

SUPPLEMENTAL MATERIAL

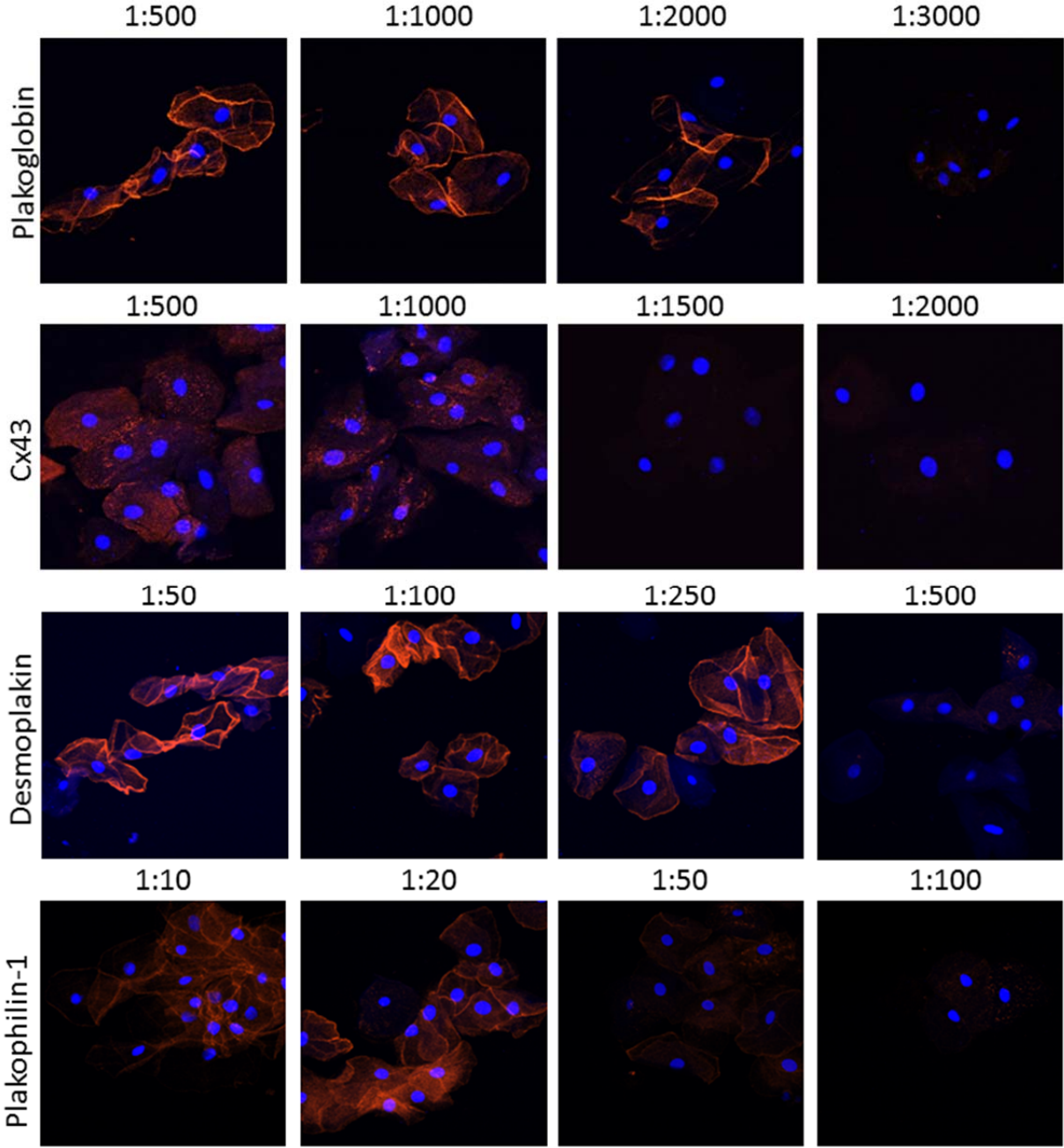
Supplementary Figure Legends

Supplementary Figure 1: Buccal mucosa cells immunostained with different concentrations of antibodies against plakoglobin, Cx43, desmoplakin and plakophilin-1. The optimal dilution for the use of each antibody in buccal smears was defined as the lowest concentration providing bright immunoreactive signal at the cell edges. Cell nuclei (blue) are stained with DAPI.

