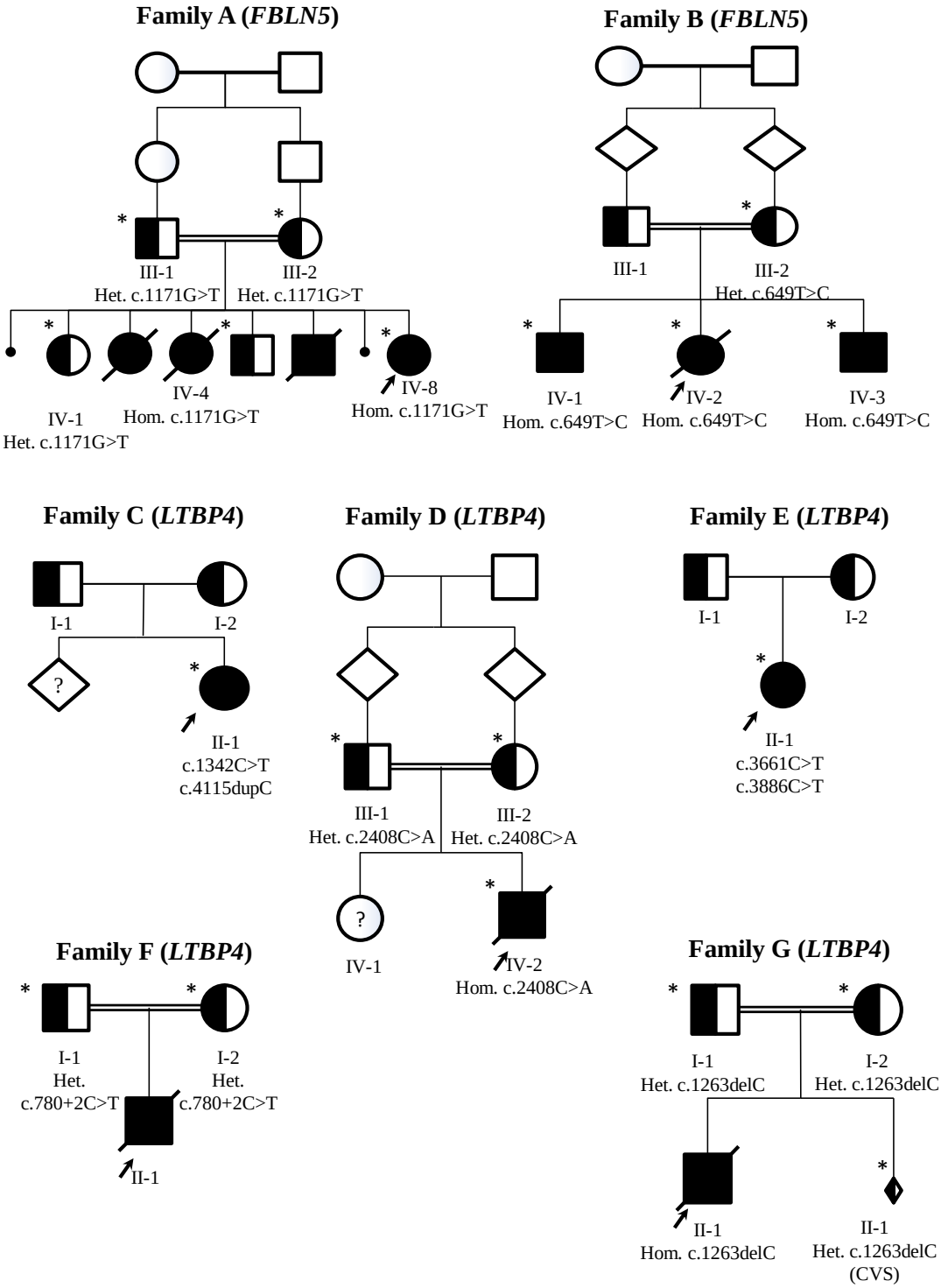
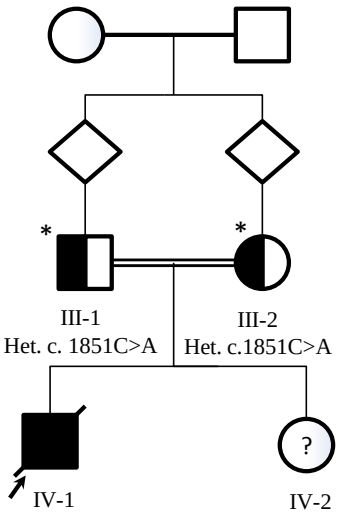
Supp. Figure S1. Pedigrees of the 12 families with autosomal recessive cutis laxa type 1 included in this study. Mutations are indicated. Square, male; round, female; Rhombus, unknown sex; Slash line, deceased, White symbol, healthy, no carrier; black symbol, affected; half-filled symbol, carrier, not affected; *, DNA was available from this family member; double line, consanguinity, small symbol, foetus. Arrow, index patient.



