutations in *ATP6V1E1* or *ATP6V1A* Cause Autosomal-Recessive Cutis Laxa

Tim Van Damme, Thatjana Gardeitchik, Miski Mohamed, Sergio Guerrero-Castillo, Peter Freisinger, Brecht Guillemyn, Ariana Kariminejad, Daisy Dalloyaux, Sanne van Kraaij, Dirk J. Lefeber, Delfien Syx, Wouter Steyaert, Riet De Rycke, Alexander Hoischen, Erik-Jan Kamsteeg, Sunnie Y. Wong, Monique van Scherpenzeel, Payman Jamali, Ulrich Brandt, Leo Nijtmans, G. Christoph Korenke, Brian H.Y. Chung, Christopher C.Y. Mak, Ingrid Hausser, Uwe Kornak, Björn Fischer-Zirnsak, Tim M. Strom, Thomas Meitinger, Yasemin Alanay, Gulen E. Utine, Peter K.C. Leung, Siavash Ghaderi-Sohi, Paul Coucke, Sofie Symoens, Anne De Paepe, Christian Thiel, Tobias B. Haack, Fransiska Malfait, Eva Morava, Bert Callewaert, and Ron A. Wevers

Supplemental Note. Case Reports

Family I

PI:1 was born as the first child of healthy first cousin Iranian parents. She was born at 39 weeks of gestation after an uneventful pregnancy and normal vaginal delivery. Birth weight (3700 grams), length (52 cm) and occipitofrontal circumference (OFC; 36 cm) were all within normal range. She had severe and generalized cutis laxa, and presented with multiple dysmorphic features including a triangular and progeroid face, a short forehead, overfolded ear helices, hypertelorism, entropion, a prominent beaked nose, a broad nasal tip and columella with narrow nostrils, a long philtrum, an open mouth and a short and pointed chin. Additional features included hypotonia, clenched hands, ulnar deviation of the fingers, congenital hip dysplasia (Graf type 4 on ultrasound), flexion contractures of the knees and club feet. Echocardiography showed a severe dilatation of the ascending aortic root and mild to moderate biventricular hypertrophy. Abdominal ultrasound was normal. She passed away at the age of four months.

First trimester ultrasound in a second pregnancy showed increased nuchal thickness and cardiac abnormalities, but karyotyping of amniocytes was normal (46, XX). The pregnancy was terminated at 21 weeks of gestation. The female fetus (PI:2) had a low weight for gestational age, hypertelorism, low-set ears, increased nuchal thickness and a right hypoplastic heart syndrome with a small and hypoplastic right ventricle, tricuspid valve stenosis and a hypoplastic pulmonary artery.

Family II

PII:1 is a 10-year-old Kuwaiti girl and the first child of a second degree, double consanguineous couple. She was born at term (37 + 2/7 weeks) with a normal birth weight (3430 grams). Shortly after birth she developed severe respiratory insufficiency secondary to bilateral pneumothorax. Her skin was very wrinkled with excessive skin folds and she also presented with bilateral entropion and hip dysplasia. The entropion was surgically corrected at the age of five months and she had surgery for the hip dysplasia at the age of 18 months. She was last seen by a clinical geneticist at the age of ten years and seven months. She suffered from disabling muscular hypotonia and was unable to climb stairs or walk long distances. Clinical examination showed an alert, lean and slender girl. Body length and weight were 134 cm (3^{rd} - 10^{th} percentile) and 23.5 kg (below 3^{rd} percentile), respectively. Her skin was wrinkled with some excessive skin folds, but not fragile. She had mild scoliosis, pedes planovalgi, hypermobile joints and marked muscular atrophy. Her facial gestalt was characterized by a long and triangular face, overfolded ear helices, hypertelorism, deepset eyes, discrete nystagmus, a broad and high nasal root, dental crowding, a highly arched palate, prominent nasolabial folds and a short and pointed chin. Intelligence was normal. Echocardiography at the age of ten years showed mild dilatation of the right ventricle in the absence of pulmonary hypertension, an atrial septal defect, mitral valve prolapse with grade I-II mitral insufficiency, grade I aortic and tricuspid insufficiency and reduced diastolic compliance, but no cardiomyopathy or aortic dilatation. ECG showed an incomplete right bundle branch block. Cerebral MRI at the ages of two and eight years and abdominal ultrasound

at the age of ten years were all normal. An X-ray of the left hand showed an age-appropriate bone age, undermineralization, overgrowth of the ulnar styloid processus, mild hypertubulation of the phalanges and sharp demarcation of their metaphyseal endplates. Laboratory results showed normal blood count, kidney function, electrolytes and hemostasis, but increased AST (109 U/L, reference value: 11-50 U/l), ALT (71 U/l, 7-31 U/l), LDH (367 U/l, 143-290 U/l), alkaline phosphatase (765 U/l, 69-325 U/l) and creatine kinase (532 U/l, 36-219 U/l; CK). Plasma concentrations of phenylalanine and tyrosine were normal. An extended neurometabolic screening using tandem mass spectrometry was unremarkable.

