Red blood cell Gardos channel (KCNN4): the essential determinant of erythrocyte dehydration in hereditary xerocytosis

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Supplemental Table 1: Molecular, clinical and hematological characteristics of the 3 HX subjects .

Patients were diagnosed through specialized consultation by a hematologist or a clinical geneticist in CHU Amiens and CHU Bicêtre. These subjects were initially diagnosed using osmolar gradient ektacytometry. Informed consents for genetic analysis were obtained for all patients, according to local institutional ethical boards. Analyses were done on fresh cells at room temperature.

RBC: Red blood cell count, Hb: Hemoglobin, Ht: Hematocrit, MCV: Mean Corpuscular Volume, MCH: mean corpuscular hemoglobin, MCCH: Mean corpuscular concentration of hemoglobin, Retic: reticulocytes count, PHK: Pseudohyperkalemia, PNE: Perinatal Edema, Y=yes, N=no.

Values outside normal range are highlighted with a blue star.

Id	Piezo1 substitution	age	Family History	RBC (10^{12} /L)	Hb (g/dL)	Ht (%)	MCV (fL)	MCH (pg/cell)	MCCH (%)	Retic. (10^9 /L)	PHK	PNE	Ektacytometry at diagnosis (IDmax, Omin and O'mOsm/kg)
1	p.V598M	40	Y	3.60	13.9	0.41	115.0*	38.7*	33.6	346*	Y	Y	Y (0,41,130*, 289
2	p.F681S	42	ND	3.66	13.6	0.37	101.1*	37.2*	36.8*	220*	Y	N	Y (0,53, 123*, 316
3	p.G782S/R808Q	33	Y	5.19	17.1	0.48	92.7	32.9	35.5	261*	Y	Y	Ref [9]

MCV normal range : 80 -100 fL
MCH normal range : 27 - 32 pg/cell
MCCH normal range : 32 -36 %
reticulocytes normal range : 20 - 80 10^9 /L

Ektacytometry normal range
ID max > 0, 4
O min 135 -145 mosmol/kg
O' > 320 mOsm/kg

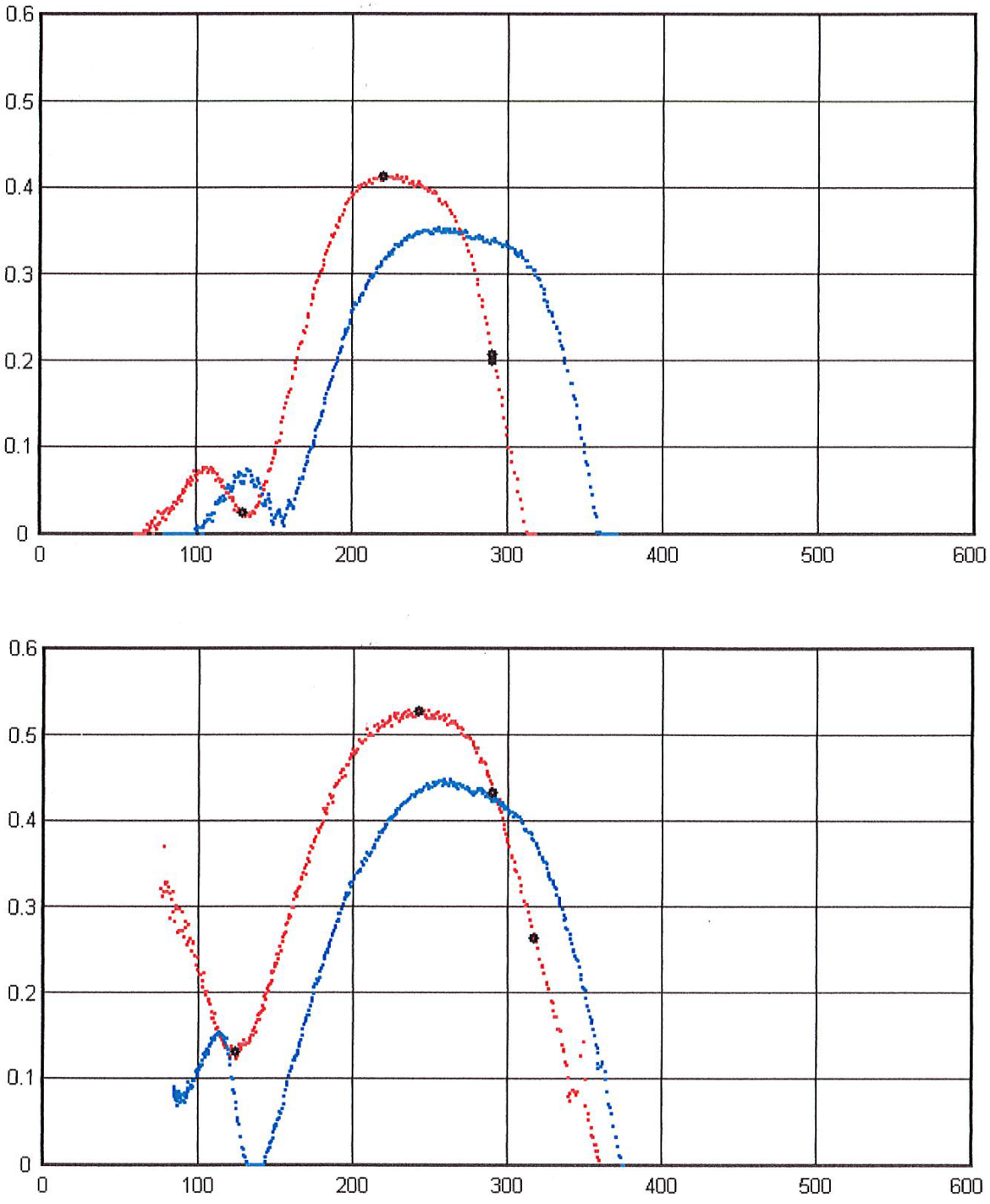
Supplemental table 2: Na^+ and K^+ contents in RBCs from patients and corresponding control at blood reception (t=0).

