Supplementary Information

Supplementary Discussion

E was significantly lower in CL patients compared to controls. We saw no correlation of E with age. This is consistent with previous reports that showed steady E values with age with an increase past 70 years (Escoffier *et al.*, 1989; Grahame and Holt, 1969), an age range not covered by either our cases or our controls. The E values obtained by the DermaLab® instrument (E = 5-14 MPa) is lower than E values obtained by ex vivo testing of human skin (E = 30-110 MPa) (Rollhauser 1950), but within the very broad range of measurements obtained by different devices utilizing suction or torsion (E = 0.06-100 MPa) (Diridollou *et al.*, 2000; Escoffier *et al.*, 1989; Grahame and Holt, 1969; Hendriks *et al.*, 2003). The wide range of E values observed with different instruments may reflect the non-linear mechanical properties of the skin, different assumptions made in the calculations of E and different part of the stress-strain curve interrogated by each type of device. E is defined as the slope of the stress-strain curve, which is less steep at low displacement (strain) values but very steep at high strain (Hendriks *et al.*, 2003).

RT showed age dependence and significant difference between cases and controls. In the calculation of VE, E is divided by a normalized RT value, as RT is thought to be proportional to the viscosity of the skin. We note, however, that RT is not only proportional to the viscosity of the skin, but also is inversely proportional to the recoil property of the skin and propose that RT is increased in CL patients because of decreased recoil in the absence of functional elastic fibers, rather than because of increased viscosity. As a result of such complex biophysical contributions to RT (and hence VE), measurements obtained with the DermaLab® suction cup device are not directly comparable to measurements of skin biomechanics with other devices.

Our study assumes uniform skin thickness (1 mm) in calculating the E and VE moduli. This is consistent with relatively constant skin thickness in the age range of 15-70 years by a variety of methods, including caliper (0.8-1.4 mm) (Grahame and Holt, 1969) or by ultrasound measurements (0.6-0.9 mm) (Escoffier *et al.*, 1989). Furthermore, previous studies found no difference in the thickness of skin between individuals with CL and controls (Grahame and Beighton, 1971).

The results presented here demonstrate that a cutoff value (2.538) of a composite variable (D) incorporating Age, E and VE can distinguish between CL patients and controls with the highest specificity and sensitivity. This cutoff value will be useful in future studies for the objective diagnosis of CL, especially in cases where extensive wrinkling, sagging or redundancy of the skin is not immediately apparent, or in individuals where natural skin aging may interfere with the diagnosis of cutis laxa by simple physical examination. The non-invasive and rapid nature of the DermaLab® measurement may provide advantages over

histological or electron microscopic examination of the skin in individuals suspected of cutis laxa.

Additional studies will be needed to determine how to best distinguish CL cases from other connective tissue syndromes. Similar studies using the DermaLab® elasticity module in patients with Williams-Beuren syndrome (Kozel *et al.*) a multiple gene deletion disorder associated with elastin haploinsufficiency, also cause lower than typical VE. Interestingly, in Williams-Beuren syndrome, affected individuals have slightly longer recoil time but the predominant factor driving the decrease in VE ($VE=E/RT_n$) is a lower E, whereas in CL a markedly prolonged RT as well as decreased E contribute to the VE difference. Consequently, a scoring system taking into account multiple biomechanical properties may ultimately be needed for diagnostic purposes. In classic forms of Ehlers-Danlos syndrome, a collagen disorder, patient skin is described as hyperelastic. Given the reported phenotype, one would predict significantly shorter RT with potentially lower E. Pilot studies in very small numbers of affected individuals by our group have supported this hypothesis (unpublished results) but additional work is needed to fully characterize the biomechanical properties in each of these disorders so as to optimize testing parameters yielding maximal sensitivity and specificity for each.

Supplementary Material and Methods

Subjects
Studies were conducted in accordance with the IRB protocols at Washington University School of Medicine and the University of Pittsburgh. Controls (n=136) were recruited from Washington University clinical and research populations. Cases (n=22) were recruited during Cutis Laxa Research Clinics at the University of Pittsburgh. At the time of evaluation, participants, or their parents if the individual was a minor, completed a questionnaire providing demographic information and skin elasticity was measured using the DermaLab® suction cup device.