Family H (*LTBP4*)



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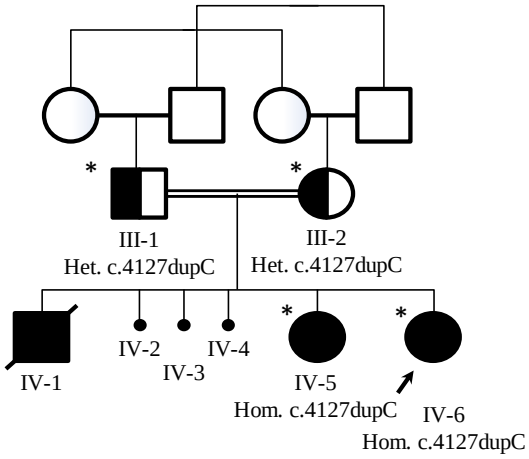
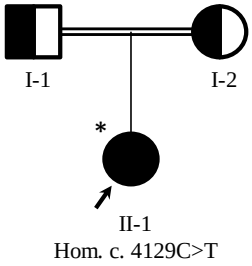
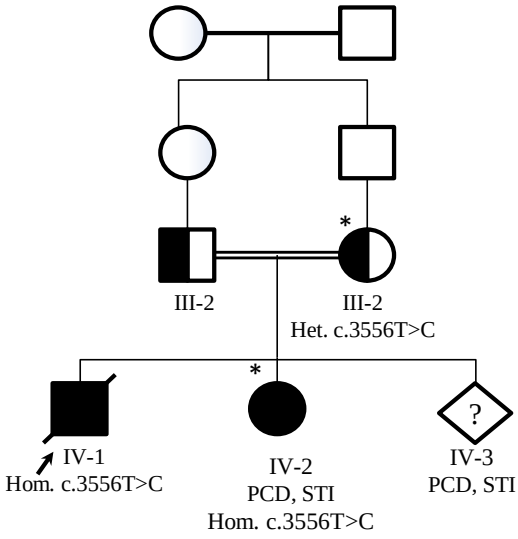


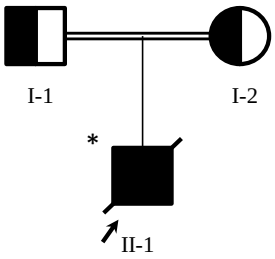
Family J (*LTBP4*)