Data are expressed in μmol per gram of dry cell weight. Data are means±sem, n=6 for V598M and G782S/R808Q and n=3 for F681S Piezo1 mutations. * $p<0.05$, Mann and Whitney test, comparison of mutant versus control, corresponding to normal volunteers.

μmol/g d.w.	Na^+			K^+		
Control	48.1±4.2	52.4±5.6	55.7±1.3	216.3±9.4	237.3±5.1	231.2±2.1
V598M	55.3±0.9			180.5±4.8*		
F681S		64.4±0.8			185.9±1.6*	
G782S/R808Q			57.1±1.6			203.4±9.4*

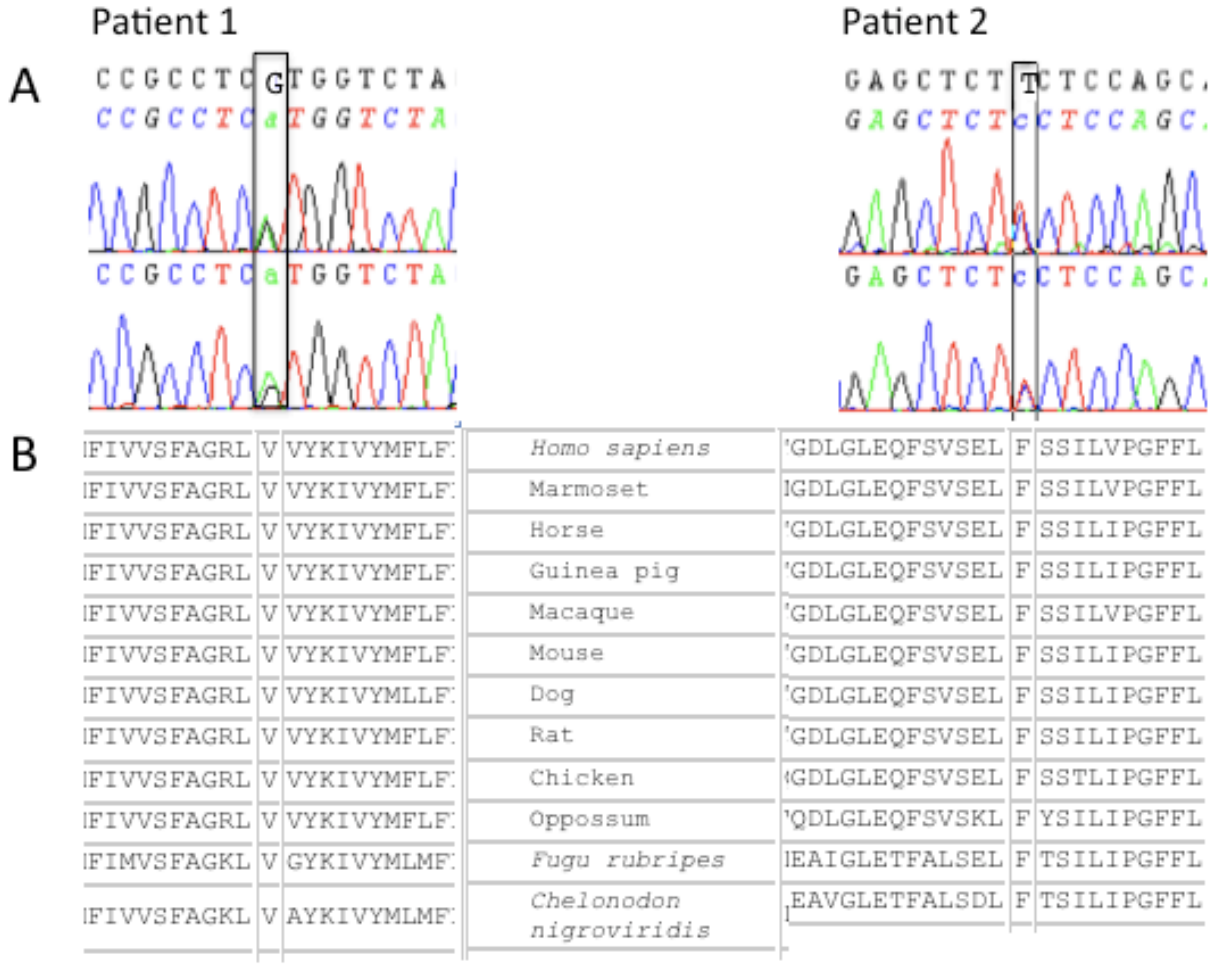
Supplemental Figure 1

Ektacytometric profile of patients 1 (top) and patient 2 (bottom), in red, compared with control, in blue, showing an overall switch of the curve to the left, in agreement with RBC dehydration.



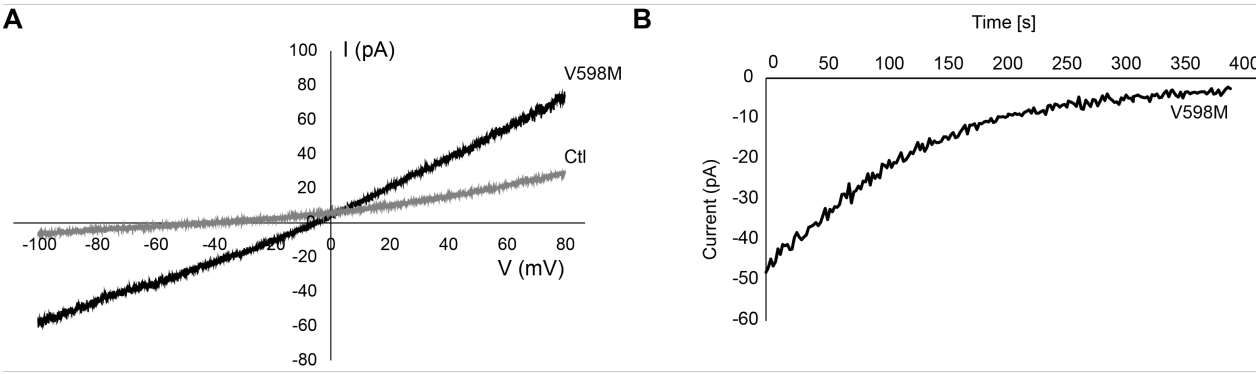
Supplemental Figure 2

PIEZO1 and *KCNN4* coding sequences and intron-exons junctions were analyzed by Sanger sequencing (AbiPrism DNA Analyser 3130 or 3730 and Big Dye Terminator cycle sequencing kit, Applied Biosystems).
A) Identification of two unreported heterozygous mutations in PIEZO1, a c1792G>A mutation in exon 14, leading to pVal598Met substitution (patient 1) and a c2042T>C mutation in exon 16 leading to Phe681Ser substitution (patient 2)
B) Val 598 and Phe681 are highly conserved amino acid residues in Piezo1.



Supplemental figure 3:

A) I/V curves of RBCs with WT Piezo1 (ctl) or V598M mutation just after whole cell configuration is reached. B) Time course of current registered at -80 mV in RBCs with V598M mutation. Representative traces.



Supplemental figure 4: Kinetic of Na^+ and K^+ movements induced by Yoda1 in control red blood cells.

In order to assess the involvement of Piezo1 channel in RBC cation permeability, Yoda1, a Piezo1 activator was used. Control RBCs were treated by 15 μM Yoda1 in presence of ouabain to prevent cation recirculation through the Na^+/K^+ ATPase pump. K^+ (A) and Na^+ (B) contents were measured with or without TRAM-34 as a function of time. Black squares: 15 μM Yoda1; red squares: 15 μM Yoda1 + 10 μM TRAM-34. Data are means±sem, n=3.