Supplemental Table 1. Hematological indices of the proband heterozygous for PIEZO1 V598M (II:1) and an unaffected family member (I:1), from 2016. Some of these indices are more severe than usually encountered in HX patients with other causative PIEZO1 mutations (Andolfo et al., Am J Hematol 2018; 93:107-121). RR, reference range.

Supplemental Figure 1. Proband's pedigree. The sporadic heterozygous PIEZO1 mutation V598M present in proband is absent from other family members. The common variant ΔK1878 previously proposed as pathogenic for HX does not cosegregate with symptomatic disease in this family, and was not reported in the French HX patient heterozygous for PIEZO1 V598M [5].

Supplemental Figure 2. **A**. Whole blood total RNA prepared from the proband and unaffected family members was reverse-transcribed to cDNA, PCR-amplified and Sanger-sequenced. The heterozygous PIEZO1 variant c.1792G>A encoding missense mutant 598V>M was detected in cDNA from the proband (II:1) only, a finding confirmed in genomic DNA (not shown). **B.** cDNA sequencing trace of the same stretch of PIEZO1 sequence from one unaffected family member (II:2) representative of that sequence in four additional unaffected family members (not shown). The sequence was confirmed in genomic DNA (not shown). **C.** Amino acid sequence alignment showing evolutionary conservation of human PIEZO1 residue V598 in PIEZO1 from mammals, birds, amphibia and fish, and in human PIEZO2.

Supplemental Figure 3. Peripheral blood smears (Wright Giemsa) of (**A**) proband (II:1) and her (**B**) Mother (I:2), from 2008.

Supplemental Figure 4. Ektacytometry scans from 2008 of red cells from proband (II.1, red), mother (I:1, purple), father (I:2, green) and a healthy control (blue), on a Lorca ektacytometer lacking capability of continuous osmotic gradient generation

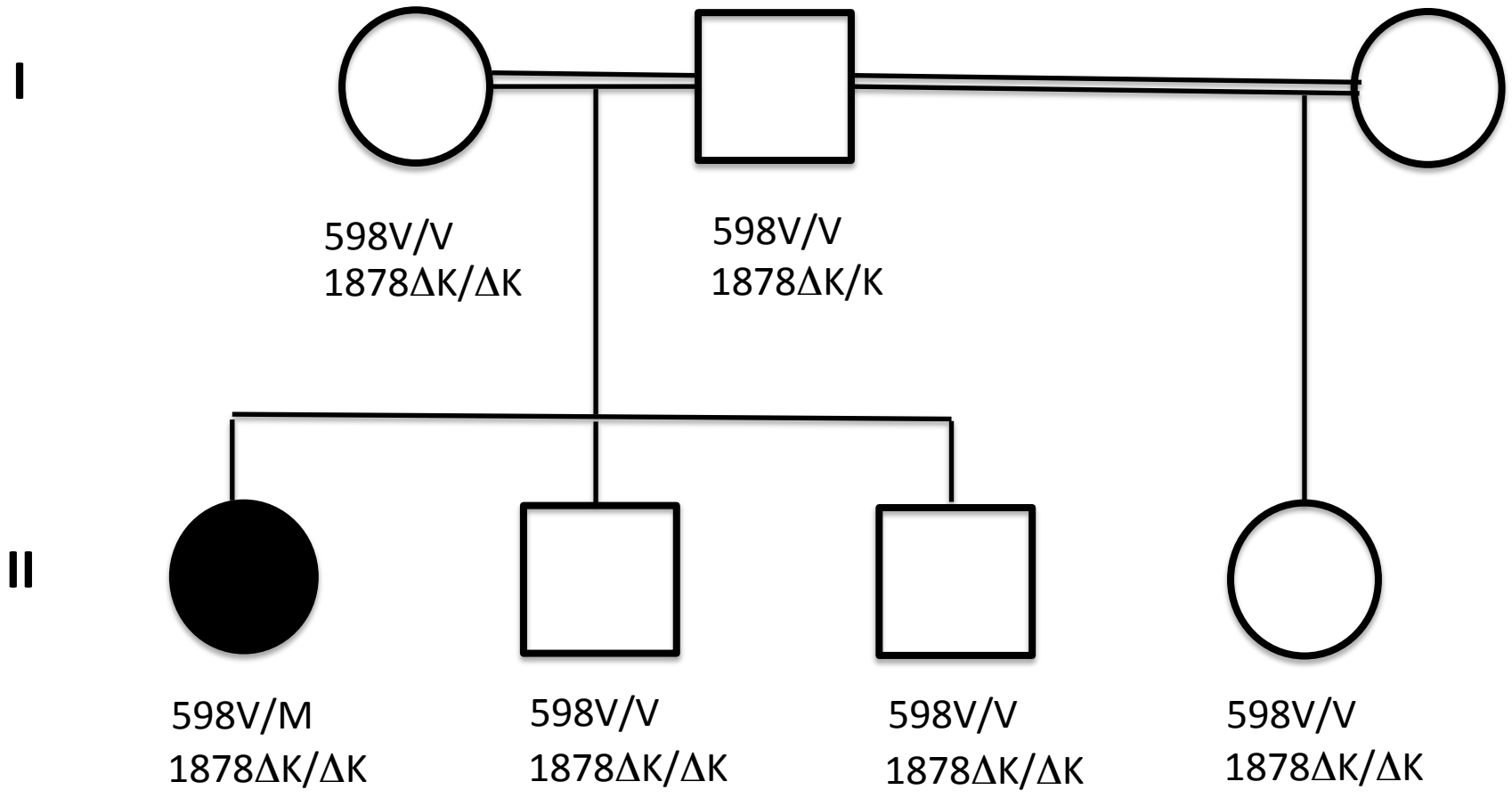
(RR Mechatronics, Netherlands). **A.** Deformability at 400 mOsm and at discrete shear stress values. **B**. Deformability at 16.86 Pa and at discrete osmolarity values.