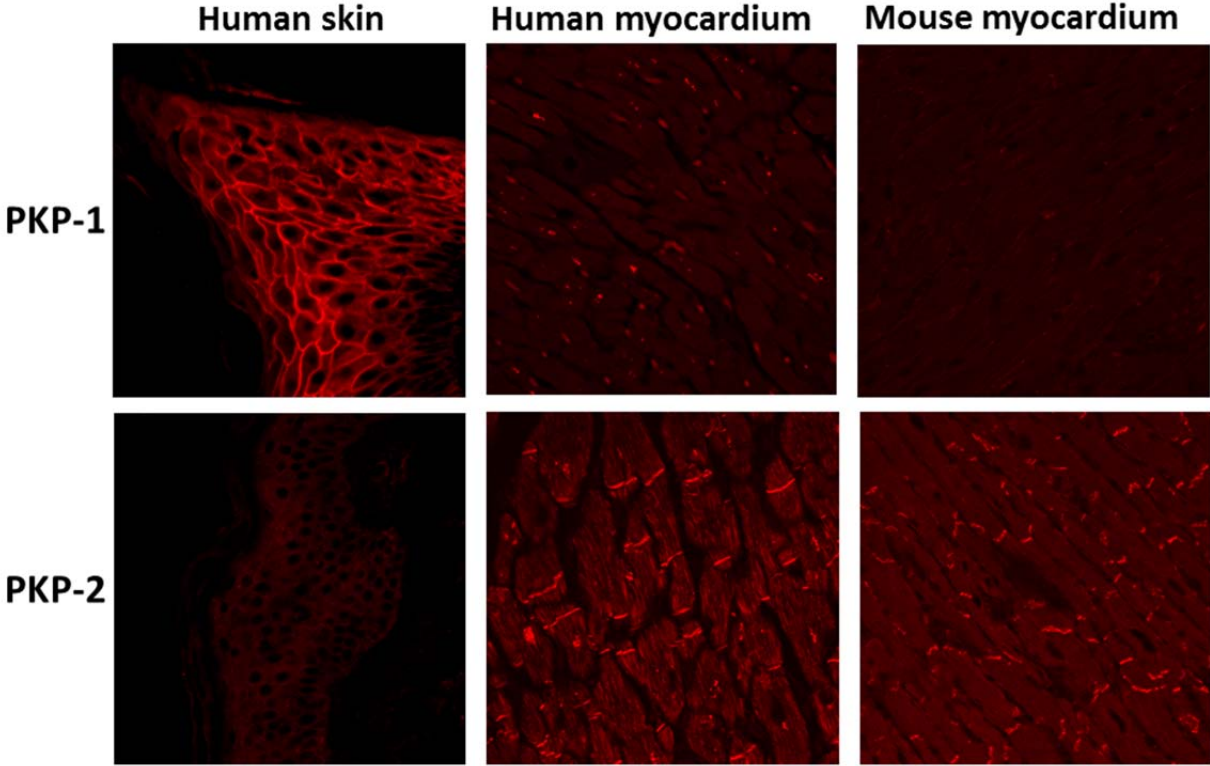
Supplementary Figure 2: Representative images of sections of fixed human skin, human myocardium and mouse myocardium immunostained with antibodies against plakophilin-1 and plakophilin-2 with the same conditions used in buccal mucosa cells smears. Strong junctional signal for plakophilin-1 is seen in only keratinocytes, whereas strong signal for plakophilin-2 is seen only at intercalated discs in myocardium.