Her brother (PII:2) presented with a very similar phenotype. He was born with cutis laxa, scoliosis, laryngomalacia, entropion, bilateral cryptorchidism, bilateral inguinal hernias and bilateral pneumothorax. At the age of seven and eight years, he had rodding surgery to correct a progressive scoliosis. He had severe muscle hypoplasia, preventing him from walking distances over 500 m or climbing stairs. His intelligence was normal. At the age of nine and a half years he measured 122 cm (below 3rd percentile) and weighed 18.6 kg (below 3rd percentile). He had a slender habitus, very soft and excessive skin, very little subcutaneous fat, prominent muscle hypoplasia and severely reduced muscle force. He also presented with a pronounced dextroconvex scoliosis, chest deformity and pedes plani. His facial appearance was remarkable due to a triangular face, hypertelorism, entropion, nystagmus, overfolded ear helices, a beaked and prominent nose with a high nasal root and broad nasal tip and columella, and narrow nostrils, prominent nasolabial folds, a short and pointed chin, an open mouth, a highly arched palate, malpositioned teeth and temporomandibular joint dysfunction with recurrent dislocations. Echocardiography and abdominal ultrasound were normal. An X-ray of the left hand showed an age-appropriate bone age, undermineralization, sharp delineation of the metaphyses and overgrowth of the ulnar styloid processus with delayed ossification and mild flaring of the ulnar epiphysis. Routine biochemical analysis revealed elevated serum levels of AST (86 U/l, 11-50 U/l), ALT (59 U/l, 7-40 U/l), LDH (351 U/l, 143-290 U/l), CK (426 U/l, 36-219 U/l) and alkaline phosphatase (549 U/l, 86-315 U/l). The plasma concentration of glycine was slightly elevated, but phenylalanine and tyrosine concentrations were normal. Urinary amino acid and organic acid concentrations were normal and homocystinuria was excluded. An extended neurometabolic screening using tandem mass spectrometry was unremarkable.

Family III

PIII:1 was born as the first child of non-consanguineous German parents. His clinical phenotype was reported earlier.[1] In summary, he was referred for metabolic and genetic work-up because of severe neonatal cutis laxa and an abnormal serum glycosylation pattern (Table S3). In addition, he presented with dysmorphic facial features, including downslanting palpebral fissures, large and prominent ears, bilateral cataract, an abnormal fat distribution and hypotonia. He failed to thrive with postnatal growth restriction and a microcephaly. Cerebral MRI showed enlarged ventricles with white matter involvement and periventricular parieto-occipital gliosis. He has a developmental delay affecting both

cognitive and motor performance. At the age of four years he developed hypertrophic cardiomyopathy, which has remained stable. He was last evaluated at the age of 14 years. He was normocephalic (25^{th} percentile), and body length and weight were on the 75^{th} percentile and 50^{th} percentile, respectively. The severe, inelastic, generalized sagging skin improved over time, but no improvement occurred in the lipodystrophy. A few months later, he suffered a generalized tonic-clonic seizure.

Family IV

PIV:1 was born as the third child of healthy, consanguineous parents of Pakistani descent. His two older sisters were healthy. Prenatal ultrasound showed bilateral cerebral ventriculomegaly, a ventricular septal defect and overriding aorta. After a spontaneous delivery, he had an irregular breathing pattern and required respiratory support. His birth weight (3745 grams) was within normal limits. His skin was lax and wrinkled. He had hypotonia and reduced spontaneous leg movements. Multiple dysmorphic features were present: he had a bulbous nose with a broad nasal bridge, simple folded auricles and a receding chin. There was a suspicion of a submucosal cleft palate. In addition, he presented with severe bilateral club feet, dislocation of the hips, flexion contractures of all joints, camptodactyly and bilateral simian creases. He had a micropenis, cryptorchidism and a right inguinal hernia. Echocardiography showed a small atrial septal defect and a dilated ascending aorta with a tortuous aortic arch. The neonatal course was further complicated by progressive cardiac failure, pneumonia, sepsis and a 36 misnute resuscitation following dislocation of an endotracheal tube. Shortly after birth, he developed epileptic seizures, which evolved to generalized and complex partial seizures at the age of two months. Initial EEGs showed a burst suppression pattern, which later evolved to hypsarrhythmia. Cerebral MRI revealed diffuse thickening of the cerebral cortex, most prominent in frontal lobes and was suggestive of polymicrogyria. The myelination pattern was abnormal and the corpus callosum thin. A basic metabolic screen did not show any abnormalities. The child passed away after a long period of intensive treatments.