Supplemental Table 1.

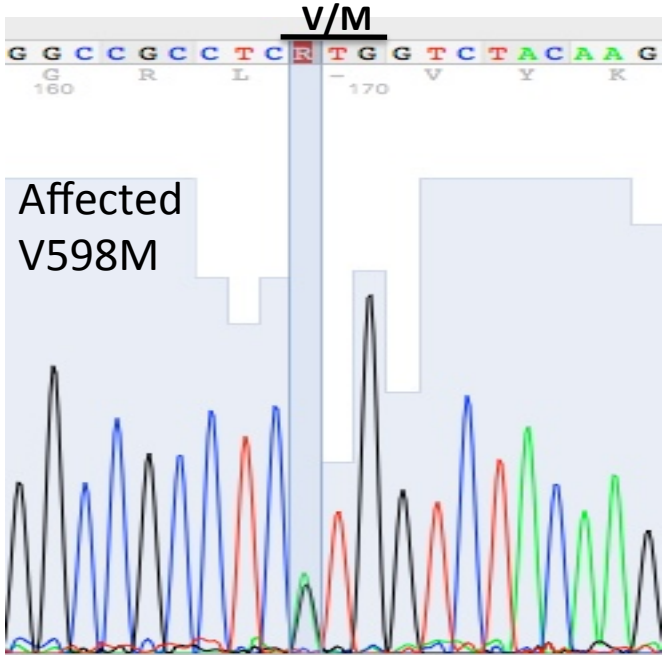
Hematological indices of PIEZO1 V598M and WT erythrocytes.

Mature Erythrocytes	**WT**	**V598M**	**RR**
Red Blood Cells (X 10^6 cells/uL)	4.30	2.86	3.75-5.07
Hematocrit (%)	40.8	32.8	31.7-43.4
Hemoglobin (g/dL)	12.4	9.9	11.4-15.1
Mean Cellular Volume (fL)	94.9	114.8	82.7-90.2
MCHC (g/dL)	30.4	30.1	33.6-35.3
CHCM (g/dL)	29.3	29.5	32.2-37.7
RDW (%)	13.3	18.1	12-15*
HDW (g/dL)	1.96	3.44	1.9-3.0*
% Hyperchromic cells	0.0	0.1	-
Reticulocytes			
Reticulocytes (X 10^6 cells/uL)	0.038	0.424	0.0406-0.140
Retics (%)	0.87	14.82	0.8-2
Mean Cellular Volume (fL)	102.8	118.7	93-121*
CHCM (g/dL)	25.1	27.5	27-34*
RDW (%)	9	11.7	-
HDW (g/dL)	2.33	3.28	-
% Hyperchromic reticulocytes	0.0	0.2	-