Skin elasticity measurement
The skin of participants was measured using the suction cup elasticity module of the DermaLab® device (Cortex Technology, Denmark). The suction cup was placed on the volar surface of each forearm, midway between the wrist and the elbow using a 2-sided adhesive sticker. Measurements were taken from each arm and the mean of the two measurements were used for analysis. The instrument applies vacuum in increasing increments to a small patch of skin (10 mm in diameter) under the suction cup, causing the skin to be lifted into the cup. The pressure is first recorded when the skin crosses a light beam emitted at the base of the suction cup (P_1). When the skin has moved 1.5 mm further into the cup, as detected by interference with a second light beam emitted within the suction cup, the final pressure is recorded (P_2) and the skin released. The device then measures the amount of time required for the skin to cross the lower light beam again.

The output from the device is ΔP (P_2-P_1, measured in mBar) and RT (retraction time, measured in ms). This cycle is repeated two subsequent times. Data from cycles 2 and 3 showed similar characteristics to cycle 1, but lower effect sizes. Differences between cycles in ΔP did not predict disease outcome and did not correlate with age (data not shown). There was a slight group and age effect for differences in RT by cycle, but this did not reach statistical significance (data not shown). Therefore, we report cycle 1 data only (ΔP_1 and RT_1).

Measurements were excluded from analysis (1) if measurements were only available on one arm, (2) if measurements from the left *vs.* right arms differed by more than two standard deviations of the age adjusted population mean, or (3) if RT measurements exceeded 10,000ms. Among 136 controls 1 individual was excluded because of missing left arm data, and 17 were excluded because the difference between right and left arm measurements were too large, yielding 118 individuals for final analysis. Among 22 cases, 3 were excluded because of the difference between left and right measurements and 2 were excluded because the RT values were larger than 10,000ms. Interestingly, both of the latter individuals were positive for the same *ELN* mutation.

The elastic modulus (E) is calculated by the DermaLab® software by solving the following equation for E: $\Delta x = \psi \times \Delta P_1 \times r^4 / (E \times s^3)$. Where Δx is skin displacement (0.0015 m for this probe), Ψ is an instrument constant, ΔP_1 is pressure difference as described above, but converted to MPa units, r is the radius of the skin patch displaced (0.005 m) and s is the thickness of the skin, estimated to be 0.001 m. Thus, E, as measured by the DermaLab® instrument, is an approximate value assuming uniform skin thickness across all participants. Using 1 mm skin thickness and other probe constants, the formula used by the instrument is $E = 0.3125 * \Delta P_1/1.5$. An approximate value for skin viscoelasticity (VE) is calculated by the instrument using the following formula: $VE = E/RT_n$ where RT_n is a normalized recoil time obtained by dividing RT_1 with 260 ms, the average control underarm recoil time.

The DermaLab® elasticity module was validated by several previous studies. One took measurements of a latex sheet stretched to various degrees. The pressure measurements by the instrument correlated with the degree of stretch, but the pressure difference, and hence E, remained constant (Grove *et al.*, 2006) resulting in reproducible measurements at various stretch conditions. Another study evaluated the Dermalab® suction cup device relative to Dermaflex®, another suction cup device (Pedersen *et al.*, 2003). The two devices have different measurement parameters, the first measuring the force required to lift the skin to a standard height, the second measuring the displacement of the skin in response to a standard amount of force. The results obtained with the two methods showed moderate, but significant correlation, consistent with the differences in the measurement approach and assumptions made in calculating comparable variables. The reproducibility of measurements obtained by the Dermalab® elasticity module has been investigated in the context of measurements on normal and scarred skin (Anthonissen *et al.*, 2013). Both intra-observer and inter-observer reliability were high with intraclass correlation coefficient (ICC) confidence intervals of 0.79-0.97 and 0.81-0.98 for normal skin. A more recent, independent study found similarly high ICC values (Gandanke *et al.*, 2014).

Statistical Analysis

Chi-square tests were used for categorical and independent t-tests for continuous data for initial analysis. Pearson's correlations were calculated among age, E, VE and RT. Body mass index did not correlate significantly with any of the skin data and therefore was not included in any regression models. Logistic regression was used to determine the strength of each individual biomechanical variable in predicting affected status. Step-wise logistic regression was used to obtain a multivariate model for affected status, with age, sex, E, VE and RT in the initial model. ROC curve analysis was used to evaluate the power of the models to determine affected status, as quantified by the area under the ROC curve (AUC). Differences between logistic regression models were tested using ANOVA. Descriptive and multivariate statistics were carried out with SPSS

software (version 21.0) and R software (version 2.14). P values < 0.05 were considered statistically significant.