Family V

This male child (PV:1) was born to consanguineous parents of Turkish descent. Laxity of the skin with extensive folding was noted at birth. He had a triangular face with hypertelorism, blepharophimosis, entropion, a bulbous nose and a small receding chin. Cardiac ultrasound revealed a ventricular septal defect. MRI brain showed an anatomic variant of the cavum septum pellucidum. Ophthalmologic evaluation revealed paucity of the optic disk. He was not hypotonic, but CK levels were increased.

Figure S1. Clinical phenotype of PI:2 (termination at 21 weeks of gestation)

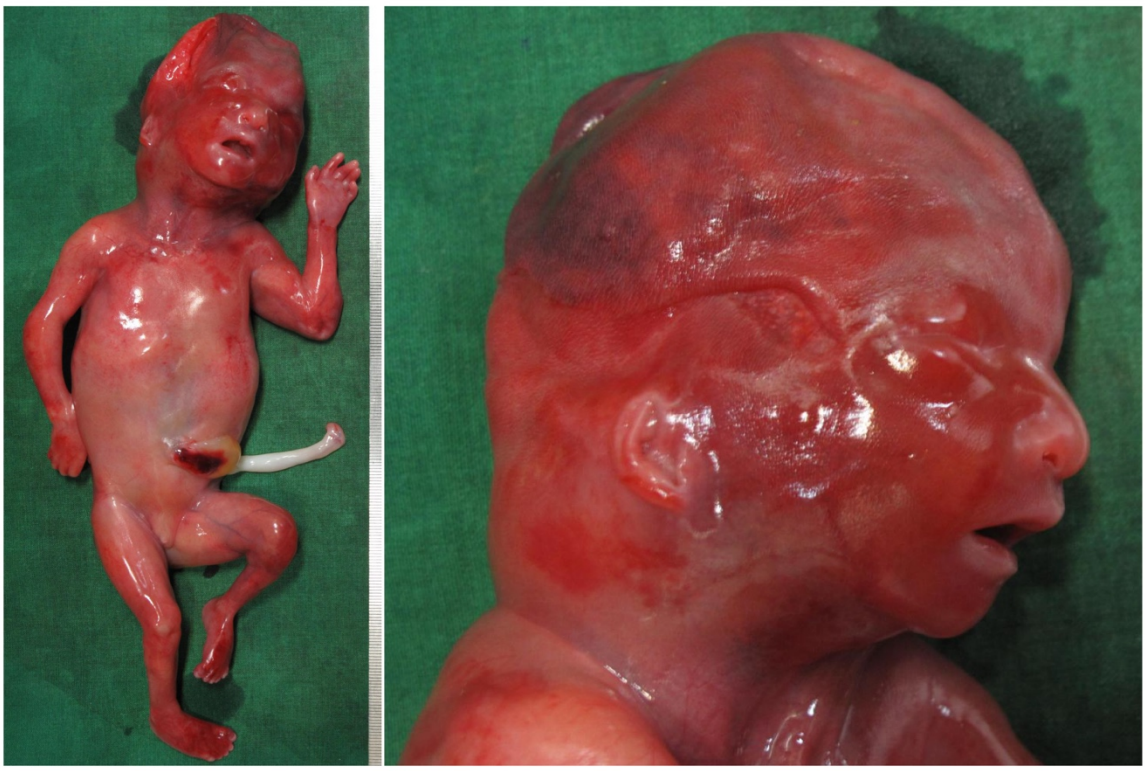
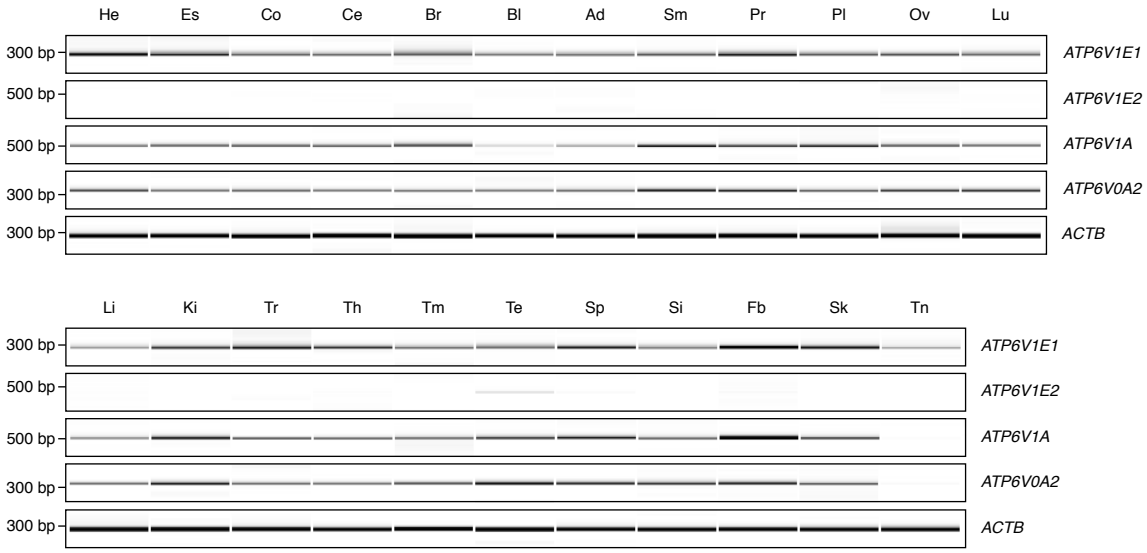
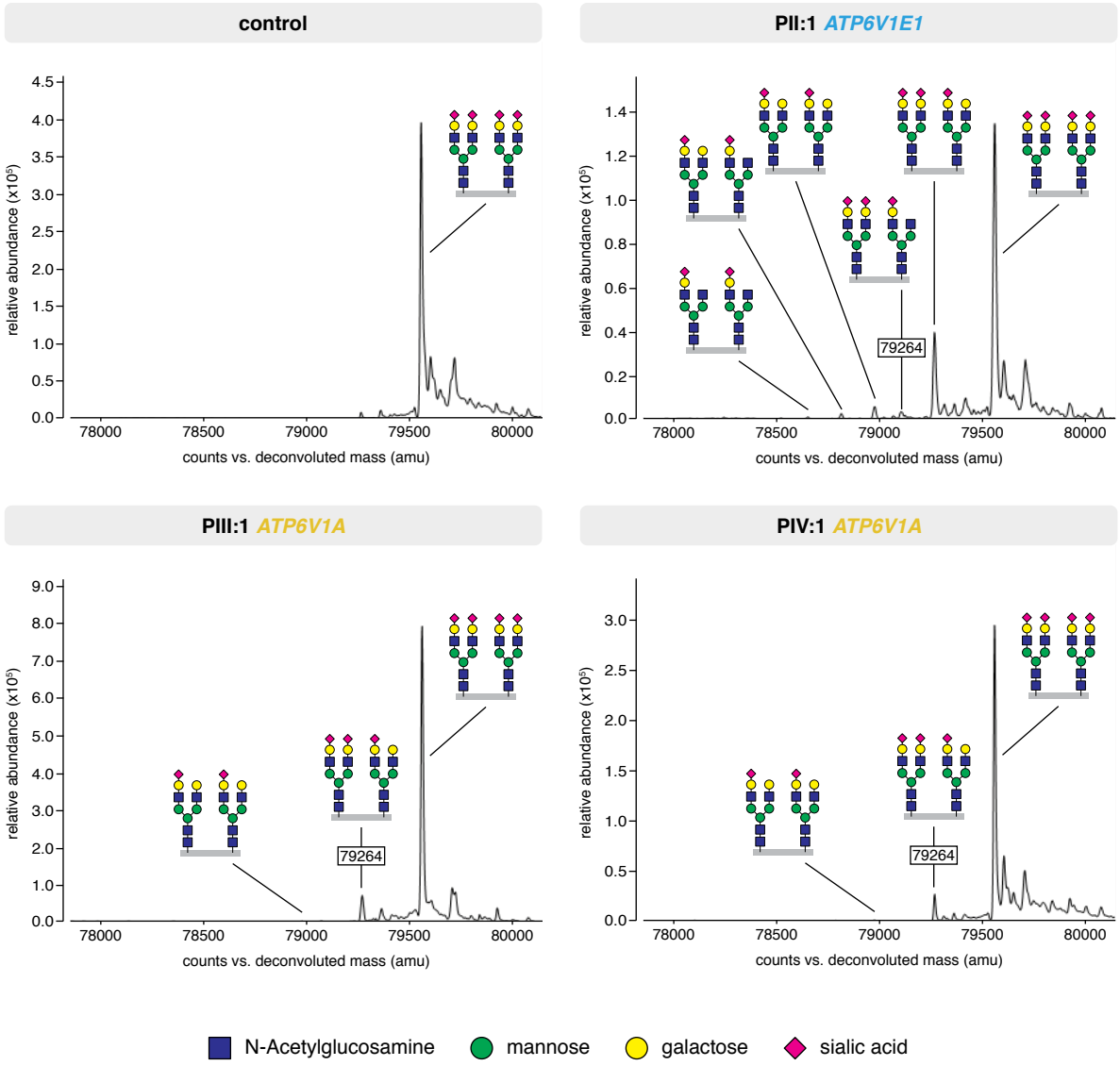


