Distribution and Expression of Non-Neuronal Transient Receptor Potential (TRPV) Ion Channels in Rosacea

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Supplementary Information

Supplementary Figure S1

Supplementary Tables S1-S3

Supplementary Figure 1 (Figure S1)

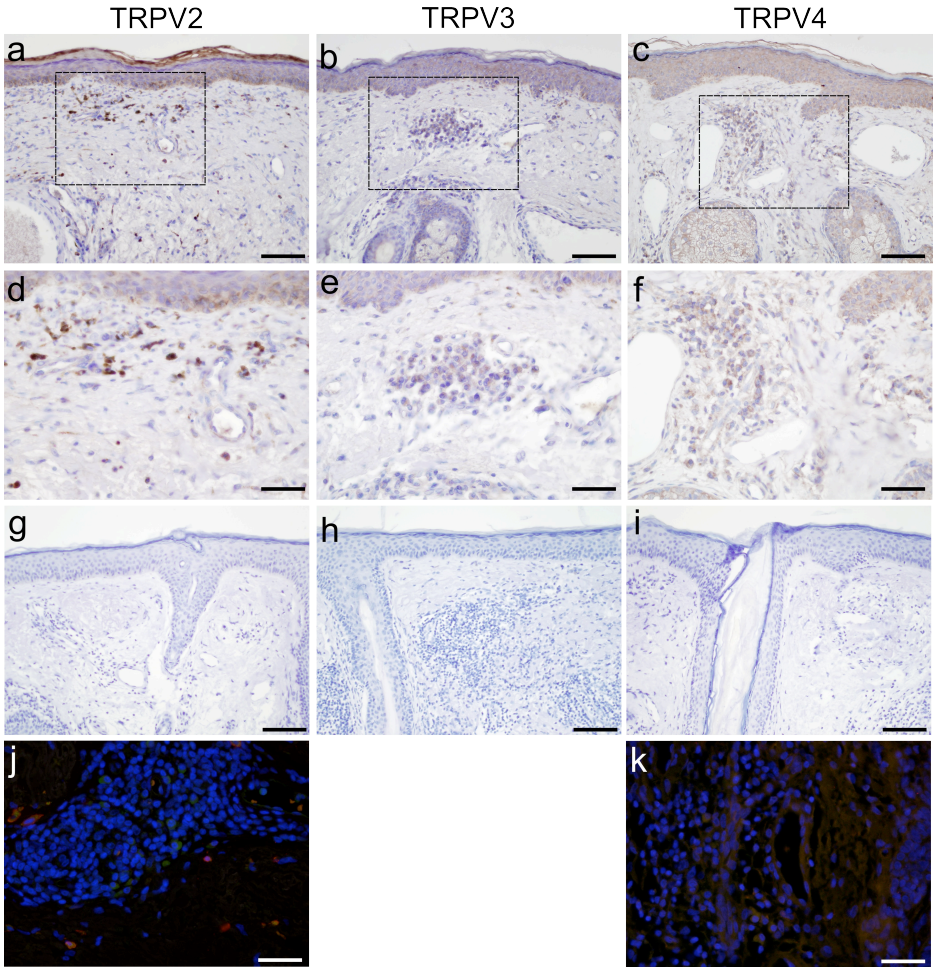

Figure S1: Demonstration of antibody specificity for TRPV2, TRPV3, and TRPV4.

We determined the specificity on the protein level by using another different primary antibody against TRPV2 (a, d), TRPV3 (b, e), TRPV4 (c, f), which all showed a similar staining pattern in PhR as compared to Figures 1-3. As negative controls, primary antibodies were omitted and immunostaining was absent for rabbit-TRPV2 in ETR (g), sheep-TRPV3 in LE (h) and rabbit-TRPV4 in ETR (i). Pre-immune absorption control for double-immunoflourescence shows absence of TRPV2 (j) and TRPV4 (k) in PPR demonstrating specificity of immunostaining. Scale bars: 100µm (a-c, g-i), 50µm (d-f), 40µm (j-k).

Supplementary Table 1: Summary of TRPV-staining in rosacea-affected skin compared to normal skin

E = semiquantitative analysis of epidermal staining intensity; D = semiquantitative analysis of immunoreactivity in the dermal compartment; qRT-PCR = gene expression. (+ = upregulation, P<0.4; ++ = P<0.2, +++ = P<0.05; - = downregulation, P<0.4; -- = P<0.2; --- = P<0.05, n.i. = not investigated/performed, N = normal/no changes)

	LE		**ETR**			**PPR**			**PhR**		
	E	D	E	D	qRT-PCR	E	D	qRT-PCR	E	D	qRT-PCR
TRPV1	n.i.	n.i.	n.i.	n.i.	+++	n.i.	n.i.	++	n.i.	n.i.	+++
TRPV2	N	+	N	+++	+	-	+++	+++	---	+	++
TRPV3	N	+	N	+++	++	-	++	++	-	+++	+++
TRPV4	N	N	N	+	N	N	+++	N	+	+++	-

Supplementary Table 2:

Antibodies used for (double-) immuohistochemistry

Antibody	Host	Dilution	Source
TRPV2 (Figure 1)	rabbit	1:300	AP07065PU-N; Acris Antibodies, Herford, Germany
TRPV2 (Figure S1)	rabbit	1:200	PAB14319; Abnova, Heidelberg, Germany
TRPV3 (Figure 2)	sheep	1:1000	GT15180; Neuromics Antibodies, Edina, MN
TRPV3 (Figure S1)	rabbit	1:100	AP11388PU-N; Acris Antibodies
TRPV4 (Figure 3)	rabbit	1:4000	ab63003; Abcam, Cambridge, UK
TRPV4 (Figure S1)	rabbit	1:1200	NB110-55614; Novus Biologicals, Cambridge, UK
CD4	mouse	1:20	anti-human CD4 IgG1; Biozol, BZL 02416, Eching, Germany
CD68	mouse	1:20	anti-human CD68; DAKO M0876, DAKO, Hamburg, Germany
Mast Cell Tryptase	mouse	1:100	Monoclonal Mouse Anti-human Mast Cell Tryptase; DAKO M7052
Secondary Anti Rabbit (IHC)	goat	1:100	EnVision+ System- HRP Labelled Polymer; DAKO
Secondary Anti Sheep (IHC)	donkey	1:100	EnVision+ System- HRP Labelled Polymer; DAKO
Secondary Anti Rabbit (DIF)	chicken	1:200	Alexa Fluor 488 chicken anti-rabbit IgG; Molecular Probes, Inc., Eugene, Oregon
Secondary Anti Mouse (DIF)	donkey	1:200	Alexa Fluor 555 donkey anti-mouse IgG; Molecular Probes, Inc.

Supplementary Table 3:

Ready-to-use Taqman® Gene Expression Assay-Numbers

Ready-to-use TaqMan® Gene Expression Assay	No.
TRPV1	Hs00218912_m1
TRPV2	Hs00275032_m1
TRPV3	Hs00376854_m1
TRPV4	Hs00222101_m1
GAPDH	Hs99999905_m1
ACTB	Hs99999903_m1
HPRT	Hs99999909_m1