Supplementary Figure 3: Representative images of buccal mucosa smears from a control subject, and from family members of ACM probands who carry mutations in *JUP*, *PKP2* and *DSP* but showed no clinical evidence of heart disease. All mutation carriers show loss of junctional signal for plakoglobin and Cx43 in their buccal mucosa cells compared to controls. Cells from the *PKP2* mutation carrier show loss of signal for plakophilin-1 but not desmoplakin, whereas cells from the *DSP* mutation carrier show loss of signal for desmoplakin but not plakophilin-1. Cell nuclei (blue) are stained with DAPI.

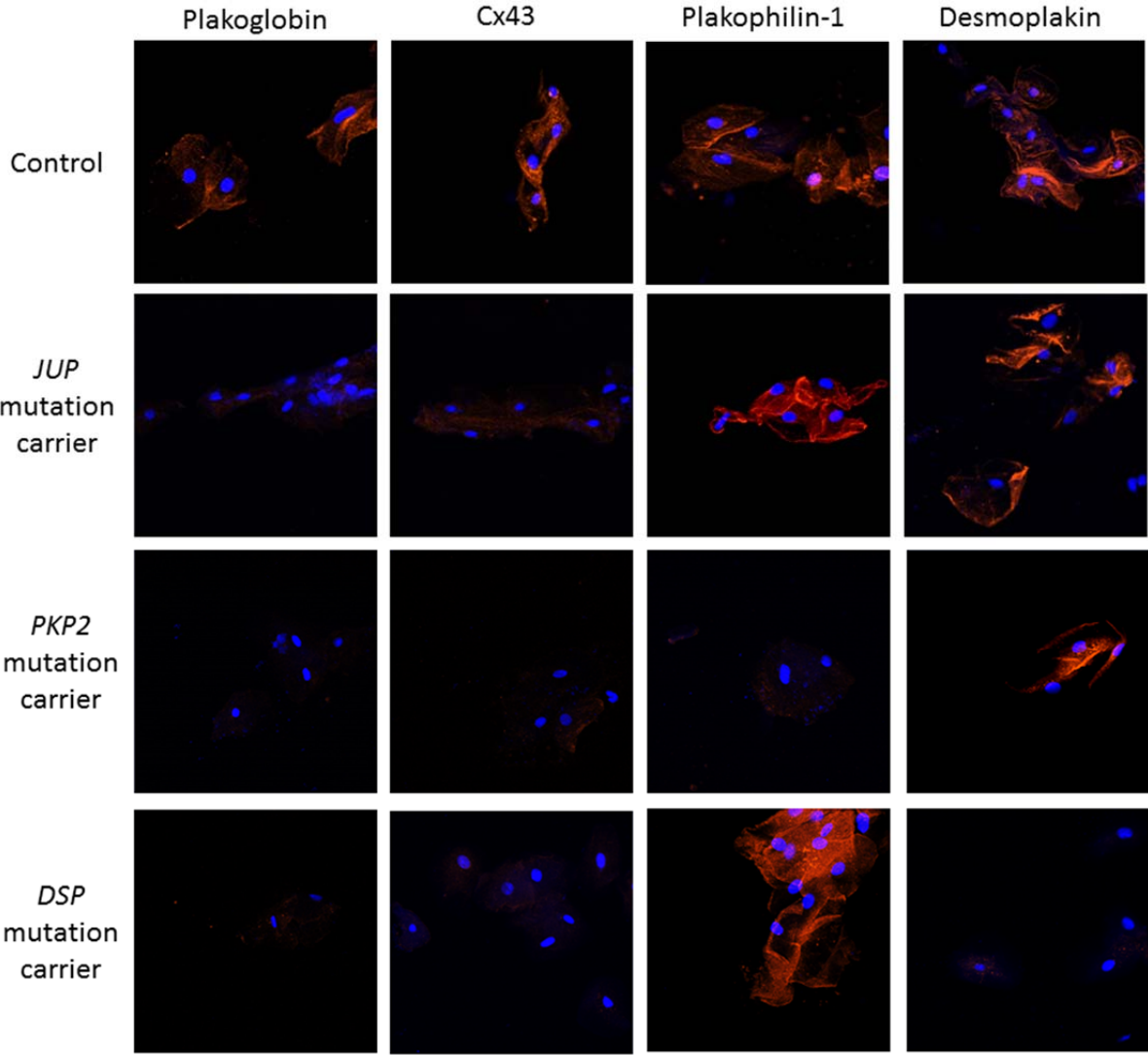
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Table 1. Desmosomal gene mutations in patients (Group 1) and carriers (Group 3).