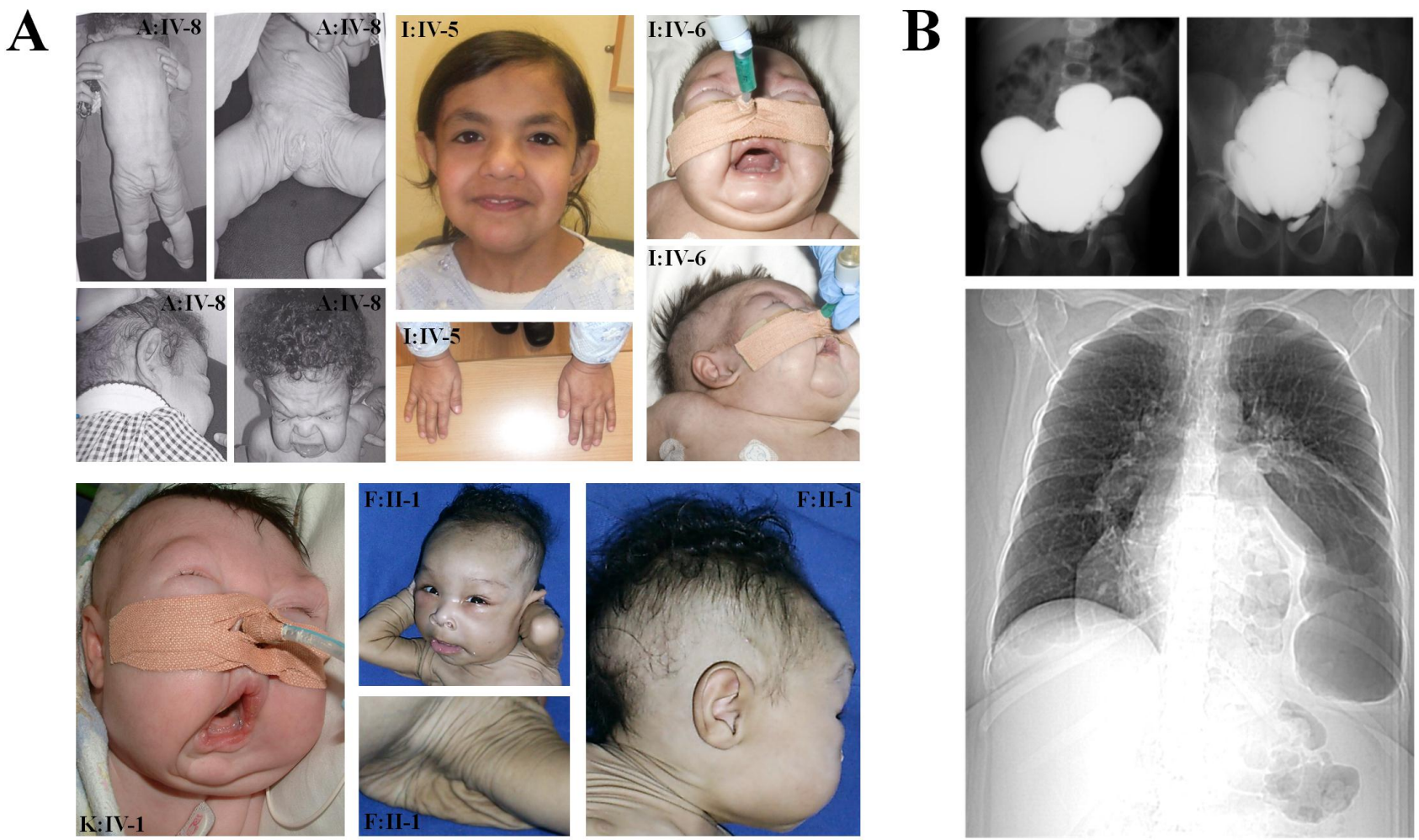


Family K (*LTBP4*)