Cross-Validation Study

We randomized 4/5 of cases and controls into a training dataset, which was used to determine the parameters of the regression models. The remaining 1/5 of the cases and controls comprised the validation or testing sub-dataset, which was used to calculate the AUC of ROC. This approach was repeated 20 times and the average analysis in model 1 and model 2 were calculated.

Supplementary References

Anthonissen M, Daly D, Fieuws S*, et al.* (2013) Measurement of elasticity and transepidermal water loss rate of burn scars with the Dermalab((R)). *Burns* 39:420-8.

Diridollou S, Patat F, Gens F*, et al.* (2000) In vivo model of the mechanical properties of the human skin under suction. *Skin Research and Technology* 6:214-21.

Escoffier C, de Rigal J, Rochefort A*, et al.* (1989) Age-related mechanical properties of human skin: an in vivo study. *J Invest Dermatol* 93:353-7.

Gandanke TU, Duke JM, Danielsen PL*, et al.* (2014) Reliability of scar assessments performed with an integrated skin testing device - the DermaLab Combo. *Burns*.

Grahame R, Beighton P (1971) The physical properties of skin in cutis laxa. *The Br J Dermatol* 84:326-9.

Grahame R, Holt PJ (1969) The influence of ageing on the in vivo elasticity of human skin. *Gerontologia* 15:121-39.

Grove GL, Damia J, Grove MJ*, et al.* (2006) Suction chamber method for the measurement of skin mechanics: the DermaLab. In: *Handbook of non-invasive methods and the skin* (Serup J, Jemec GBE, Grove GL, eds) 2 ed., Boca Raton, FL: CRC Press, 593-9.

Hendriks FM, Brokken D, van Eemeren JT*, et al.* (2003) A numerical-experimental method to characterize the non-linear mechanical behaviour of human skin. *Skin Research and Technology* 9:274-83.

Kozel BA, Bayliss SJ, Berk DR*, et al.* (2014) Skin findings in Williams-Beuren syndrome. *Am J Med Genet A* (in press).

Pedersen L, Hansen B, Jemec GB (2003) Mechanical properties of the skin: a comparison between two suction cup methods. *Skin research and technology : official journal of International Society for Bioengineering and the Skin* 9:111-5.

Supplementary Tables

Supplementary Table S1. CL cases with known mutations

Patient	Sex	Onset	Gene	Type	OMIM
P1	F	Congenital	*LTBP4*	ARCL1C	613177
P2	F	Congenital	*LTBP4*	ARCL1C	613177
P3	M	Congenital	*ELN*	ADCL1	123700
P4	M	Congenital	*ELN*	ADCL1	123700
P5	F	Late-onset	*ELN*	ADCL1	123700
P6	M	Congenital	*ATP6V0A2*	ARCL2A	219200
P7	F	Congenital	*ATP6V0A2*	ARCL2A	219200
P8	M	Congenital	*ATP6V0A2*	ARCL2A	219200

ARCL1C: autosomal recessive cutis laxa type 1C, ADCL1: autosomal dominant cutis laxa type 1, ARCL2A: autosomal recessive cutis laxa type 2A, OMIM: Online Mendelian Inheritance in Man phenotype ID number.

Supplementary Table S2. CL cases with unknown mutations

Patient	Age (years)	Sex	Onset
P9	30	M	Late-onset
P10	4	F	Congenital
P11	42	F	Late-onset
P12	68	F	Late-onset
P13	1.6	F	Congenital
P14	41	M	Late-onset
P15	17	F	Congenital
P16	58	F	Late-onset
P17	58	F	Late-onset

Supplementary Table S3. Pearson's correlation (r) among age and dermal elasticity parameters