Supplemental
Figure 1



A



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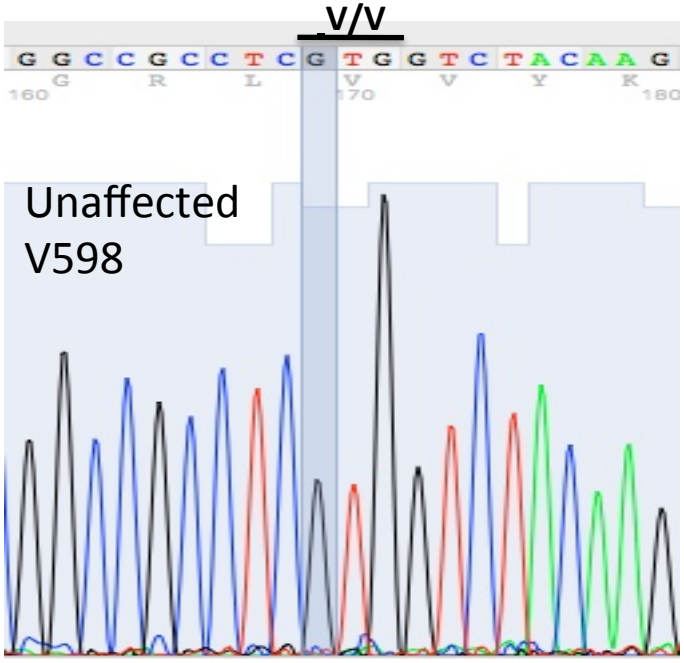