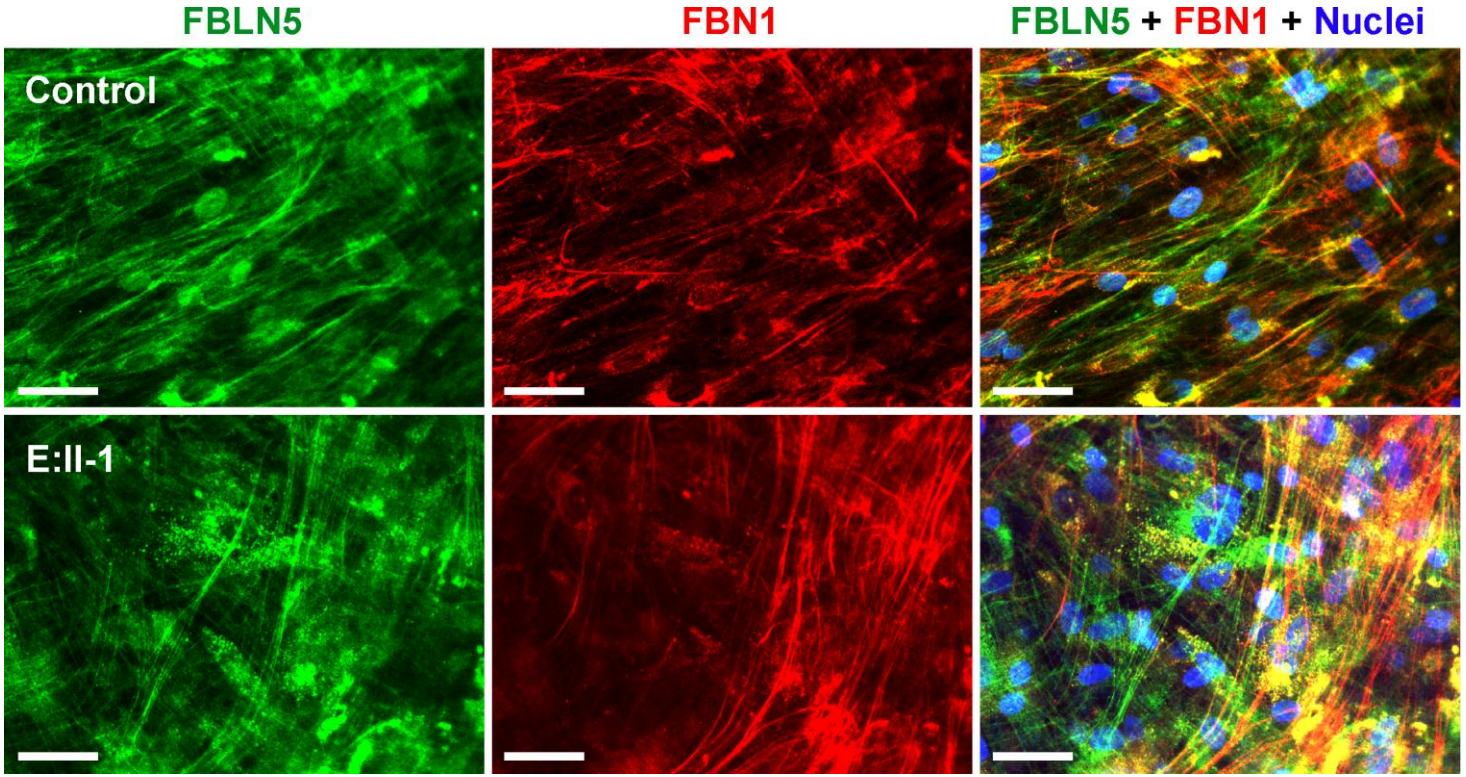


Family L (Mutation negative)