GROUP 1 (Patients)	Gene (n)	Nucleotide Change	Amino Acid Change	No. of Patients n=39
	PKP2 (25)	c.148_151delACAG	p.Thr50SerfsX61	2
		c.253_256delGAGT	p.Glu85MetfsX26	1
		c.971_980del10	p.Ala324GlyfsX25	2
		c.1237C>T	p.Ala324GlyfsX25	1
		c.1271T>C	p.Phe424Ser	2
		c.1307_1315delins8	p.Leu436HisfsX11	1
		c.1643delG	p.Gly548ValfsX15	1
		c.1821dupT	p.Val608Cysfs	1
		c.1849C>T	p.Gln617X	1
		c.1951C>T	p.Arg651X	1
		c.2013delC	p.Lys672ArgfsX12	2
		c.2145+1G>C	Abnormal splice product	1
		c.2146-1G>C	Abnormal splice product	2
		c.2197_2202delinsG	p.His733AlafsX8	1
		c.2484C>T (homozygous)	Abnormal splice product	1
		c.2489+1G>A	Abnormal splice product	3
		c.2509delA	p.Ser837ValfsX94	2
		DSG2 (4)	c.1038_1040delGAA	p.Lys346del
	c.1520G>A		p.Cys507Tyr	1
	c.2358delA		p.Asp787MetfsX21	2
	DSP (2)	c.3474_3475insA	p.Glu1159ArgfsX3	1
		c.478C>T	p.Arg160X	1
	DSC2 (2)	c.1112-1113delTG	p.Val371GlyfsX8	1
		c.1276G>A	p.Glu426Lys	1
	JUP (6)	c.2157delTG (homozygous)	p.Trp680GlyfsX11	6

GROUP 3 (Carriers)	Gene (n)	Nucleotide Change	Amino Acid Change	No. of Carriers (n=15)
	DSP (2)	c.478C>T	p.Arg160X	1
		c.538G>A	p.Trp180X	1
	DSC2 (1)	c.1276G>A	p.Glu426Lys	1
	JUP (12)	c.2157delTG (heterozygous)	p.Trp680GlyfsX11	12

Abbreviations: *DSC2*: Desmocollin-2; *DSG2*: desmoglein-2; *DSP*: Desmoplakin; *PKP2*: Plakophilin-2; *JUP*: Plakoglobin.

Supplemental Table 2: Summary of sources of primary antibodies and dilution factors used for optimized immunostaining of human buccal mucosa smears.

Protein	Antibody Description	Commercial Source	Catalog Number	Dilution factor
Plakoglobin	Mouse monoclonal	Sigma Aldrich	P8087	1:2000
Plakophilin-1	Mouse monoclonal	Santa Cruz Biotechnology	3G250, sc-71900	1:20
Desmoplakin	Mouse monoclonal	Fitzgerald Industries	10R D108A	1:250
Cx43	Rabbit polyclonal	AbCam	ab11370	1:1000
Plakophilin-2	Mouse monoclonal	Fitzgerald Industries	10R-P130b	undiluted