C

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                                        *
Human PIEZO2      685   KYWIYVCGGMFFFVSFEGKIVMYKIIYMVLFLFCVALYQVHY 726
                        KYWIYVC GMF  VSF G++V+YKI+YM LFL C+ L+QV+Y
Human PIEZO1      578   KYWIYVCAGMFIVVSFAGRLVVYKIVYMFLFLLCLTLFQVYY 619
M. musculus       583   KYWIYVCAGMFIVVSFAGRLVVYKIVYMFLFLLCLTLFQVYY 624
C. porcellus      586   KYWIYVCAGMFIVVSFAGRLVVYKIVYMFLFLLCLTLFQVYY 627
C. lupus          590   KYWIYVCAGMFIVVSFAGRLVVYKIVYMLLFLLCLTLFQVYY 631
B. taurus         383   KYWIYVCAGMFIVVSFAGRLVVYKIVYMLLFLLCLILFQVYY 424
G. gallus         566   KYWICVCAGMFIVVSFAGRLVVYKIVYMFLFLLCLTLFQVYY 607
X. laevis         584   KYWIYVCGGMFIVVSFAGRLVVYKIVYMLLFLLCLVSFQVYY 625
D. rerio          597   KYWIYVCGGMFIMVSFAGKLVAYKIVYMLLFLLCMCLYQVYY 638
O. niloticus      602   KYWIYVCGGMFIMVSFAGKLVAYKIIYMLLFLLCMCLNQVYY 643
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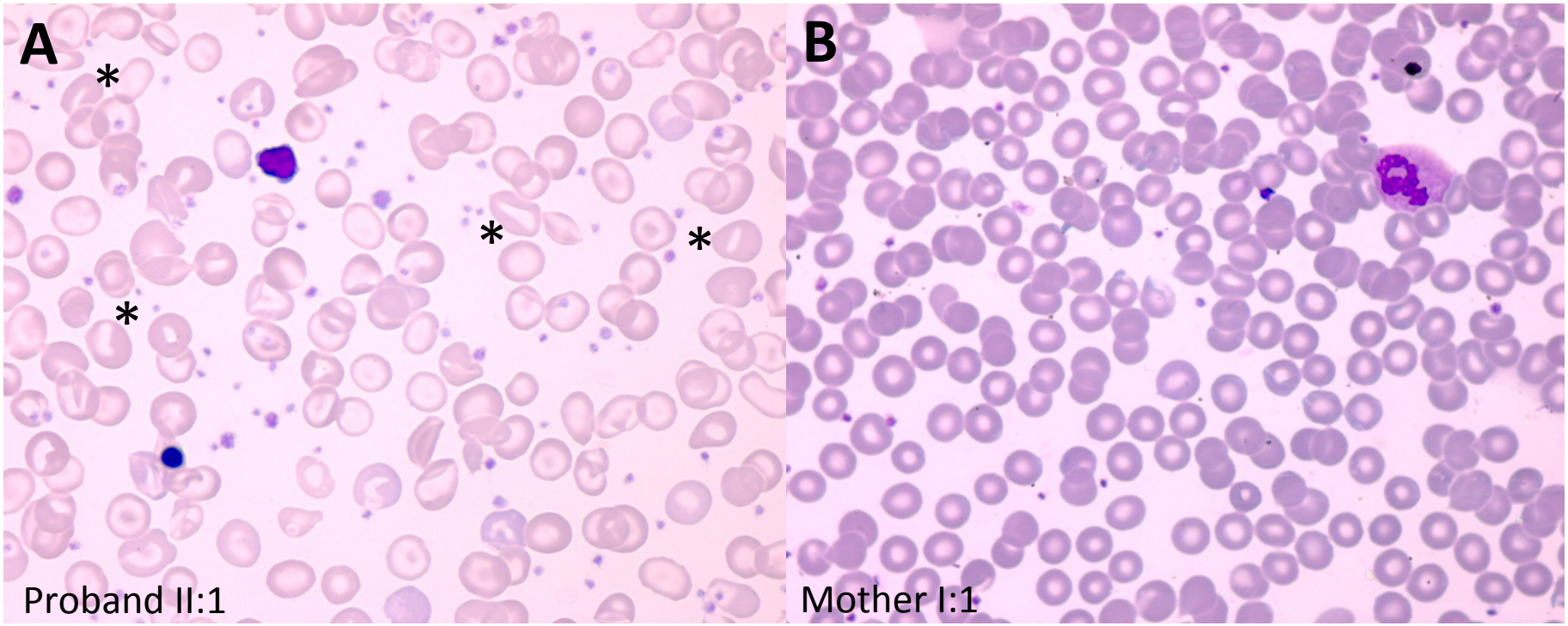
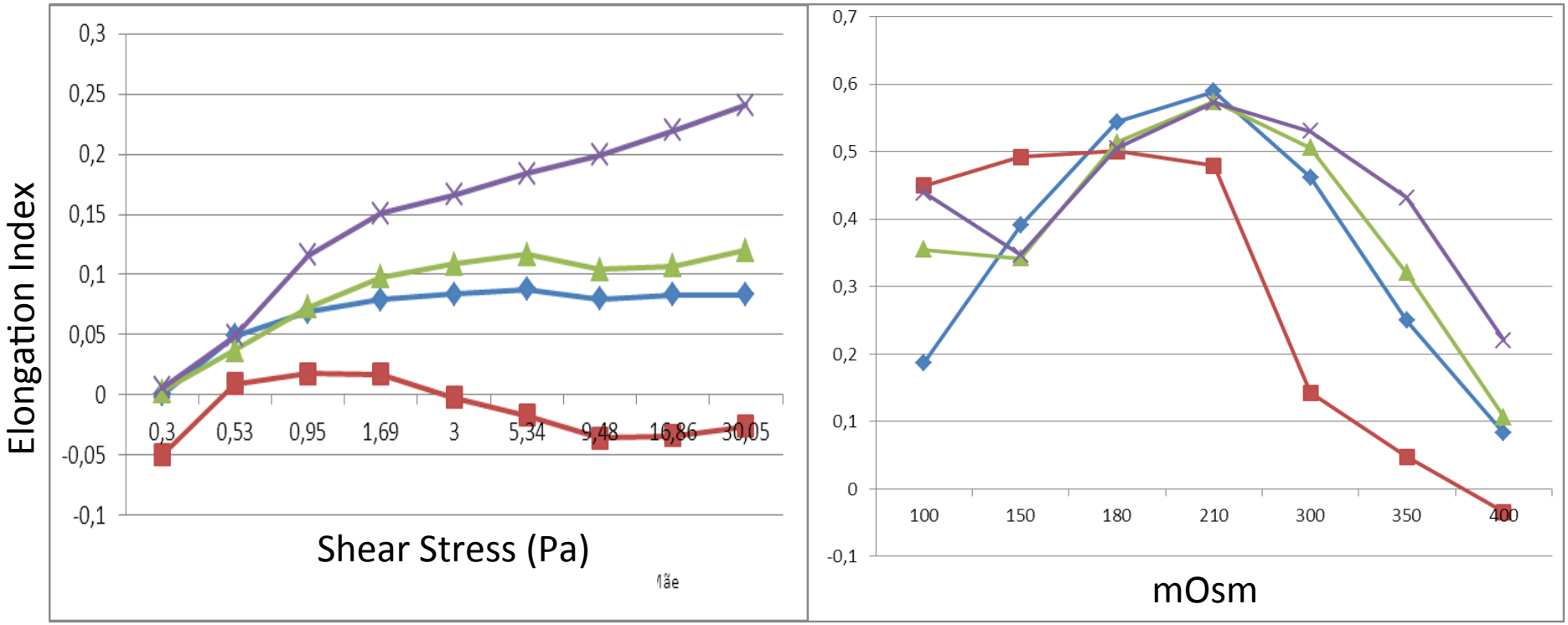
Supplemental
Figure 3



Supplemental
Figure 4

A. Deformability at 400 mOsm with varying shear stress

B. Deformability at 16.86 Pa with varying osmolarity



Control subject
Proband (II:1)
Unaffected Father I:2
Unaffected Mother 1:1