Figure S2. Tissue-specific Gene Expression of V-ATPase Subunits



Tissue-specific expression of *ATP6V1E1*, *ATP6V1E2*, *ATP6V1A* and *ATP6V0A2* was assessed using the FirstChoice Human Total RNA Survey Panel (Life Technologies), human tendon (OriGene) and adult skin RNA (BioGenomics) and pooled RNA from three human dermal fibroblast cultures. cDNA was synthesized with the iScript cDNA Synthesis kit (Bio-Rad Laboratories). For each gene of interest, reverse transcription PCR (RT-PCR) was performed using two primer pairs (only one is shown) and an aliquot of each reaction was analyzed on the Caliper LabChip GX (Caliper Life Sciences). *ACTB* was included as loading control. The darker the bands the higher the expression of the gene of interest in a tissue. RT-PCR showed ubiquitous expression of *ATP6V1E1*, *ATP6V1A* and *ATP6V0A2*. The expression of *ATP6V1E2* was restricted to testicular tissue. Abbreviations are as follows: bp, base pairs; He, heart; Es, esophagus; Co, colon; Ce, cervix; Br, brain; Bl, bladder; Ad, adipose; Sm, skeletal muscle; Pr, prostate; Pl, placenta; Ov, ovary; Lu, lung; Li, liver; Ki, kidney; Tr, trachea; Th, thyroid; Tm, thymus; Te, testes; Sp, spleen; Si, small intestine; Fb, fibroblasts; Sk, skin; Tn, tendon.

Figure S3. Quadrupole-Time-of-Flight Mass Spectrometry of Intact Transferrin



Quadrupole-time-of-flight (Q-TOF) mass spectrometry (MS) of intact transferrin showed a consistent increase of mass 79264 in all evaluated affected individuals (PII:1, at age 10; PIII:1, at age 13; PIV:1, before the age of 1), corresponding to the loss of one sialic acid. In addition, a minor lack of galactose was observed in Family II (PII:1).

Table S1. V-ATPase Subunits Identified by Complexome Profiling

subunit	gene	GI accession #	annotation	M_r (kDa)	peptides	sequence coverage (%)
V_1 cytosolic domain						
A	*ATP6V1A*	19913424	V-type proton ATPase subunit A	68.303	40	83
B2	*ATP6V1B2*	19913428	V-type proton ATPase subunit B, isoform 2	56.500	27	69
C1	*ATP6V1C1*	4502315	V-type proton ATPase subunit C, isoform 1	43.941	25	65
D1	*ATP6V1D*	7706757	V-type proton ATPase subunit D	28.262	14	57
E1	*ATP6V1E1*	4502317	V-type proton ATPase subunit E, isoform 1	26.145	15	58
F	*ATP6V1F*	20357547	V-type proton ATPase subunit F, isoform 1	13.370	6	68
G1	*ATP6V1G1*	4757818	V-type proton ATPase subunit G, isoform 1	13.757	9	75
H	*ATP6V1H*	47717100	V-type proton ATPase subunit H, isoform 2	54.151	12	34
V_0 transmembrane domain						
a1	*ATP6V0A1*	19913418	V-type proton ATPase subunit a, isoform 1	95.755	34	44
a2	*ATP6V0A2*	42741679	V-type proton ATPase subunit a, isoform 2	98.081	15	22
a3	*TCIRG1*	19924145	V-type proton ATPase subunit a, isoform 3	92.967	35	53
c	*ATP6V0C*	4502313	V-type proton ATPase subunit c	15.736	2	32
d1	*ATP6V0D1*	19913432	V-type proton ATPase subunit d, isoform 1	40.329	18	54
accessory subunits						
Ac45	*ATP6AP1*	17136148	V-type proton ATPase subunit S1 precursor	52.025	18	40
M8-9	*ATP6AP2*	15011918	renin receptor precursor	39.008	13	42