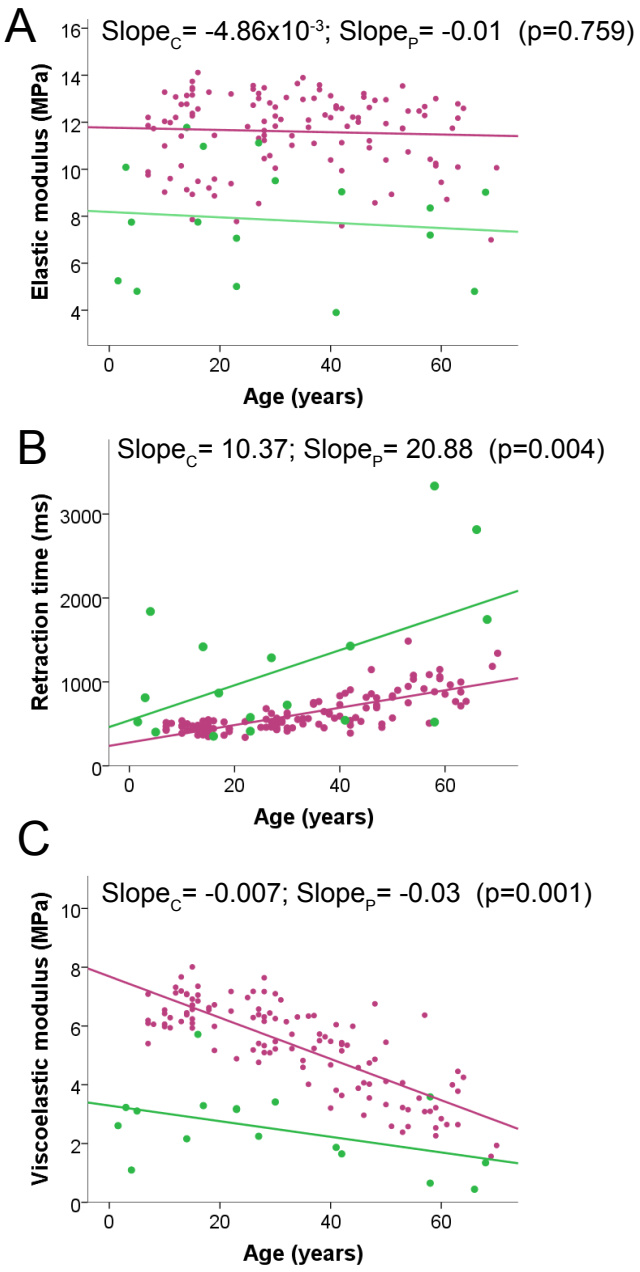
		Controls			**Cases**		
		E	**RT**	**VE**	**E**	**RT**	**VE**
Age	**r**	-0.052	**0.770**	**-0.801**	-0.105	**0.541**	-0.461
	p	0.574	**<0.001**	**<0.001**	0.689	**0.025**	0.063
E	**r**		0.090	**0.246**		0.160	0.068
	p		0.333	**0.007**		0.541	0.795
RT	**r**			**-0.898**			**-0.806**
	p			**< 0.001**			**< 0.001**

Significant values are shown in bold.

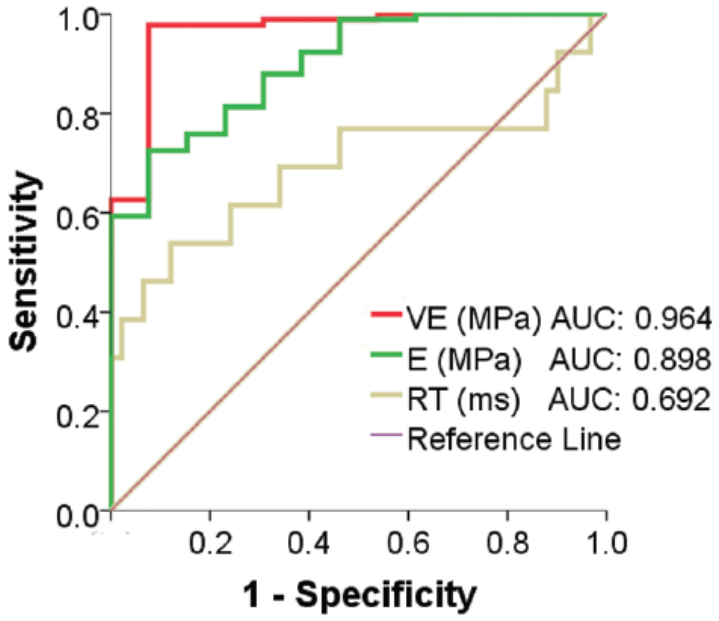
Supplementary Table S4. Results of the cross-validation study