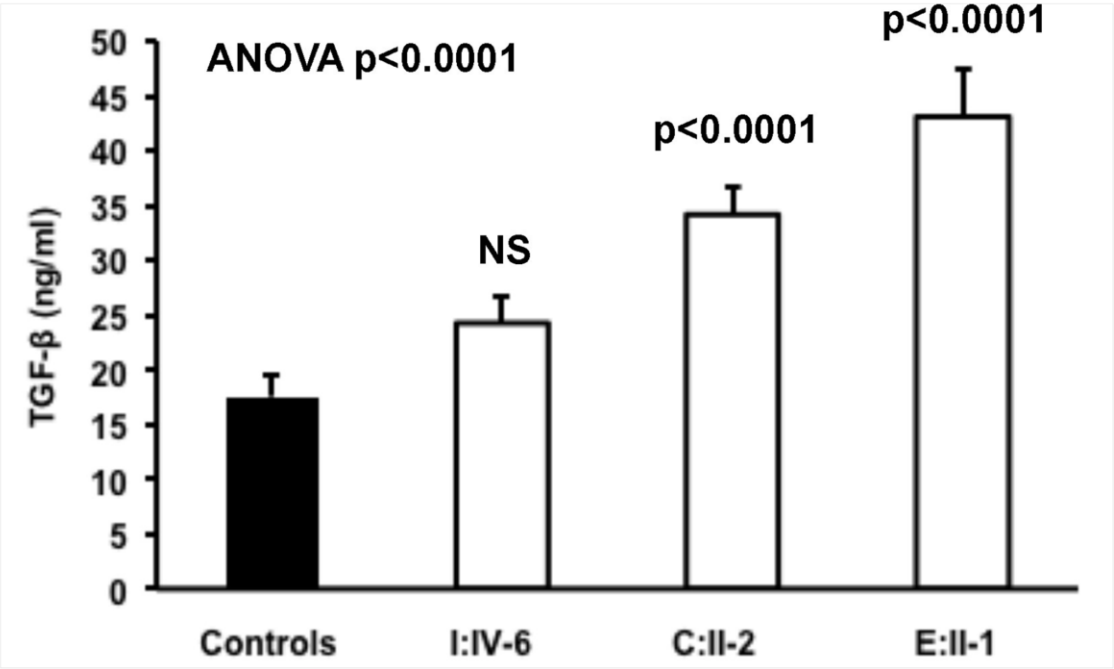




Supp. Figure S2. Clinical presentation of patients with *FBLN5* or *LTBP4* mutations. **A**: Clinical manifestations of patients with *FBLN5* or *LTBP4* mutations. Patient A:IV-8 with a homozygous *FBLN5* mutation presents with generalized cutis laxa, curly hair, a square face, large prominent ears, a broad forehead, Blepharochalasis and ptosis, sagging cheeks, and full lips. Patient I:IV-6 and F:II-1 with a homozygous *LTBP4* mutation showed generalized cutis laxa, sparse hair on the temporal sides, a sloping forehead, large ears, blepharochalasis and periorbital fullness, mild hypertelorism, a bulbous and beaked nose (with anteverted nostrils in F:II-1), a long philtrum, and a wrinkled skin on hands and feet. **B**: Internal manifestations in patient C:II-2 with *LTBP4* mutations included bladder diverticula, and diaphragmatic hernia.



Supp. Figure S3. LTBP4 mutation does not affect fibulin-5 localization. The fibulin-5 (FBLN5; green) and fibrillin-1 (FBN-1; red) immunofluorescent staining of skin fibroblasts from control (A) and from LTBP4 mutant (patient E:II-1) individuals revealed similar fibulin-5 staining of the extracellular matrix. Magnification bar: 50 µm.



Supp. Figure S4. Measurements of TGFβ1 activity. TGFβ1 reporter cells are co-cultured with fibroblasts from patients C:II-2, E:II-1 and I:IV-6 and four controls (four replicates each). Error bars show standard errors of means and *p*-value (ANOVA) is significant among groups (Controls, Patients C:II-2, E:II-1 and I:IV-6). P values from pairwise comparisons between controls and each set of patient samples are shown above the bars. NS=not significant.

Supp. Table S1. Antibodies used for immunoblotting and immunofluorescent staining