Table S2. *In Silico* Prediction of Pathogenicity for Missense Variants

	cDNA	protein	PolyPhen-2	SIFT	MutationTaster	Align GVGD	MAPP
ATP6V1E1	c.383T>C	p.Leu128Pro	probably damaging	deleterious	disease causing	C0	bad
	c.634C>T	p.Arg212Trp	probably damaging	deleterious	disease causing	C0	bad
ATP6V1A	c.1012C>T	p. Arg338Cys	probably damaging	deleterious	disease causing	C0	bad
	c.215G>A	p.Gly72Asp	probably damaging	deleterious	disease causing	C65	bad

The following reference sequences were used: *ATP6V1E1* (MIM: 108746; GenBank: NM_001696.3) and *ATP6V1A* (MIM: 607027; GenBank: NM_001690.3). Abbreviations are as follows: PolyPhen-2, Polymorphism Phenotyping v2; MAPP, Multivariate Analysis of Protein Polymorphism.

Table S3. Glycosylation Screening

	age	transferrin isofocusing						ApoCIII isofocusing						MS
		disialo		trisialo		tetrasialo		ApoCIII-0		ApoCIII-1		ApoCIII-2		
	years	value (%)	ref. (%)	value (%)	ref. (%)	value (%)	ref. (%)	value (%)	ref. (%)	value (%)	ref. (%)	value (%)	ref. (%)	value (%)
PII:1	10	**14.4 ↑**	3.3 – 7.6	**28.9 ↑**	4.9 – 0.6	**32.2 ↓**	47.3 – 62.7	**8.7**	0 – 11.6	**50.2**	33.1 – 66.9	**41.1**	27.4 – 60.0	**29.3 ↑**
PII:2	9	**12.9 ↑**	3.3 – 7.6	**29.6 ↑**	4.9 – 10.6	**35.4 ↓**	47.3 – 62.7	**13.1 ↑**	0 – 11.6	**55.1**	33.1 – 66.9	**31.7**	27.4 – 60.0	**27.4 ↑**
PIII:1	6	**3.6**	3.3 – 7.6	**12.9 ↑**	4.9 – 10.6	**62.5**	47.3 – 62.7	**NA**		**NA**		**NA**		**9.1 ↑**
	7	**5.7**	3.3 – 7.6	**15.9 ↑**	4.9 – 10.6	**50.2**	47.3 – 62.7	**8.1**	0 – 11.6	**57.9**	33.1 – 66.9	**34.0**	27.4 – 60.0	**15.9 ↑**
	13	**3.2 ↓**	3.3 – 7.6	**11.8 ↑**	4.9 – 10.6	**71.0 ↑**	47.3 – 62.7	**5.4**	0 – 11.6	**70.2 ↑**	33.1 – 66.9	**24.4 ↓**	27.4 – 60	**14.9 ↑**
PIV:1	<1	**3.6**	3.3 – 7.6	**11.0 ↑**	4.9 – 10.6	**62.1**	47.3 – 62.7	**2.6**	0.2 – 4.5	**40.9 ↓**	42.7 – 69.9	**56.5**	26.2 – 56.7	**9.2 ↑**

Quantification of transferrin and apolipoprotein CIII (ApoCIII) isoelectric focusing and the loss of sialic acid on quadrupole-time-of-flight (Q-TOF) mass spectrometry (MS) analysis represented as relative abundance of the main peak of the normal tetrasialic transferrin (ref. 3.5 ± 1.9%). Abbreviations are as follows: NA, not available; ref., reference value.

Supplemental References

1. Van Asbeck, E., Wolthuis, D.F.G.J., Mohamed, M., Wevers, R.A., Korenke, C.G., Gardeitchik, T., and Morava, E. (2014). A novel phenotype associated with cutis laxa, abnormal fat distribution, cardiomyopathy and cataract. Am. J. Med. Genet. A *164A*, 1049–1055.