	Model 1	Model 2
Test 1	0.992	1
Test 2	0.96	0.98
Test 3	0.993	0.972
Test 4	1	1
Test 5	0.92	0.8
Test 6	0.556	0.542
Test 7	0.969	0.985
Test 8	1	1
Test 9	0.92	0.8
Test 10	0.917	0.75
Test 11	1	1
Test 12	0.85	0.75
Test 13	1	1
Test 14	1	1
Test 15	0.97	0.985
Test 16	0.983	1
Test 17	0.992	0.992
Test 18	1	1
Test 19	0.993	0.972
Test 20	1	0.5
Average AUC	0.95075	0.9014

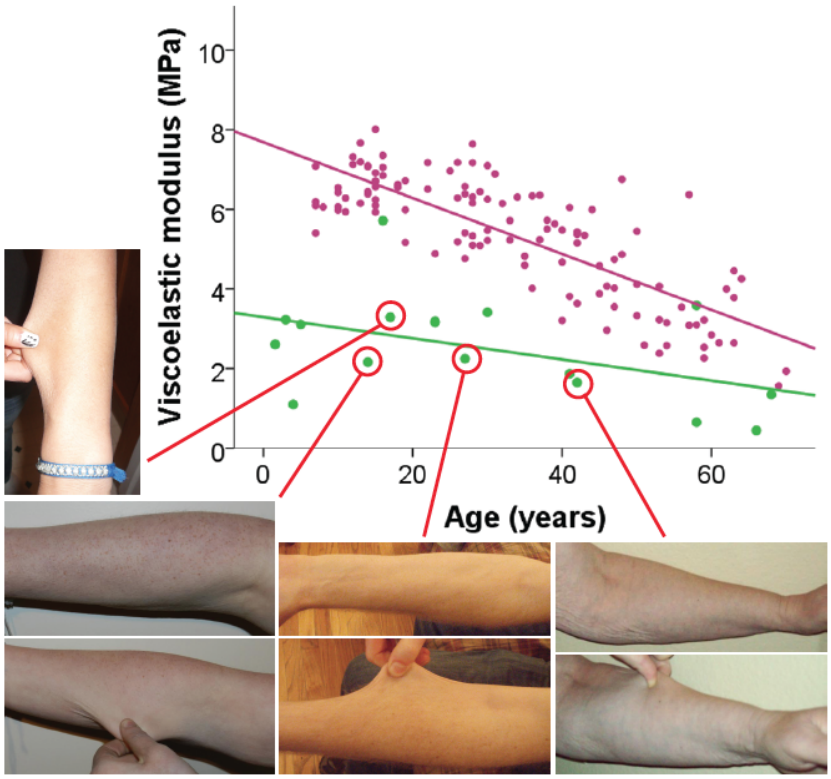
Note. Area under the curve (AUC) of the receiver operating characteristic (ROC) is shown in 20 tests where the data were randomized to separate training and test sets.



Supplementary Figure S1. Mechanical properties of the skin in controls and individuals with CL. Elastic modulus (a), retraction time (b) and viscoelastic modulus (c) are plotted as a function of age for controls (magenta dots) and CL cases (green dots). Linear regression lines are shown for each variable in each group (cases: green, controls: magenta). The slopes of the regression lines are shown above each chart with p values for statistical analysis of covariance. Retraction time (B) changes faster with age in patients than controls, whereas the viscoelastic modulus (C) changes slower with age in patients compared to controls. $Slope_C$ and $Slope_P$: slopes of the regression lines for controls and patients, respectively.



Supplementary Figure S2. ROC analysis of biomechanical variables in individuals 47 years old or younger.
Viscoelastic modulus remains more effective than elastic modulus or retraction time in differentiating cases from controls as indicated by receiver operating characteristic (ROC) curves.



Supplementary Figure S3. Viscoelastic modulus in relation to visual skin laxity. Images showing loose and wrinkled skin at the inner lower arm of several representative cases. Red tie-lines identify the data points on the viscoelastic modulus (VE)/age plot corresponding to each set of images.