Antibody	Dilution	Source	Application	Catalog Number
Mouse anti-human fibrillin-1 (monoclonal)	1:500	Chemicon	IF	MAB1919
Fibrillin-1(mAb69**)**	1:1000	Dr. Lynn Y. Sakai	IF	N/A
Rabbit anti-human fibrillin-1 (polyclonal)	1:1000	Dr. Robert Mecham	IB	N/A
Goat anti-human LTBP-4 (polyclonal)	1:1000	R&D Systems	IB	AF2885
Goat anti-human LTBP-4 (polyclonal)	1:500	Santa Cruz Biotechnology	IB	SC-30359
Goat anti-human LTBP-4 (polyclonal)	1:200	R&D Systems	IF	AF2885
Mouse anti-mouse LTBP-1 (polyclonal)	1:100	R&D Systems	IF	AM388
Rabbit anti-human fibulin-5 (polyclonal)	1:200	Dr. Elaine Davis	IF	N/A
Rabbit anti-human fibronectin (polyclonal)	1:1000	Sigma	IF	89062001
Goat anti-rabbit IgG-HRP	1:20000	Thermo Scientific	IB	PAI-29391
Donkey anti-goat IgG-HRP	1:2000	Santa Crux Biotechnology	IB	SC-2020
Donkey anti-mouse Alexa Fluor 594	1:500	Invitrogen	IF	A21203
Donkey anti-goat Alexa Fluor 594	1:250	Invitrogen	IF	A11058
Donkey anti-rabbit Alexa Fluor 488	1:1000	Invitrogen	IF	A21206

IB: immunoblotting;

IF: immunofluorescence; HRP: horseradish peroxidase; N/A: not applicable

Supp. Table S2. Clinical manifestations of probands included in the study

Individual	Age/ Sex	Cons.	Fam. hist.	Cause of Death	Skin				Lungs	Cardiovascular					BD	GID	Hypotonia	Dysmorphism	Other
					CL	FI	IH	DH	PE	PPAS	SVAS	Valvular stenosis	Valvular insuff.	other					
***FBLN5* MUTATION POSITIVE PROBANDS**																			
A: II-8	19m/ F	+	+ (1)		++	++	-		+								+	frontal bossing, low and broad nasal bridge, beaked nose large dysplastic ears, sagging cheeks, everted lower lip	photophobia
B: IV-2	11m/ F	+	+ (2)	PE / bronchiolitis	++	++	+		++	+			A						pyloric stenosis
Summary FBLN5		**2/2**	**2/2**	**1/2**	**2/2**	**2/2**	**1/2**	**0/2**	**2/2**	**1/2**			**1/2**		**0/2**		**1/2**		
***LTBP4* MUTATION POSITIVE PROBANDS**																			
C: II-2	23y/ F	-	+ (3)		++	+/-		+	+/-	+					++			Large ears, beaked nose	Rectal prolapse, joint hypermobility, artificial bladder
D:IV-2	4w/ M	+	+	PE	+	+			++	++							+		prominent veins, bronchiomalacia, dilated pelvis of the left kidney
E: II-1	3m/ F	-	-	bronchiolitis/ PE	++	+			+++						+			large ears, low nasal bridge	prominent veins
F: II-1	2y/ M	+	-	PE	+	+/-		+ (surgery)	++	+							+	frontal bossing	Thin hair growth
G: II-1	10y/ M	+	-	PE	+	+	+	+ (surgery)	++				M, T, A, P (dyspl–prol)	atrial septum aneurysm	+				Rectal prolapse, abdominal wall herniae, prominent veins
H: IV-1	6m/ M	+	-	PHrT/PE	+	+			++	Systemic HrT, severe PHrT									Late onset GBS sepsis, unilateral pyelic dilatation
I: IV-6	6m/ F	+	+ (4)	PHrT postop gastric perf.	+	+		+	+	+		P, A (dysplastic)	A	ASD, PHrT		+	+		polyhydramnion, esophageal tortuosity, gastric perforation, tissue fragility

Supp. Table S2, continued

Individual	Age/ Sex	Cons.	Fam. hist.	Cause of Death	Skin				Lungs	Cardiovascular					BD	GID	Hypo-tonia	Dysmorphism	Other
					CL	FI	IH	DH	PE	PPAS	SVAS	Valvular stenosis	Valvular insuff.	other					
J: II-1	13y/ F	+	-	brain abcesses	+/- (late onset)	-			+/-	++			T	PHrT, PFO	+			prominent forehead, blepharochalasis, epicanthal folds, short nose, elongated marked philtrum, low set large ears, webbed neck, low posterior hairline	Pelvic insufficiency, brain abscesses
K: IV-1	6w/ M	+	+ (5)	PE	++	+		hiatus hernia	++				T	PHrT	+		+	large ears, broad low nasal bridge, frontal bossing, small fontanel	Hydronephrosis, tracheomalacia, joint hypermobility
Summary LTBP4		**7/9**	**4/9**	**8/9**	**9/9**	**8/9**	**1/9**	**5/9**	**9/9**	**5/9**	**0/9**	**1/9**	**4/9**		**5/9**	**1/7**	**4/9**		
MUTATION NEGATIVE PROBAND																			
L: II-1	6y/ M	+	-		+	+	+	-				M			+	-			
Overall summary		**10/12**	**6/12**	**9/12**	**12/12**	**11/12**	**3/12**	**5/12**	**11/12**	**6/12**	**0/12**	**2/12**	**6/12**		**5/12**	**1/12**	**5/12**		

(1) Two sisters and one brother with cutis laxa died, father has colitis ulcerosa, mother had 2 spontaneous abortions.
(2) B: II-1, male 9 years old, generalized CL, inguinal hernias, emhysema probable, PPAS, aortic stenosis and insufficiency, Pyloric stenosis
B:II-3, male, aged 4, generalized CL, inguinal herniae, mild emphysema, PPAS, aortic insufficiency, pyloric stenosis
(3) One affected sib died at age 2
(4) Mother had 3 spontaneous abortions.
First child: male, generalized CL, died following a bowel rupture at day 5.
Second child:, female, generalized cutis laxa, failure to thrive, Bowel perforation (surgery), mitral valve prolapse, palpitations, syncopes, joint hypermobility. At 8 years of age: Nissen fundoplasty and placement of a gastrostomy for a volvulus through a massive hiatus herniae
(5) Second child, female, generalized cutis laxa, died due to respiratory failure at 2 weeks of age. She also presented with primay ciliary dyskinesia and situs inversus totalis
A third child had primary ciliary dyskinesia and situs inversus totalis without cutis laxa or emphysema.

Abbreviations:
Cons., consanguinity; Fam. Hist., family history; CL, cutis laxa; FI, Facial involvement; IH, inguinal hernia; DH, diaphragmatic hernia; PE, pulmonary emphysema; PPAS, peripheral pulmonary artery stenosis; SVAS, supravalvular aortic stenosis; insuff., insufficiency; BD, bladder diverticula; GID, gastrointestinal diverticulae; M, male; F, female; y, year; m, months; w, weeks; M, mitral; T, tricuspid; A, aortic; P, pulmonary; ASD, atrium septum defect; PHrT, pulmonary hypertension; HrT, hypertension; PFO, patent foramen ovale

Supp. Table S3. Differential diagnosis in type I recessive cutis laxa

	FBLN4	***FBLN5***	***LTBP4***	***SLC2A10***
Cutis laxa	+ (hyperextensible)	+++	+++	+ (hyperextensible)
Dysmorphology	High forehead Hypertelorism Marfanoid skeletal features	High forehead, broad and beaked nose, large dysplastic ears, sagging cheeks	Sparse hair on temporal sides, large forehead, large ears, long phitrum	Long face, Hypertelorism, Periorbital fullness, beaked nose, large ears, sagging cheeks, Marfanoid skeletal features
Emphysema	+	+++	+++	+
Aortic root dilatation	+++	-	-	+
Arterial tortuosity	++	-	-	+++
SVAS	-	+	-	-
PPAS	+	+	++	++
Valvular disease	+	+	+	+
Gastro-intestinal diverticula/tortuosity	?	+	++	+
Gastrointestinal fragility	-	-	++	-
Genitourinary diverticula	?	+	+++	-
Early mortality	++	+++	+++	+

?, not known; +, feature with rare or mild occurrence, ++, frequent feature; +++, severe and frequent feature. Based on data in this study and the following papers: (Callewaert, et al., 2008; Hucthagowder, et al., 2006; Renard, et al., 2010; Urban, et al., 2009).

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